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Modelling the transmission of and effectiveness of control measures for *Mycobacterium avium* subsp. *paratuberculosis* in dairy herds

Transmission de *Mycobacterium avium* subsp. *paratuberculosis* en troupeau bovin laitier et efficacité de mesures de maîtrise : une approche par modélisation

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General introduction

In our modern societies, farming has transformed into a business, where farmers have to look at improving the efficiency of their productions. Since they have little control on the price of the products they sell, one of their principle mean of action to improve profit margins is to control and reduce costs. This can be achieved by improving animal health thanks to the decrease in both production losses and medicine use. This is one significant progression track in dairy production where the need of competitiveness is increased by the fact that dairy cattle farmers currently have to face a decrease in the price of milk. Animal health is all the more important to the farming business that, nowadays, consumers want to eat 'healthy products from healthy animals'. There is here a large stake for the dairy industry in order to keep the positive image of milk as healthy food (Noll and Vignal, 2007).

Paratuberculosis (also called Johne's disease) is a chronic and progressive intestinal inflammatory disease in cattle and other ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (*Map*). It is widely distributed in the world (Kennedy and Benedictus, 2001). There is currently no treatment for this chronic enteritis in ruminants. It induces a decrease in protein absorption which results in a decrease in milk production, weight loss, profuse diarrhoea and death if the animal is not culled before (Patterson, *et al.*, 1967; Julian, 1975; Chiodini, *et al.*, 1984). In affected herds, the losses can be so large that profitable farming cannot be carried on (Chiodini, *et al.*, 1984; Benedictus, *et al.*, 1987). There is thus a real need for the implementation of adapted disease control or eradication programmes.

A modelling approach is relevant when studying *Map* spread. Epidemiological models are indeed suitable to study the transmission of the bacteria within a herd and the impact of control programmes. Since the development of the disease takes several years subsequent to a young animal becoming infected, it is difficult to carry out field studies assessing the transmission of the disease. This is all the more difficult as the diagnostic tests currently available for defining infection status are imperfect, with a sensitivity of either direct or indirect tests between 0.13 and 0.94 depending on the infection stage (Nielsen and Toft, 2008). Also, clinical signs due to *Map* infection are not specific and differential diagnostic has to be made in field studies. Furthermore, several control measures can be combined and their efficacy can differ depending on the management of the farm (contacts between animals and hygiene, especially): assessing the different combinations of possible control measures in different herd contexts would thus require comparing many different situations. Modelling enables to perform such a task in a limited and reasonable amount of time and money. A modelling approach is helpful for decision making in the development of control programmes.

In order to reach both the demand of the consumers on healthy products and on low prices, it appears pivotal to organise the most profitable control programme in the industry today. The aim of this thesis is to support decision makers in the design and development of control programmes for

paratuberculosis. Decisions makers can be defined at different levels: mainly farmers for their own herd (management at herd level) when it comes to paratuberculosis which is usually not regulated, and sometimes farmers' organisation in a region. Here, the objective is thus to evaluate the epidemiological and economic effectiveness of selected control programmes of paratuberculosis in dairy herds. Herd level control programmes based on surveillance, systematic testing and culling of infected animals are studied. Systematic testing in a dairy herd is especially assessed in terms of effectiveness to prevent *Map* persistence and reduce prevalence. This is done thanks to the use of modelling at a herd level.

1 Paratuberculosis in dairy cattle

1.1 Definitions

Different infection conditions can be differentiated. As there is only limited consistency in the literature about the definitions of the terms employed, we provide here a definition of terms that will be used throughout the manuscript of the thesis. Susceptible and resistant animals are animals who are not infected. Susceptible animals can get the infection while resistant animals cannot. When infected, cattle can be transiently infectious, latently infected, subclinically infected, or clinically affected. Transiently infectious cattle are infectious animals shedding *Map* only during a limited period of time. Latently infected cattle are infected but not infectious cattle. Subclinically infected cattle are infected and infectious animals that do not present clear clinical signs and therefore are hard to observe. Finally, clinically affected cattle are infected, infectious and affected. Infected, infectious and affected terms are infection conditions defined by Nielsen & Toft (Nielsen and Toft, 2008).

1.2 Distribution of the disease

The infection has been described in most of the intensive cattle production systems around the world (Kennedy and Nielsen, 2007; Guicharnaud, 2009). The proportion of infected herds reported by different studies varies from 10 to 70% and the estimated prevalence of infected animals is generally below 5% (McNab, *et al.*, 1991; Collins, *et al.*, 1994; Thorne and Hardin, 1997; Boelaert, *et al.*, 2000; Gasteiner, *et al.*, 2000; Muskens, *et al.*, 2000; Pak, *et al.*, 2003; Van Schaik, *et al.*, 2003; Nielsen and Toft, 2009). A recent review of paratuberculosis prevalence in Europe reports that the animal level prevalence is around 20% (estimate based on different studies using different diagnostic tests), or a minimum of 3 to 7% if based on methods detecting *Map* directly or based on occurrence of pathological changes in tissues (Nielsen and Toft, 2009). Another recent study aiming at estimating the true prevalence (infected animal prevalence, herd prevalence and within-herd prevalence) of paratuberculosis in dairy herds in the world while assessing the reliability of

the results reports that true individual prevalence can be very high, especially in America (Figure 1).

In Europe, this corrected individual prevalence remains at a medium level, except for Sweden and Norway where it is low (almost zero in dairy cattle) (Figure 2). At the herd level, the corrected prevalence is still high in a large number of countries in America while it is variable in Europe. There is little data on distribution of within-herd prevalence estimates. It appears that within-herd prevalence is on average low (1-30%) but can reach high values in some herds (60% and more). Globally, estimates of prevalence are probably under-estimated as the sensitivity and specificity of the diagnostic tests used are low and high, respectively, for the levels of prevalence studied. For the herd prevalence, the lack of sensitivity is particularly compensated by repeated testing of all the animals.

1.3 Economic impact

Worldwide, paratuberculosis is of economic importance for dairy producers. In affected herds, economic losses result from reduced milk production, mortality or premature culling, and lower slaughter value of clinically affected cows (Benedictus, *et al.*, 1987; Johnson-Ifearulundu and Kaneene, 1997; Lombard, *et al.*, 2005). Additionally (although there is no clear evidence yet), *Map* possible involvement in Crohn's disease in humans is still under consideration. Humans could be exposed to *Map* via several routes such as milk and meat products, or water contamination (Eltholth, *et al.*, 2009). One way or the other, the wholesome image of the dairy industry might be threatened by paratuberculosis. All of these parameters increase the need for effective and economically attractive control programmes against paratuberculosis.

Paratuberculosis can have substantial effects at the farm, region and country level. An economic loss of almost 95€ per cow is reported for positive herds compared to negative herds due to reduced productivity; this loss being up to 190€ per cow when the prevalence of clinically affected animal is above 10% in the positive herds (Ott, *et al.*, 1999). Other studies reported average losses per cow on infected farms per year varying from 33€ to more than 67€ in The Netherlands (Groenendaal and Galligan, 1999), 32€ in Canada (Tiwari, *et al.*, 2008), 7€ for parity 1 cows, 122€ for parity 2 cows, and 221€ for parity 3 cows in the United-States (Wilson, *et al.*, 1995). Overall estimates of the economic loss vary widely depending on production and pricing systems. At a national level, a loss of 21€ to 25€ per cow or 190 to 234 million of euros per year was reported for the US dairy industry (Ott, *et al.*, 1999), 0.58€ million for Canadian maritime provinces (Chi, *et al.*, 2002; McKenna, *et al.*, 2006), 40€ million for The Netherlands (Kennedy and Benedictus, 2001), 8€ million for the New Zealand (Kennedy and Benedictus, 2001), and around 2.3€ million for Australia (Kennedy and Benedictus, 2001) (currency converted to € values at 15 May of the year of the publication of the papers). It is likely that these estimates are underestimated as it is difficult to estimate the direct costs of subclinical infection when available diagnostic tests have a very low

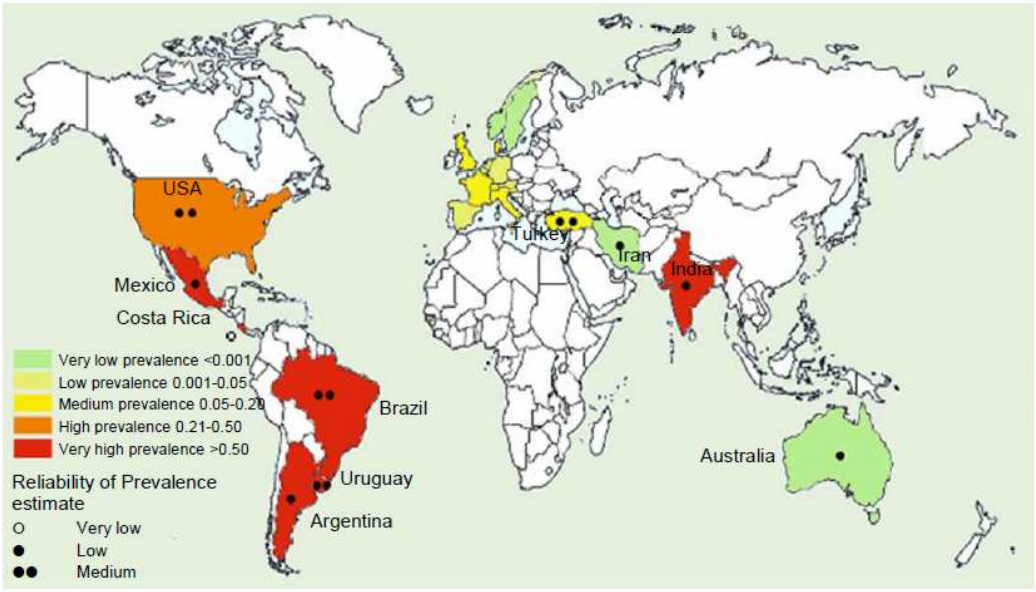

Figure 1: Individual animal prevalence of paratuberculosis infected dairy cattle in the world (Guicharnaud, 2009): summary of published estimates.

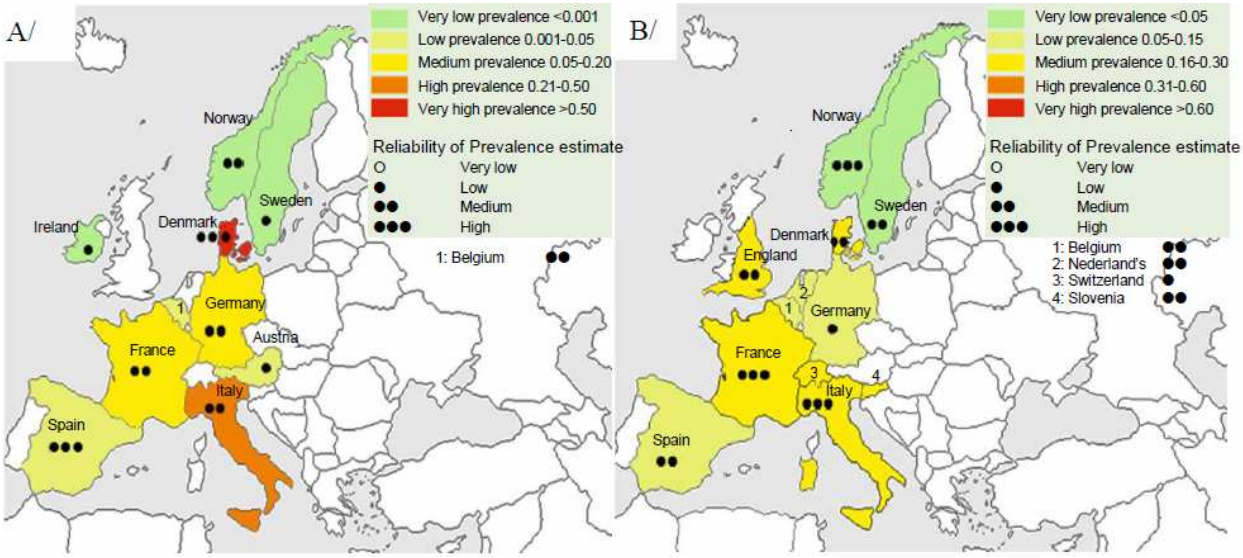

Figure 2: A/ Prevalence of paratuberculosis infected dairy cattle herds in Europe, B/ Individual animal prevalence of paratuberculosis infected dairy cattle in Europe (Guicharnaud, 2009): summary of published estimates.

sensitivity. It is also difficult to estimate indirect inapparent costs such as the loss of genetic potential through early culling and trading restrictions (Kennedy and Benedictus, 2001).

2 Control of paratuberculosis

The control of a disease usually necessitates different measures to reduce the persistence, prevalence or incidence of an infection or a disease in a known infected population. Controlling a disease can be here understood as reducing the frequency of *Map* infection to a level biologically or economically justifiable (Martin, *et al.*, 1987; Dowdle, 1998). Sometimes the elimination of the disease is targeted (no clinically affected animals on the farms), while sometimes even infected but not affected animals are targeted. Elimination of the infection from the population, which is the reduction to zero of the incidence of infection caused by a specific pathogen in a defined geographical area as a result of deliberate efforts (Dowdle, 1998), is not necessarily targeted or possible.

There is no treatment available for paratuberculosis. A possible way to control the disease is then to protect susceptible animals from *Map*-exposure by controlling the transmission of *Map* and to eliminate infectious animals (Kennedy and Benedictus, 2001; McKenna, *et al.*, 2006). Several European countries have been trying for several years to eliminate the infection based on organized control programmes mainly using test-and-cull measures. But these programmes are not sufficiently effective for clearing farms (Kennedy and Benedictus, 2001) and are very costly for the whole industry: their profitability is unsatisfactory. Programmes are now oriented towards control and not eradication. These measures are for example to separate neonates and dams within 12-24 hours after birth, to ensure that neonates receive colostrum only from their negative-tested mother or from animals that have tested negative, to rear young calves in a clean environment, to use milk replacer or pasteurised / sterilised milk (Kennedy and Benedictus, 2001). The efficacy and effectiveness of these measures probably vary depending on herd characteristics. However, neither their epidemiological effectiveness (capacity to decrease disease frequency) nor their economic effectiveness (positive return on investment in an acceptable amount of time) has yet been thoroughly assessed in the field to provide advice.

2.1 Existing programmes

Programmes are implemented in different countries but their objectives differ. Two recent studies show through questionnaire implementation at national or regional levels that the objectives associated to national or regional programmes can be very different (large variety of initiatives) and are often not clearly or explicitly defined (Coursaget, 2009; Nielsen, 2009). Announced objectives could be i/ to describe the situation of the country, ii/ to control the disease or *Map* transmission, iii/ to eradicate *Map*; iv/ as a precaution for food

safety. The lack of a clear definition of target conditions is reported to confuse the interpretation of the information collected in the questionnaires (Coursaget, 2009; Nielsen, 2009). There is also little information on the participation level in the different programmes and a lack of documentation of the results of these programmes.

In Japan, Norway, Sweden and Austria, active surveillance programmes are implemented as paratuberculosis is a notifiable disease. These countries have implemented mandatory national programmes. They are mainly focusing on animals with clinical disease. In other countries in which paratuberculosis is also a notifiable disease (some countries of Eastern and Northern Europe, Australia, New Zealand, New Caledonia, Mozambia, Namibia, South Africa, Swaziland, Mexico, Argentina, Chile, Iran, Israel, Korea, Malaysia, Taiwan), there is no obligation to test animals with clinical signs. Some countries such as Australia, Austria, Canada, Czech Republic, Denmark, Italy, Japan, Norway, The Netherlands, Sweden, and The USA implement control programmes in infected herds (Benedictus, *et al.*, 2000; Kennedy and Allworth, 2000; U.S.D.A., 2006; Nielsen, 2007; Ferrouillet, *et al.*, 2009). These programmes are mainly voluntary and targeted animals differ depending on the country. In France as well as in Spain, such programmes exist only at a regional level. Finally, accreditation or certification programmes (non infected herds) are also implemented in several countries such as Austria, Canada, France, Italy, The Netherlands, The United-Kingdom, and The USA. In other countries, reasons for not implementing any programmes vary: it is reported not to be a priority in the country (economic issue), the diagnostic is considered too difficult and not reliable, paratuberculosis is not considered to be a problem, there is no disease in the country, or the prevalence is not known.

2.2 Difficulties encountered

When control programmes have been implemented, the compliance to recommended measures has often been reported to be poor (Wraight, *et al.*, 2000; Muskens, *et al.*, 2003; Ridge, *et al.*, 2005; Coursaget, 2009; Taisne, 2009; Nielsen and Toft, 2010). Technical and material restraints, lack of knowledge and farmer's perception and belief have been identified as playing a role in this lack of compliance (Coursaget, 2009; Taisne, 2009). Low tests sensitivity and the difficulty of their interpretation make *Map* infection control difficult. The definition of objective criteria for the evaluation of the effectiveness of control programmes are lacking and the results of implemented control programmes cannot be expected within a short time frame (long time necessary for the development of the disease) which can be disheartening for the farmer or the industry as a whole. Moreover, depending on the control programme implemented, the cost of the implementation can be high, with a lot of constraints due to the test-and-cull programme in which culling can be too frequent to be economically sustainable.

To increase the level of compliance, communication and farmers' consciousness raising should be improved (Sorge, *et al.*, 2010). Vet

practitioners should be more involved in the control programmes and advices should be coordinated amongst all the stakeholders. As farmers usually do not implement all advised control measures but the two or three easiest one, control measures should be prioritised and adapted to each farm management (Ridge, *et al.*, 2005; Coursaget, 2009; Taisne, 2009; Benjamin, *et al.*, 2010; Sorge, *et al.*, 2010).

3 Objective of the thesis

Because of the economic losses due to *Map* infection and food safety concerns, the need for the development of effective and economically viable control programmes against paratuberculosis is real. There is particularly a need in ranking the different control measures that can be implemented in a specific farm. The final objective of the thesis is thus to evaluate the epidemiological and economic effectiveness of paratuberculosis selected control programmes in infected dairy cattle herds, or in other words to investigate potential improvements in the control of *Map* infection leading to a decrease in the losses due to the infection at the herd level whilst keeping the costs of such programmes at a reasonable level. Systematic testing of a herd is particularly studied in order to assess its impact on *Map* persistence, on the prevalence of infectious adults, and on gross margins and return on investment. The increase of control costs and the decrease of production losses can indeed be taken into account with these outputs, as well as the time necessary to reach a definite level of prevalence or an economically viable investment.

To reach this final objective, different prior objectives are defined. They are gathered in Figure 3.

A review of the different models of *Map* within-herd transmission is first performed in order to assess whether it is necessary to build a new model to reach our final objective (Chapter 1). In order to represent *Map* indirect transmission via the environment and calf-to-calf transmission, a new epidemiological model is built (Chapter 2). This model is then used to better understand the transmission of *Map* within a dairy herd, especially the relative contribution of the different routes of transmission, and why sometimes the infection spontaneously fade out while sometimes a persistent infection occurs when no control measure is implemented. The population dynamics of a dairy herd is represented. It is indeed necessary to consider the structure of contact when indirect transmission via the environment exists. In dairy herds, calves and adults are generally housed separately. This results in the separation of susceptible animals (calves) and the main shedding animals (adults). The main dairy calf housing systems across Europe are assessed (Chapter 3). This allows validating the choices performed in the epidemiological model in terms of calf housing facilities. The impact of contact structure on *Map* indirect transmission is assessed with the new epidemiological model (Chapter 4). Finally, a bioeconomic model is built from the epidemiological model and a pre-existent economic herd simulator.

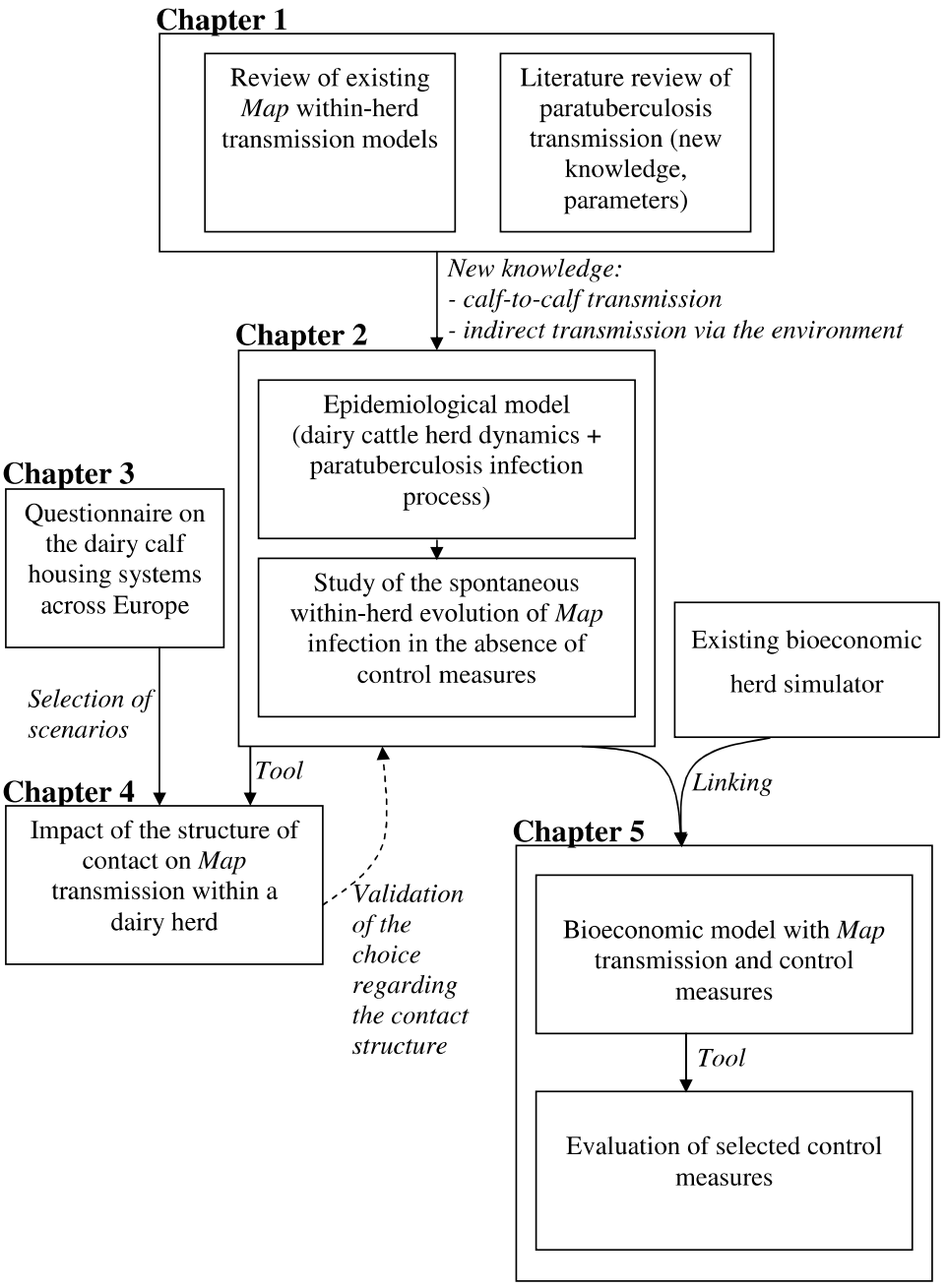

Figure 3: Outline of the PhD project

This final model is used to assess the effectiveness of selected control measures of *Map* infection (Chapter 5). While it is expected to obtain epidemiological results such as a decrease in the prevalence when control measures are implemented, this cannot come at any price. Both the results in terms of prevalence and persistence, and gross margin (taking simultaneously into account the increase of control measures and the decrease of production losses) of implemented control measures need to be assessed.

4 Outline of the thesis

Chapter 1 is a review of existing models of within-herd transmission of *Map* in dairy herds. Current scientific knowledge about *Map* transmission is summarized and then used to evaluate the validity of the models described in the scientific literature (Marcé, *et al.*, 2010b).

Chapter 2 describes the development and structure of an epidemiological stochastic simulation model. This model is used in this chapter to study the spontaneous within-herd evolution of *Map* infection in the absence of control measure (Marcé, *et al.*, 2010c).

Chapter 3 describes dairy calf housing systems across Europe and how the diversity of described housing systems influences the transmission of calf infectious diseases (Marcé, *et al.*, 2010d).

Chapter 4 describes the effects of within-herd contact structure on *Map* spread in a persistently infected dairy herd in which no control measures are implemented; these effects being studied thanks to the model presented in chapter 2 (Marcé, *et al.*, 2010a).

Chapter 5 describes the development and structure of an economic stochastic herd simulation model. In addition, the epidemiological and economic effectiveness of a range of possible control strategies based on a Test-&-Cull programme and/or improved hygiene are evaluated. The interest and impact of active and passive surveillance on limiting persistent infection and high prevalence at a reasonable cost are indeed studied.

Finally, chapter 6 provides a general discussion on the PhD project. It presents the main results related to the objectives of the thesis and their field application. The modelling approach chosen is discussed as well as the evaluation of the results. Prospects are also presented.

A summary of the thesis both in English and in French is available at the end of the thesis.

References

United States Department of Agriculture (USDA), Uniform program standards for the voluntary bovine Johne's Disease control program, [on line] (2006), http://www.aphis.usda.gov/animal_health/animal_diseases/johnes/downloads/johnes-umr.pdf [consulted 15/09/2009].

Benedictus, G., Dijkhuizen, A. A. and Stelwagen, J., Economic losses due to paratuberculosis in dairy cattle, Vet. Rec. (1987) 121:142-146.

Benedictus, G., Verhoeff, J., Schukken, Y. H. and Hesselink, J. W., Dutch paratuberculosis programme history, principles and development, Vet. Microbiol. (2000) 77:399-413.

Benjamin, L. A., Fosgate, G. T., Ward, M. P., Roussel, A. J., Feagin, R. A. and Schwartz, A. L., Attitudes towards biosecurity practices relevant to Johne's disease control on beef cattle farms, Prev. Vet. Med. (2010) 94:222-230.

Boelaert, F., Walravens, K., Bironf, P., Vermeersch, J. P., Berkvens, D. and Godfroid, J., Prevalence of paratuberculosis (Johne's disease) in the Belgian cattle population, Vet. Microbiol. (2000) 77:269-281.

Chi, J., VanLeeuwen, J. A., Weersink, A. and Keefe, G. P., Direct production losses and treatment costs from bovine viral diarrhoea virus, bovine leukosis virus, *Mycobacterium avium* subspecies *paratuberculosis*, and *Neospora caninum*, Prev. Vet. Med. (2002) 55:137-153.

Chiodini, R. J., Van Kruiningen, H. J. and Merkal, R. S., Ruminant paratuberculosis (Johne's disease): the current status and future prospects, Cornell Vet. (1984) 74:218-262.

Collins, M. T., Sockett, D. C., Goodger, W. J., Conrad, T. A., Thomas, C. B. and Carr, D. J., Herd prevalence and geographic distribution of, and risk factors for, bovine paratuberculosis in Wisconsin, J. Am. Vet. Med. Assoc. (1994) 204:636-641.

Coursaget, S., Review of paratuberculosis action programmes in Western France, Doctorate in Veterinary Medicine Thesis, Nantes, (2009).

Dowdle, W. R., The principles of disease elimination and eradication, Bull. world Health Organ. (1998) 76:23-25.

Eltholth, M. M., Marsh, V. R., Van Winden, S. and Guitian, F. J., Contamination of food products with *Mycobacterium avium paratuberculosis*: a systematic review, J. Appl. Microbiol. (2009) DOI: 10.1111/j.1365-2672.2009.04286.x.

Ferrouillet, C., Wells, S. J., Hartmann, W. L., Godden, S. M. and Carrier, J., Decrease of Johne's disease prevalence and incidence in six Minnesota, USA, dairy cattle herds on a long-term management program, Prev. Vet. Med. (2009) 88:128-137.

Gasteiner, J., Awad-Masalmeh, M. and Baumgartner, W., *Mycobacterium avium* subsp. *paratuberculosis* infection in cattle in Austria, diagnosis with culture, PCR and ELISA, Vet. Microbiol. (2000) 77:339-349.

Groenendaal, H. and Galligan, D. T., Economic consequences of Johne's disease control programs, School of Veterinary Medicine, University of Pennsylvania. (1999) Center of Animal Health and Productivity Technical Report, pp. 52.

Guicharnaud, M., Prevalence of paratuberculosis in dairy cattle herd worldwide: review and analysis [in French], Doctorate in Veterinary Medicine Thesis, Nantes, (2009).

Johnson-Ifearulundu, Y. J. and Kaneene, J. B., Epidemiology and economic impact of subclinical Johne's disease: a review, Vet. Bull. (1997) 67:437-447.

Julian, R. J., A short review and some observations on Johne's disease with recommendations for control, Can. Vet. J. (1975) 16:33-43.

Kennedy, D. and Nielsen, D. D., Report from the first IDF ParaTB forum, Bulletin of the International Dairy Federation. (2007) 410:3-7.

Kennedy, D. J. and Allworth, M. B., Progress in national control and assurance programs for bovine Johne's disease in Australia, Vet. Microbiol. (2000) 77:443-451.

Kennedy, D. J. and Benedictus, G., Control of *Mycobacterium avium* subsp. *paratuberculosis* infection in agricultural species, Rev. sci. tech. Off. int. Epi. (2001) 20:151-179.

Lombard, J. E., Garry, F. B., McCluskey, B. J. and Wagner, B. A., Risk of removal and effects on milk production associated with paratuberculosis status in dairy cows, J. Am. Vet. Med. Assoc. (2005) 227:1975-1981.

Marcé, C., Ezanno, P., Seegers, H., Pfeiffer, D. U. and Fourichon, C., Within-herd contact structure and spread of *Mycobacterium avium* subspecies *paratuberculosis* in a persistently infected dairy herd, Prev. Vet. Med. (2010a) Submitted.

Marcé, C., Ezanno, P., Seegers, H., Pfeiffer, D. U. and Fourichon, C., Modeling within-herd transmission of *Mycobacterium avium paratuberculosis* in dairy cattle: a review, J. Dairy Sci. (2010b) DOI: 10.3168/jds.2010-3139.

Marcé, C., Ezanno, P., Seegers, H., Pfeiffer, D. U. and Fourichon, C., Modelling the spread of *Mycobacterium avium* subsp. *paratuberculosis* towards fadeout or endemic infection in a dairy herd (2010c) Submitted.

Marcé, C., Guatteo, R., Bareille, N. and Fourichon, C., Dairy calf housing systems across Europe and risk for calf infectious diseases, Animal. (2010d) 4:1588-1596.

Martin, S. W., Meek, A. H. and Willeberg, P., Veterinary Epidemiology, Iowa State University Press, Iowa, USA, (1987), pp. 245-258.

McKenna, S. L. B., Keefe, G. P., Tiwari, A., VanLeeuwen, J. and Barkema, H. W., Johne’s disease in Canada Part II: Disease impacts, risk factors, and

control programs for dairy producers, Can. Vet. J. (2006) 47:1089-1099.

McNab, W. B., Meek, A. H., Duncan, J. R., Martin, S. W. and Van, D. A. A., An epidemiological study of paratuberculosis in dairy cattle in Ontario : study design and prevalence estimates, Can. J. Vet. Res. (1991) 55:246-251.

Muskens, J., Barkema, H. W., Russchen, E., Van, M. K., Schukken, Y. H. and Bakker, D., Prevalence and regional distribution of paratuberculosis in dairy herds in the Netherlands, Vet. Microbiol. (2000) 77:253-261.

Muskens, J., Elbers, A. R. W., Van, W. H. J. and Noordhuizen, J. P. T. M., Herd management practices associated with paratuberculosis seroprevalence in Dutch dairy herds, J. Vet. Med. B. (2003) 50:372-377.

Nielsen, S. S., Danish control programme for bovine paratuberculosis, Cattle pract. (2007) 15:161-168.

Nielsen, S. S. and Toft, N., Ante mortem diagnosis of paratuberculosis: A review of accuracies of ELISA, interferon-γ assay and faecal culture techniques, Vet. Microbiol. (2008) 129:217-235.

Nielsen, S. S. and Toft, N., A review of prevalences of paratuberculosis in farmed animals in Europe, Prev. Vet. Med. (2009) 88:1-14.

Nielsen, S. S., Programmes on Paratuberculosis in Europe, Proceedings of the 10th International Colloquium on Paratuberculosis, Minneapolis, Minnesota, USA, (2009), pp. 7.

Nielsen, S. S. and Toft, N., Management practices reducing the test-prevalence of paratuberculosis in Danish dairy herds, Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine, Nantes, France, (2010), pp. 189-200.

Noll, M. and Vignal, J., The view of a food company, Bulletin of the International Dairy Federation 410/2007. (2007):40-41.

Ott, S. L., Wells, S. J. and Wagner, B. A., Herd-level economic losses associated with Johne's disease on US dairy operations, Prev. Vet. Med. (1999) 40:179-192.

Pak, S. I., Kim, D. and Salman, M., Estimation of paratuberculosis prevalence in dairy cattle in a Province of Korea using an enzyme-linked immunosorbent assay: application of Bayesian approach, J. Vet. Sci. (2003) 4:51-56.

Patterson, D. S. P., Allen, W. M. and Lloyd, M. K., Clinical Johne's disease as a protein losing enteropathy, Vet. Rec. (1967) 81:717-718.

Ridge, S. E., Baker, I. M. and Hannah, M., Effect of compliance with recommended calf-rearing practices on control of bovine Johne's disease, Aust. Vet. J. (2005) 83:85-90.

Sorge, U., Kelton, D., Lissemore, K., Godkin, A., Hendrick, S. and Wells, S., Attitudes of Canadian dairy farmers toward a voluntary Johne's disease control program, J. Dairy Sci. (2010) 93:1491-1499.

Taisne, D., A control programme for paratuberculosis in infected dairy herds

in the Ille-et-Vilaine county [in French], Doctorate in Veterinary Medicine Thesis, Nantes, (2009).

Thorne, J. G. and Hardin, L. E., Estimated prevalence of paratuberculosis in Missouri, USA cattle, Prev. Vet. Med. (1997) 31:51-57.

Tiwari, A., VanLeeuwen, J. A., Dohoo, I. R., Keefe, G. P. and Weersink, A., Estimate of the direct production losses in Canadian dairy herds with subclinical Mycobacterium avium subspecies paratuberculosis infection, Can. Vet. J. (2008) 49:569-76.

Van Schaik, G., Schukken, Y. H., Crainiceanu, C., Muskens, J. and VanLeeuwen, J. A., Prevalence estimates for paratuberculosis adjusted for test variability using Bayesian analysis, Prev. Vet. Med. (2003) 60:281-295.

Wilson, D. J., Rossiter, C., Han, H. R. and Sears, P. M., Financial effects of *mycobacterium paratuberculosis* on mastitis, milk production, and cull rate in clinically normal cows, Agri-Pract. (1995) 16:12-18.

Wraight, M. D., McNeil, J., Beggs, D. S., Greenall, R. K., Humphris, T. B., Irwin, R. J., Jagoe, S. P., Jemmeson, A., Morgan, W. F., Brightling, P. and Anderson, G. A., Compliance of Victorian dairy farmers with current calf rearing recommendations for control of Johne's disease, Vet. Microbiol. (2000) 77:429-442.

Chapter 1

Modelling within-herd transmission of *Mycobacterium avium* subspecies *paratuberculosis* in dairy cattle: a review

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Abstract

Epidemiological models have been developed in order to test hypotheses on *Mycobacterium avium* subspecies *paratuberculosis* (*Map*) transmission in a herd, and to compare different paratuberculosis control strategies and alternatives for certification-and-surveillance schemes. They are simplified representations of existing biological processes tailored to the questions they are intended to answer. Such models depend on available knowledge about the underlying processes, notably in relation to pathogen transmission. All decisions relating to integration of specific aspects of the herd structure and transmission mechanisms as well as modelling objective will influence model behaviour and simulation results. This paper examines assumptions on pathogen transmission and risk mitigation represented in 8 epidemiological models of within-herd *Map* transmission in dairy cattle. We describe available models' structure and examine them in the context of current knowledge about host infection and pathogen transmission pathways. We investigate how population structure and herd management are modelled as regards to their influence on contact structure and pathogen transmission. We show that assumptions about the routes of transmission and their contribution within a herd vary greatly among models. Gaps of knowledge which are pivotal to defining transmission equations and parameters, such as variation of susceptibility with age and variability of pattern of shedding, are identified. Quantitative estimates of this incomplete information should be targeted by future research. Existing models could be improved by considering indirect transmission via the environment taking account of *Map* survival and contact structure between animals in a herd, and by including calf-to-calf transmission which has recently been proven as being important.

***Keywords*:** Mycobacterium avium subspecies paratuberculosis, dynamic model, transmission, review

1 Introduction

Paratuberculosis is of significant economic importance for dairy producers as it results in a decrease in milk production, mortality or premature culling of sick cattle, and reduction in slaughter value of clinically affected cattle in affected dairy herds (Benedictus, *et al.*, 1987; Johnson-Ifearulundu and Kaneene, 1997). Furthermore, the zoonotic importance of *Mycobacterium avium* subspecies *paratuberculosis* (*Map*) in the pathogenesis of Crohn's disease is still controversial (European Commission, 2000; Frank, 2008; Shafran and Burgunder, 2008). These factors justify the development of effective and economically viable control programmes against paratuberculosis. However, there is no treatment available currently. Up to now, certification and control programmes implemented in several countries have only had limited success. A better understanding of *Map* transmission is required to implement appropriate measures for protecting susceptible cattle.

Field studies on *Map* transmission are difficult because infection occurs mainly in young stock and clinical signs arise after a long incubation period (1 to $\geq$ 15 years). Such studies are further complicated by the low and varying sensitivity of diagnostic tests. Modelling thus appears to be an appropriate tool to study paratuberculosis. Indeed, epidemiological models can be used to identify the main factors influencing pathogen transmission within a herd, and to assess *ex-ante* control strategies of *Map* spread. Relevance of model outputs depends on the modelling approach used, the assumptions made (including parameterization) and the level of simplification of the biological mechanisms modelled.

For *Map* transmission, several models have been developed to i) investigate *Map* spread in a herd and its economic consequences (Van Roermund, *et al.*, 2002; Pouillot, *et al.*, 2004); ii) test transmission hypotheses such as representing indirect transmission via the environment (Humphry, *et al.*, 2006) or considering transiently shedding young animals (Mitchell, *et al.*, 2008); iii) compare strategies for control of *Map* spread in infected herds (Collins and Morgan, 1991a; Kudahl, *et al.*, 2007a; Lu, *et al.*, 2008), or in populations with infected and non-infected herds (Groenendaal, *et al.*, 2002; Van Roermund, *et al.*, 2002; Groenendaal, *et al.*, 2003); iv) compare certification-and-surveillance programmes aimed at low-risk pathogen transmission associated with cattle trade (Kalis, *et al.*, 2004; Weber, *et al.*, 2004; Ezanno, *et al.*, 2005), or at quality assurance of dairy products (Van Roermund, *et al.*, 2002; Groenendaal and Zagmutt, 2008; Weber, *et al.*, 2008).

Our objective is to describe and discuss assumptions used in *Map* transmission models within dairy cattle herds with particular emphasis on representation of infection status, routes of transmission, and exposure at the herd level. Current scientific knowledge about *Map* transmission is summarized and then used to evaluate the validity of the models described in

the scientific literature. Assumptions dealing with infection at the host level, transmission from infected to susceptible cattle, and population structure influencing transmission in a dairy herd are discussed in particular. As a conclusion, we provide recommendations for future modelling studies or for improvement of existing models.

2 Selection of relevant papers

Peer-reviewed papers and conference proceedings dealing with *Map* transmission models in dairy cattle were systematically selected using electronic search engines. The search was conducted using the following scientific literature electronic databases: CAB (CAB International, Oxon, UK), Medline (National Library of Medicine, Rockville Pike, USA), ISI Web of Knowledge[SM], and one conference proceedings website: the International Colloquiums on Paratuberculosis. The search was conducted on March 30, 2008 and regularly repeated until February 4, 2010. Studies from conference proceedings were included if they had not been published in peer-reviewed journals. Search terms used were "paratub*", "model*" occurring in titles, abstracts, subject headings, keywords or descriptors. Only articles written in the English language and describing models that aimed at representing the transmission of *Map* within cattle herds were considered. Papers using models previously described in other peer-reviewed articles were not retained unless they represented novel approaches to the study of *Map* transmission. Consequently, economic models that aimed at assessing the cost of the disease or cost of different control programmes without providing any information on the dynamics of *Map* spread or without relying on *Map* transmission modelling were excluded (Groenendaal, *et al.*, 2003; Kalis, *et al.*, 2004; Weber, *et al.*, 2004; Dorshorst, *et al.*, 2006; Tavornpanich, *et al.*, 2008; Weber, *et al.*, 2008).

Based on the above criteria, six models were selected from peer-reviewed papers (Collins and Morgan, 1991a; Groenendaal, *et al.*, 2002; Pouillot, *et al.*, 2004; Humphry, *et al.*, 2006; Kudahl, *et al.*, 2007a; Mitchell, *et al.*, 2008) and two from conference proceedings (Van Roermund, *et al.*, 2002; Van Roermund, *et al.*, 2005) (Table I).

All models represent *Map* transmission within a dairy herd, except one which does so within a beef herd (Humphry, *et al.*, 2006). This 'beef' model has been kept in our review because specific assumptions on transmission are used which may be relevant for models in dairy cattle.

3 Technical characteristics of the selected models

The selected models not only differed with respect to the biological hypotheses on which they are based, but also in relation to the technical

Table I: Modelling options of eight Mycobacterium avium paratuberculosis *(*Map*) transmission models in cattle herds*

	Reference	Type of herd	Effect of chance	Treatment of time (interval)	Length of the simulations (in years)[1]	Output
N°1	(Collins and Morgan, 1991a)	Dairy	Deterministic	Discrete (1 year)	50	Prevalence of infected animals
N°2	(Groenendaal, *et al.*, 2002)[2]	Dairy	Stochastic	Discrete (6 months)	20	Distribution of within-herd prevalence of infection Proportion of herds in various statuses of certification-and-surveillance programmes (Weber, *et al.*, 2004; Weber, *et al.*, 2008) Distribution of the concentration of *Map* in bulk milk (Weber, *et al.*, 2008)
N°3	(Van Roermund, *et al.*, 2002)	Dairy	Deterministic	Continuous model	ng	R_h = Number of newly infected farms by one infected farm in a naïve metapopulation
N°4	(Pouillot, *et al.*, 2004)	Dairy & Beef	One stochastic and one deterministic	Discrete (1 year) for the stochastic model Continuous for the state transition model	30	Yearly incidence rate Yearly prevalence of infected animals Cumulated yearly disease extinction probability
N°5	(Van Roermund, *et al.*, 2005)	Dairy	Deterministic	Continuous model	10	Prevalence in infected herds Average number of *Map* bacteria in bulk milk Number of infected and free dairy herds in the country Number of herd in various status of a certification-and-surveillance programme
N°6	(Humphry, *et al.*, 2006)	Beef	Stochastic	Discrete (6 months)	ng	Number of subclinical and clinical animals
N°7	(Kudahl, *et al.*, 2007a)	Dairy	Stochastic	Discrete (1 week)	10	Number of animals in each state Prevalence of infected animals
N°8	(Mitchell, *et al.*, 2008)	Dairy	Deterministic	Discrete (1 month)	25	Prevalence of infected animals

[1] ng: not given; [2]: this model was later adapted to study beef herds (Groenendaal, *et al.*, 2003)

characteristics of the models, such as discrete-time (appreciable period of time such as a day or a year) *versus* continuous-time or deterministic *versus* stochastic implementation (Table I). Hypotheses on the model system, such as type of herd, herd structure or routes of pathogen introduction are discussed later in this review.

Four models are discrete-time stochastic models which use random sampling from input parameter distributions or binomial distribution of event probability to represent variability in *Map* transmission (models N°2, 4, 6 & 7). Three models are continuous-time deterministic models (models N°3, 4 & 5) and one is a discrete-time deterministic model (model N°8) (links between references and model numbers are given in all tables).

In models based on a short time step or assuming a continuous time process, representation of biological mechanisms can be more precise than in models based on a longer time step, since processes operating at a time scale shorter than the time step cannot be considered. In the selected models, for example, choosing a time step of six months will not allow testing different lengths of the susceptible period (other than a multiple of six months), testing different lengths of the shedding period, or differentiating infection during the first weeks after birth from infection after weaning. However, choosing a longer time step is more convenient when studying long-term effects or year-round management, because the model then becomes computationally more tractable given the reduced parameter set.

Deterministic models represent the mean behaviour of the system modelled. Such a representation is generally suited for a large population. In contrast, stochastic models take into account variability in event occurrence and therefore allow representation of likely variation within small populations. Several replications are indeed implemented based on the same inputs and initial conditions in order to assess the distribution of model outputs besides their average.

4 Modelling the infection in the host

4.1 Host susceptibility

Map infection is known to occur mainly in newborn calves (Hagan, 1938). It is generally acknowledged that calves can become infected until one year of age, especially during the first weeks after being born. Resistance to infection increases up until one year, especially after one to four months of age (Taylor, 1953; Windsor and Whittington, 2010). After one year, cattle are considered resistant except when exposed to repeated infection with large amounts of bacteria (Doyle, 1953; Taylor, 1953; Larsen, *et al.*, 1975).

In the models, most authors assume that susceptibility to *Map* is age-related and define a maximum age of infection at 0.5 (model N°3) or one year of age (models N°1, 2, 4, 5, 7 & 8) (Figure 1). Cattle that do not become infected by

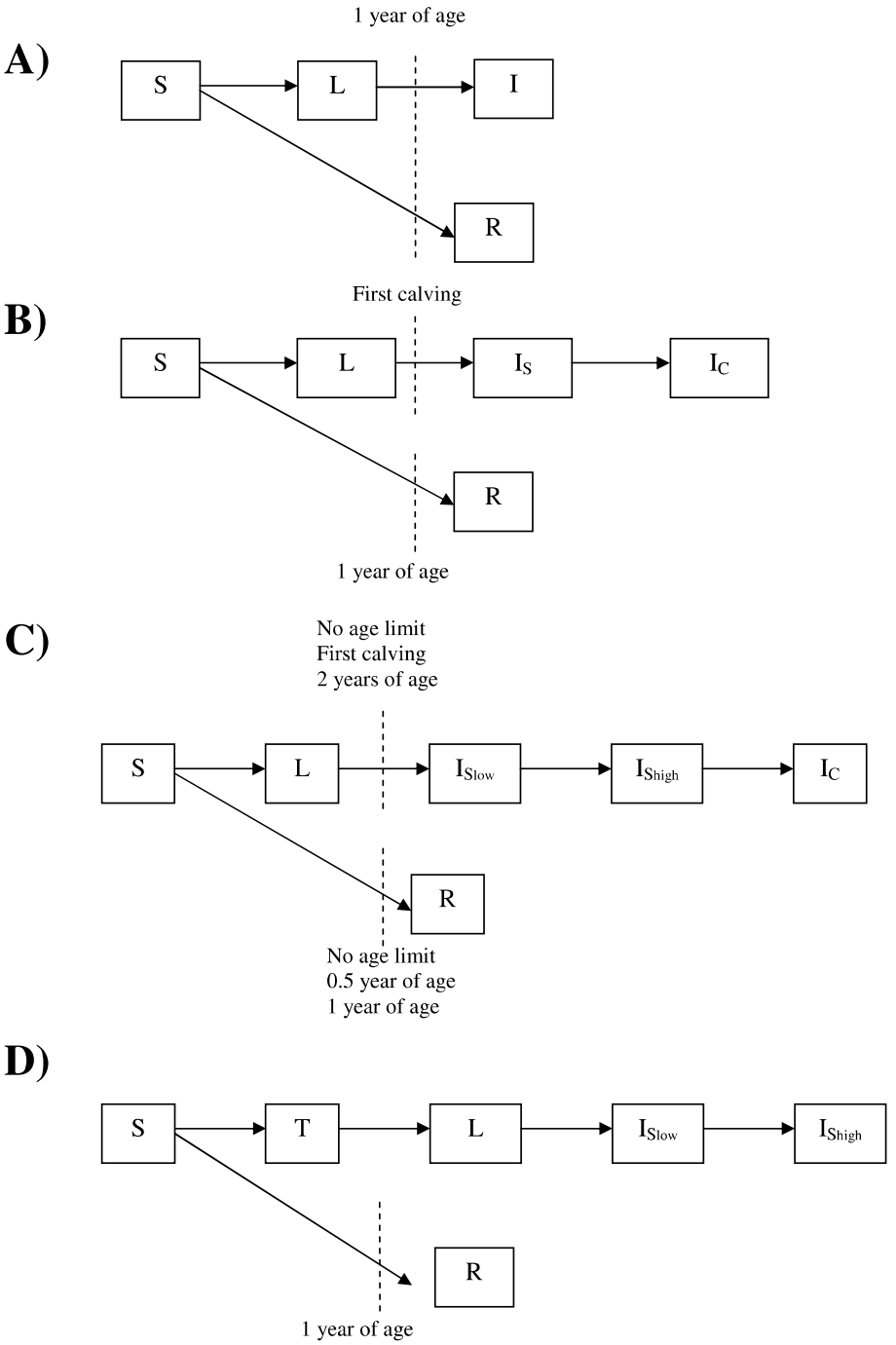

Figure 1: Graphical representation of health statuses and transitions in Mycobacterium avium paratuberculosis *transmission model of A) Collins & Morgan (1991a), B) Pouillot (2004), C) Kudahl (2007a), Groenendaal (2002), Humphry (2006), and Van Roermund (2002, 2005), and D) Mitchell (2008)*

Legend: S = susceptible, T = transient, L = latent, I = infected, I_S = subclinically infected, I_{Slow} = subclinically infected low shedder, I_{Shigh} = subclinically infected high shedder, I_C = clinically affected, R = resistant,------- = minimal age before entering the following status

the chosen age limit are assumed to be resistant (Table II). Only one model assumes that adults can become infected but with a much lower probability (model N°6) (Figure 1). In young stock, susceptibility to *Map* either remains constant (models N°1, 3, 4 & 8) or varies with age (models N°2, 6 & 7). Model N°2 compares two different approaches for modelling the decrease of susceptibility with age (model N°2, http://cahpwww.vet.upenn.edu/field/johne_report.pdf). When a linear decrease of the susceptibility of young calves to paratuberculosis is assumed, the true prevalence is higher than when an exponential decrease is assumed. However, the difference between the true prevalence under both assumptions is small. Assuming an exponential decrease necessitates defining only one parameter while a linear decrease necessitates defining two parameters. It is thus preferable to choose the simplest representation: the exponential one.

Several experimental studies indicate genetic variation of susceptibility amongst cows (Koets, *et al.*, 2000; Mortensen, *et al.*, 2004; Gonda, *et al.*, 2006; Gonda, *et al.*, 2007). This so far has not been taken into account in *Map* transmission models. It is thus not possible to consider selectively culling the most susceptible calves based on genetic markers, a method which could be both an earlier and less expensive control measure than a test-and-cull option.

4.2 Development of the disease within the host and *Map* shedding

4.2.1 Infection stages

Following infection, calves are able to shed the bacteria without any clinical signs (Bolton, *et al.*, 2005; Weber, *et al.*, 2006; Van Roermund, *et al.*, 2007). The incubation period can last from less than two to more than ten years (Van Roermund, *et al.*, 2007; Weber, *et al.*, 2009), during which time adult cattle may or may not shed bacteria in their faeces (at different levels of shedding) (Chiodini, *et al.*, 1984; Whitlock and Buergelt, 1996; Crossley, *et al.*, 2005). Later, clinical signs are characterized by losses of milk production, significant weight loss and diarrhoea (Smythe, 1950; Clarke, 1997) leading to death, if cattle are not culled before. During the clinical stage of the pathogenesis, massive numbers of bacteria are excreted in the faeces.

In the models, infection stages taken into account differ (Figure 1) and are associated with different levels of shedding. The simplest representation considers latently infected cattle (not shedding) *versus* infected cattle shedding all the same amount of bacteria (model N°1) (Figure 1,A). In contrast, the most recently published models define six infection stages (models N°5, 6, 7 & 8) (Figure 1,D). This evolution in terms of number of infection stages considered is mainly associated with the progress made in development of new *Map* knowledge. However, two models do not consider explicitly clinically affected animals, while they play a specific role in terms

Table II: Mycobacterium avium paratuberculosis *(*Map*) infection statuses and bacterial shedding in faeces in bacteria per gram or per day or colony forming unit (cfu) per tube as represented in eight transmission models in cattle herds*

	Reference	Possible infection statuses				
		Transiently infectious[1-3]	Latent (not shedding)[2]	Subclinically infected (low shedder)[3]	Subclinically infected (high shedder)[3]	Clinically affected[3]
N°1	(Collins and Morgan, 1991a)	-	NSh[4]	Only one infected status Sh (animals > 2 years, no distinction of infection statuses or level of shedding)		
N°2	(Groenendaal, *et al.*, 2002)[2]	-	NSh	Sh (2 months after calving, with a minimum of 2 years of age)	Sh (2 lactations after becoming subclinically infected low shedder)	Sh (3 months maximum in this status before culling)
N°3	(Van Roermund, *et al.*, 2002)	-	NSh	Sh (linear increase of infectivity from 2 years until 6.5 years)	Sh (linear increase of infectivity from 2 years until 6.5 years)	Sh (linear increase of infectivity from 2 years until 6.5 years)
N°4	(Pouillot, *et al.*, 2004)	-	NSh	Sh (shedding starts after the first calving)	-	Sh (culled within a year)
N°5	(Van Roermund, *et al.*, 2005)	-	NSh	Sh [5]	Sh [5]	Sh [5]
N°6	(Humphry, *et al.*, 2006)	-	NSh	Only one subclinically infected status Sh (9.10^{11} *Map*/day)		Sh (3.10^{12} *Map*/day - no minimum age limit)
N°7	(Kudahl, *et al.*, 2007a)	-	NSh	Sh (begins between first and second calving)	Sh (begins between second and third calving)	Sh (begins between third and fourth calving)
N°8	(Mitchell, *et al.*, 2008)	NSh or Sh	NSh	Sh (<300 cfu/tube)	Only 1 high shedder status Sh (>300 cfu/tube)	

[1] -: status not represented in the model; [2] NSh: the compartment exists but there is no shedding, [3] Sh: the compartment exists with shedding, [4]: infected calves below 2 years of age, [5]: the quantity of bacteria shed in the faeces was used in the model to calculate the concentration of *Map* in milk, but not to calculate transmission.

of transmission (higher load of *Map* shed, higher risk of *in utero* transmission, higher probability of being culled) (models N°1 & 8). In all models, the individual progresses through the various stages of the infection-and-disease process with increasing age. However, within a specific stage of the infection-and-disease process, shedding is considered to be independent of age. So if an animal sheds *Map* and if an appropriate test is used, detection of an infected animal will occur with a constant probability.

In contrast, in the field, shedding appears to vary in time for an infected animal; a test performed one day can miss an infected animal which was possibly shedding the day before and could possibly shed *Map* the day after. The mean probability to cull an infected animal potentially shedding should be equivalent in the model and in the field. However, the variability in the field can be expected to be more important. Simulated scenarios are probably more homogeneous in terms of probability of being culled, with less extreme values. While model N°8 considers a uniform and deterministic disease progression with cattle progressing through the same disease stages regardless of the age of infection, model N°1 considers that once infected, all calves become infectious at two years of age. Other authors assume that progress to the next infectious stage and increase in infectivity depend on the age of animals (models N°3, 4, 5, 6 & 7) or the route of infection (*in utero*, at birth, through colostrum/milk, through faeces ingestion approximated by the number of contacts between animals) (model N°2). Animals of the same age can shed different amounts of *Map* only for this last option; and the route of infection then has an impact on test and cull efficacy for every animal. The incubation period considered in the models varies between models (with a range of two to 22 years, generally around five years). If the delay before becoming clinically affected is long, the prevalence is expected to increase as the direct detection (clinical signs) occurs less often. However, the incidence could decrease as there are fewer animals shedding a very high amount of *Map* in faeces. The consequence on the achievement of persistence is not known. The overall effect of the length of the incubation period is difficult to predict without comparing the different situations by simulation with the same model, everything else being constant.

4.2.2 Factors influencing the course of infection

There is little information available in the scientific literature on factors influencing the course of infection. Some studies indicate that age at infection is a major determinant of occurrence of clinical signs and of shedding of detectable levels of *Map* : the younger that cattle are when infected, the quicker they develop clinical signs (Rankin, 1961; Whitlock and Buergelt, 1996) and the more likely they shed detectable levels of *Map* (Kostoulas, *et al.*, 2010). In experimental infection, a relationship between dose given to the animals and occurrence of lesions and clinical signs was demonstrated (Begg and Whittington, 2008), with larger doses resulting in earlier disease

development. It is also acknowledged that factors such as stress may influence the development of the disease (Chiodini, *et al.*, 1984).

These characteristics have been integrated differently in the models studied (Table II, Figure 1). Only model N°2 considers age at infection as influencing the course of infection (the earlier the infection occurs, the quicker the progression of the disease is), while the others do not. Young animals could thus shed large doses of *Map* in model N°2. This may influence the impact of control measures. More biological studies are necessary to assess whether this assumption is realistic. The number of infectious animals characterizes exposure, and thus the probability of becoming infected, but not the evolution from one status to the following one when infected (models N° 4, 5, 6 & 8). Stressors such as calving or change of feed are taken into account in one model as factors influencing disease progression (model N°7). The underlying assumption is that stress situation accelerates the development of clinical signs for infected animals. In that model, animals can become clinically affected and thus be detected earlier. Not considering such factors implies that only a mean behaviour is studied, with all cows being exposed to the same stressors.

4.2.3 Shedding characteristics

Shedding of *Map* mainly occurs in faeces. *Map* is also found in colostrum and milk of subclinically infected or clinically affected cows (Taylor, *et al.*, 1981; Sweeney, *et al.*, 1992a; Streeter, *et al.*, 1995). Several studies have shown inter- and intra-individual variability in the number of excreted bacteria (Whitlock, *et al.*, 2000; Crossley, *et al.*, 2005). *Map* faecal shedding has also been described in young stock (Bolton, *et al.*, 2005; Antognoli, *et al.*, 2007; Van Roermund, *et al.*, 2007) but culture data indicates that calves do not shed as frequently or as much as adults (Rankin, 1961; Nielsen and Ersboll, 2006; Van Roermund, *et al.*, 2007). High faecal shedders are more likely to shed the bacteria in their colostrum and milk than low faecal shedders (Sweeney, *et al.*, 1992b; Streeter, *et al.*, 1995). Furthermore, high faecal shedders are more likely to be detected with currently available tests.

In the models, cattle are usually categorized depending on their level of shedding (Table II). Levels of shedding influence the infectiousness (models N°2, 3, 4, 7 & 8) either because the probability of infection is different depending on health status, or because the number of infected cattle is balanced by allocating to the less infectious cattle a shorter time period of *Map* shedding (in the same quantity) than to high shedders (model N°2) (Table III). However, little information is generally available on the quantity of bacteria shed by animals depending on their infection stage (Table II). Only one model does not consider that levels of shedding influence the probability of transmission (model N°1) as it only considers one level of shedding. This simplification does not allow studying control measures targeting animals that shed the highest levels of *Map*. Only one model assumes that calves are able to shed the bacteria (model N°8). In that model, shedding is substantially

Table III: Probability of infection and underlying assumptions used to model Mycobacterium avium paratuberculosis *horizontal transmission in eight models (intrauterine infection, infection at birth and infection through milk and colostrum excluded)*

Model / Reference		Probability of infection[1]	Categories of animals to which susceptible animals are exposed to	Type of contact	Probability of transmission if infectious contact
N°1	(Collins and Morgan, 1991a)	$1-(1-\frac{k}{N_c})^{I_a}$	Number of infected adults (I_a)	Direct contact between calves and adults	Constant = 1
N°2 & N°5	(Groenendaal, *et al.*, 2002; Van Roermund, *et al.*, 2005)[2]	$1-\left(1-\frac{k(age)}{N_a}s(age)\right)^{I_a}$	Number of infected adults in the last 6 months (I_a)	Direct contact between calves and adults	Varies with calf susceptibility, which depends on age I_a: weighted sum in order to take account of the shorter shedding of lowly infectious animals
N°3	(Van Roermund, *et al.*, 2002)	$\int_{age}\beta(age)\frac{I_{age}}{N}$	Proportion of infected animals per age	Direct contact between calves and adults	Frequency-dependent, varies with infectivity, which depends on age
N°4	(Pouillot, *et al.*, 2004)	$1-\prod_i(1-\tau_{(i)})^{I_i}$	Number of infected cows (I)	Direct contact between calves and adults	Varies with adults infectious statuses
N°6	(Humphry, *et al.*, 2006)	$1-(1-p(age))^{b(I_i,t)}$	Bacterial density in the environment (b)	Indirect contact via the environment	Varies with age of susceptible animals
N°7	(Kudahl, *et al.*, 2007a)[3]	$1-\left(1-\frac{k(age)}{N_a}s(age)\rho\right)^{I_a}$	Number of infectious cows (I_a)	Direct contact between calves and adults	Varies with calf susceptibility, which depends on age
N°8	(Mitchell, *et al.*, 2008)	$\sum_i\beta(i)\frac{I_i}{N}$	Proportion of infected animals per infectious status	Direct contact between calves and adults, or among calves	Frequency-dependent, varies with adults infectious status

[1] I_x: number of infected animals related to state x (x=a: adult; x=i: infectious status i; x=age: adult age), N_x: total number of animal related to state x (-: animals of all ages; x=c: calves; x=a: adults), b: bacterial density in the environment; β: transmission parameter, k: number of effective contacts, p: probability of infection, ρ: weighting factor depending on calves age, s: calf susceptibility, τ_i: pathogen transmission probability related to adult infectious status i, t: time.

[2] for more details on this model, see also the report found on: http://cahpwww.vet.upenn.edu/field/johne_report.pdf

[3] An error in the formulation of the probability of infection in the original paper has been detected; the formula is here corrected and validated by the authors.

lower for calves than for low-shedding adults. Studying between-calf *Map* transmission is here possible as is evaluating the contribution of the different routes of transmission and studying control measures specifically targeting young shedding cattle.

Level of shedding also influences management and production parameters. Higher levels of shedding are associated with a higher probability of being culled if test-and-cull is used for control. Models distinguishing several levels of shedding allocate sensitivity and specificity of diagnostic tests depending on these levels of shedding. Finally, models aiming at quantifying *Map* in bulk tank milk as an output (bacteria per liter) also rely on accurate representation of levels of shedding in both milk and faeces (Van Roermund, *et al.*, 2005; Weber, *et al.*, 2008).

5 Modelling the transmission of the pathogen

Vertical or direct *in utero* transmission from dam to calf has been reported (Whittington and Windsor, 2009). In a meta-analysis, Whittington and Windsor (2009) estimated that 9% of foetuses born from subclinically infected cows and 39% from clinically affected cows were infected with *Map*. Other potential sources of vertical transmission could be semen, or embryo transplants but these routes seem to be rare (Kruip, *et al.*, 2003).

Horizontal transmission is due to ingestion of *Map* from contaminated sources, especially from faeces (Chiodini, *et al.*, 1984), but also from milk and colostrum. Actually, *Map* is shed directly in milk and colostrum and faecal contamination of milk and colostrum occurs (Nauta and van der Giessen, 1998). For a long time it was thought that transmission occurs only from adults to calves, but calf-to-calf transmission has been reported recently albeit at a lower level than adult-to-calf transmission (Van Roermund, *et al.*, 2007). It seems that *Map* is unable to multiply in the environment (Whittington, *et al.*, 2004). *Map* can survive in the environment for several months and up to 55 weeks in faeces (Lovell, *et al.*, 1994; Whittington, *et al.*, 2004; Whittington, *et al.*, 2005). Soil desiccation and exposure to direct sunlight shorten survival (Larsen, *et al.*, 1956; Whittington, *et al.*, 2004).

In the reviewed models, the number of transmission pathways considered varies (Table IV). Out of 5 models explicitly representing faecal-oral transmission, 4 assume direct contacts between susceptible and infected animals (models N°1, 2, 7 & 8) (Table III). A further assumption in these models is that animals are raised together, which is not always correct. Furthermore, it is difficult to estimate the frequency of contacts and the probability of infection given contact. Two types of force of infection (transmission rate per susceptible animal) are used: density and frequency-dependent (Table III). In the first case (density-dependent), the number of cases is usually considered, while in the latter (frequency-dependent), it is the

Table IV: Transmission pathways of Mycobacterium avium paratuberculosis *considered in 8 transmission models in cattle herds*

Reference		Overall transmission[1]	*In utero* transmission	Adult-to-calf transmission			Calf-to-calf transmission
				Milk and colostrum ingestion	Faeces ingestion through contacts	Faeces ingestion through the environment	
N°1	(Collins and Morgan, 1991a)	X					
N°3	(Van Roermund, *et al.*, 2002)	X					
N°4	(Pouillot, *et al.*, 2004)	X					
N°5	(Van Roermund, *et al.*, 2005)	X					
N°2	(Groenendaal, *et al.*, 2002)		X	X	X		
N°7	(Kudahl, *et al.*, 2007a)		X	X	X		
N°6	(Humphry, *et al.*, 2006)		X			X	
N°8	(Mitchell, *et al.*, 2008)		X		X		X

[1] X: included in the model; empty cell: not included in the model

proportion of infected cattle in the population (Begon, *et al.*, 2002; Hoch, *et al.*, 2008). The consequence of such a choice is discussed below when the impact of herd size is addressed. A link between the extent of animal confinement and pathogen transmission is considered in model N°4: transmission is more likely if disease onset occurs during the calving period, assumed to always be indoors in this model, than if it occurs when animals are kept on pastures.

The only model that does not consider faecal-oral transmission by direct contact explicitly includes indirect transmission via the environment by taking into account the density of *Map* and their survival in the environment (model N°6). A constant survival rate of the bacteria leads to an exponential decay model. Susceptible animals are exposed to a specific bacterial density present in the environment, these bacteria being shed by infectious animals that are or were present. Whatever their age, cattle are exposed to the same contact rate with *Map* within a specific environment (homogeneous contacts). Infectious areas are larger outdoor and are consequently associated with a lower exposure.

Survival of *Map* in the environment should be considered to account for the potential delay between shedding and exposure to *Map* that can be followed by infection, which may change the predictions when modelling different control options. As *Map* transmission mainly occurs via ingestion of faeces, the probability that a contact involves a shedding animal is likely to under-estimate transmission since transmission can occur even if no shedding animals are present in the herd as the bacteria may persist for a long time in the environment. Calf-to-calf transmission is generally not accounted for, except in model N°8. In this model, the sensitivity of transmission dynamics in the herd to calves shedding is explored. Accounting for age-dependent contacts, calf-to-calf transmission has been shown to be required for *Map* to persist in the herd (model N°8). Such a result is in agreement with observations from a field study, which were better explained by a statistical model when including calf-to-calf transmission (van Roermund and de Jong, 2002).

6 Modelling population structure and herd management

6.1 Type of herd

One of the most important factors influencing *Map* transmission is whether it is a dairy or a beef farm. Contacts between animals differ between these two types of farm, as does transmission via calf feeding. In beef cow-calf herds, calves are raised in the same environment as their dam until at least seven months of age, whereas separation from adults occurs within a few hours or days after birth in most of the dairy farms. Due to the higher susceptibility of

young calves, raising calves with adults should strongly influence *Map* transmission. Moreover, beef cows often have a lower replacement rate than dairy cows, therefore tending to be older at culling. As a result, the chance for beef cows to reach an infectious stage is higher.

Three papers investigate disease dynamics in both dairy and beef herds (model N°2 and one associated paper analyzing this model (Groenendaal, *et al.*, 2003) & model N°4). However, in model N°4, only replacement rate and herd size differ between the two types of herd; contact structures are the same for the two farm types in the model. Outputs of both models are different and thus cannot be directly compared. Adapting contact structure to that of a beef herd should account for both increased direct contacts between the dam and its calf before weaning and for indirect faecal-oral transmission through *Map* survival in the environment.

6.2 Herd management

6.2.1 Contact structure

In a farm, animals are often grouped by age or production status. Therefore, contacts between animals are not homogeneous within a herd. Animal locations both influence local bacterial density, especially when adults are considered, and probability of infection, especially when calves are considered. Allowing contacts between young stock and adults or even among young stock enhances *Map* transmission. Furthermore, calving management, i.e. seasonal calving *versus* all-year-round calving, influences the mixing of animals of different age groups; all-year-round calving increases the likelihood of raising calves of different age in the same pen (collective housing facility).

In the models, age of animals is always considered because it influences infection related factors (e.g. susceptibility), but most often not because of preferential contacts between animals of the same age. Model N°8 as an exception considers different contact rates whether animals are of the same age or not, assuming a much greater intra-group than inter-group rate of contact. In this model, calf-to-calf transmission could therefore be higher than adult-to-calf transmission depending on the value of the parameter tested. Inter-class rate of contact appears to be always the same. In model N°2, a separation of age groups is modelled by a reduction of the contact rate *k* in the formula (Table III). No calf-to-calf transmission is possible (i.e. no specific intra-class rate of contact), but adult-to-calf transmission varies with the age of the calf (different inter-class rate of contact).

Models could be improved by including a more detailed contact structure between animals in a herd because contact structure may play a considerable role in pathogen transmission as has been shown for some other cattle diseases (Ezanno, *et al.*, 2008). The number of shedding animals, the type of

housing facilities and the management of the herd in terms of space utilization should therefore be taken into account.

6.2.2 Herd size

In models N°2 & 4, increasing herd size has been reported to increase the speed of the transmission and the infection prevalence at which equilibrium is reached, assuming a similar herd management. From model N°4, authors conclude that it is not possible to assess if this is linked to a greater risk of introducing infected animals into a larger herd, or if it is linked to different within-herd disease dynamics. On the contrary, in model N°1, increasing herd size decreases the prevalence after the introduction of one infected animal. The authors explain this phenomenon by the increase in the number of susceptible animals in larger herds, in association with the decreased probability of effective contact. This finding contrasts with observed data from which the seroprevalence of paratuberculosis appears to be positively correlated to herd size (Wells and Wagner, 2000; Muskens, *et al.*, 2003).

Herd size is likely to influence the results when modelling *Map* transmission within a cattle herd. However, conclusions are still controversial. More precise information about the effect of herd size on within-herd infection levels would thus be useful while also taking account of transmission via the environment. Improving our knowledge about *Map* density, *Map* survival in the environment and the role of fomites in *Map* spread within a herd is required to do so. Furthermore, results could differ depending on the transmission function used (Hoch, *et al.*, 2008). In a frequency-dependent model, the force of infection is indeed constant, whereas the number of infected animals increases with herd size in a density-dependent model, leading to the increase of the force of infection.

6.2.3 Control of herd size

Sales and culling can influence the spread of the pathogen in two contrasted ways. On the one hand, if the culling rate is higher than the renewal rate, farmers have to purchase animals to keep herd size constant. This increases the risk of introducing infected animals. On the other hand, if the culling rate is low, infected but undetected animals remain in the herd and the chance to contaminate the environment increases.

In the selected models, either closed (models N°4, 5, 6 & 8) or open herds (models N°1, 2, 3 & 7) are modelled. In open herds, purchased heifers are either assumed to be uninfected (model N°4) and thus introduction does not contribute to *Map* spread, or infected (models N°1, 2 & 7) resulting in maintaining *Map* in the herd. However, because of the low sensitivity of diagnostic tests, it is difficult to be certain that purchased animals are truly uninfected. Calves born to either infected or uninfected dams have the same probability to be kept in the herd unless there is an active intervention on the

former (model N°8). Culling due to paratuberculosis infection is infection stage-dependent (models N°2, 4, 7 & 8) or age-dependent (model N°1) when represented. Model N°1 assumes that there is no variation of the infection stage for a given age. This assumption is acceptable for a simple model but not truly realistic for paratuberculosis knowing the variability of the incubation period. It is furthermore known that the culling rate of clinically affected animals is higher than for other stages of infection. This is taken into account in models N°2, 4, 7 & 8. In these models, animals can become clinically diseased at any age.

6.2.4 Other routes of pathogen introduction

In addition to the purchase of infected cattle, between-herd transmission occurs through transfers of faeces, manure, slurry, soiled forage and use of soiled fields for pasture, and thus is dependent on herd management. Pathogen introduction into a cattle herd can also originate from other farmed ruminants such as sheep and goats, or from wildlife (Beard, *et al.*, 2001; Manning, 2001; Daniels, *et al.*, 2003). However, the strain of *Map* isolates from wildlife has not been confirmed to be the same as in domestic ruminants (Daniels, *et al.*, 2003; Anderson, *et al.*, 2007). Furthermore, the causal link and the direction of potential causality between environmental contamination from wildlife or cattle, and infection in cattle or wildlife are difficult to prove under field conditions mainly because of the long incubation period of the disease and the resulting difficulty of excluding other potential sources of infection (Daniels, *et al.*, 2003). Moreover, even if *Map* transfers were possible between wildlife and domestic cattle, under typical farm management conditions, the frequency of contacts between young susceptible animals and wildlife, or environments contaminated by wildlife, are low. This is particularly the case in dairy farms where calves are kept indoors. Moreover, the contribution of wildlife is likely to be small once the disease becomes established in a herd compared to the challenge from infected cattle present within the same herd.

None of the reviewed models consider other sources of infection but cattle.

7 Modelling control of *Map* infection

One of the most important control measures is to prevent the exposure of young animals to *Map*. Both *in utero* transmission and ingestion of *Map* in faeces, milk or colostrum have to be controlled. The only possible control for *in utero* infection is to cull infected cows that are likely to infect their foetus. After birth, protection of susceptible cattle implies hygienic measures such as preventing calf contamination by early separation from the dam, using milk and colostrum from non-infected dams, milk replacer or pasteurized milk, calving in a separate pen, cleaning the calving pen, preventing contact between cattle of different ages, improving the general cleanliness of the cattle and housing, using non-contaminated feed, water and pasture for rearing

young cattle, and raising separately calves born from infected and non-infected cows (Gay and Sherman, 1992; Rossiter and Burhans, 1996).

Decreasing the number of infectious cattle in the herd in order to decrease the contamination of the environment is an alternative to the control of exposure. Therefore, possible measures are to cull detectable infectious animals, to purchase animals in herds known to be clear of infection, to cull calves born from infected dams, or to reduce stressful events that trigger progression of the infection and shedding of *Map* (McKenna, *et al.*, 2006). Several test-and-cull strategies have been proposed, always accounting for the low sensitivity of diagnostic tests (Groenendaal, *et al.*, 2003). Furthermore, culling cattle positive to a culture-based test assumes that isolation of *Map* indicates established infection and not simple transit of *Map* through the gastro-intestinal tract. It also assumes that recovery from infection cannot occur and that the culture-based tests are 100% specific (Sockett, *et al.*, 1992).

Vaccination is also possible to reduce the number of faecal shedders (Kormendy, 1994) and the number of clinically affected cattle in a herd (Wentink, *et al.*, 1994). However, the vaccine has been prohibited in several countries such as Sweden, United Kingdom, and Ireland because it interferes with diagnostic tests. Moreover, although vaccination reduces the economic losses of paratuberculosis (Van Schaik, *et al.*, 1996), it does not reduce *Map* transmission (Kalis, *et al.*, 2001). Therefore, hygienic practices remain essential in herd management.

All these measures are more or less strictly applied depending on the constraints of the farms (such as housing facilities). Their effect on disease dynamics within a herd are not known in detail. The association between management-related risk factors and (apparent) prevalence of *Map* has been studied broadly, but these studies do not take into account the time between the implementation of the preventive measures and the disease dynamics (Obasanjo, *et al.*, 1997; Jakobsen, *et al.*, 2000; Dieguez, *et al.*, 2008).

A modelling approach is relevant for evaluating and comparing control strategies of *Map* spread in a cattle herd. Most reviewed models have been used for such a purpose, especially for evaluating test-and-cull options when diagnostic tests are poorly sensitive (Collins and Morgan, 1991b; 1992; Groenendaal and Galligan, 2003; Groenendaal, *et al.*, 2003; Dufour, *et al.*, 2004; Weber, *et al.*, 2004; Kudahl, *et al.*, 2007b; Kudahl, *et al.*, 2008; Weber, *et al.*, 2008). The only strategies predicting a drop in the prevalence are the ones including actions targeting infection routes (such as improved hygiene, improved milk feeding management); test-and-cull strategies alone (considering imperfect tests) have no or little effect on the prevalence (model N°2 & 7). In model N°2, the more management measures a farmer takes, the more effective a control programme is. It is not possible to rank studied control measures according to their effectiveness as results of individual management control measures are not given. If tests had a sensitivity of 1, the impact of a test-&-cull option would be more important. The reviewed models cannot be used to evaluate the impact of contact structure on *Map*

transmission, as contact structure is not specifically represented. However, it could be of interest knowing whether limiting indirect contacts between adults and calves or between calves would have an effect on *Map* transmission.

8 Model validation

Validation of a model is the step which assesses if the model accurately represents the real system. Qualitative or quantitative validation is performed by checking that appropriate assumptions have been made, by verifying that observed behaviours can be mimicked with the model, or by comparing model outputs with observed data. Validation is generally partial (Oreskes, *et al.*, 1994) as it is usually difficult to compare strictly similar situations. For example it is rare to collect data for a situation without any control measure when it comes to paratuberculosis. To evaluate model behaviour, a sensitivity analysis can be performed on uncertain variables. Sensitivity analysis aims to quantify how models outputs vary with a change in parameter values (Saltelli, *et al.*, 2000). Uncertain parameters strongly influencing model outputs are suitable targets for further research.

The consistency between model outputs and real system behaviour was assessed for model N°2 via the study of the transmission parameter. Model N°2 simulates an overall transmission parameter similar to estimates from a field study (Groenendaal, *et al.*, 2002). Moreover, the results of a certification-and-surveillance scheme were comparable in outcomes of a simulation with model N°2 and in a field study in 90 dairy herds (Weber, *et al.*, 2004). The small number of such comparisons is associated with the lack of comprehensive data about *Map* dynamics. The seasonal hypothesis (exposure differs between winter housing *versus* summer grazing) made in model N°6 was examined against data collected for this purpose (Kudahl *et al.*, 2007a). No effect of the season of calving was found in field data.

In five models (N°2, 4, 6, 7 & 8), a sensitivity analysis is performed on uncertain parameters. Studied parameters related to exposure are bacterial survival rate, density of bacteria in the faeces of shedding cattle, size of the infectious area (these 3 first parameters being studied in model N°6), number of contacts to potentially infected cattle (models N°7 & 8), and initial herd-level prevalence (one associated paper of model N°2 analyzing this model: Weber *et al.*, 2008). Model N°6 appears to be highly sensitive to changes in bacterial density and to size of the exposure area. Other models are also sensitive to the parameters related to exposure they studied. Studied parameters related to transmission are pathogen transmission probability (model N°2 and one associated paper analyzing this model: Weber *et al.*, 2008 & model N°4), probability of introducing infected cattle (model N°2 and one associated paper analyzing this model: Groenendaal *et al.*, 2003), and probability of infection (model N°6). All models are sensitive to all the parameters related to the particular transmission mechanism they studied. Finally, studied parameters related to infection consequences are the

probabilities of disease onset (models N°2 & 4). Again, models are sensitive to all the parameters related to infection consequences they studied. The sensitivity analyses did not enable identifying any parameter related to *Map* transmission and consequences for which uncertainty has little effect on the model results.

9 Discussion

The review has shown that the models described in the published literature are constrained by the limitations of the epidemiological knowledge at the time of their development. The maximum age at which cattle can become infected, and the dose-response relationship between the quantity of *Map* organisms and infection probability of cattle at different ages are currently unknown. Whether dose or age at infection has an influence on disease progression is also unknown. It should be kept in mind that the identification of important areas for future research is one of the purposes of modelling. Moreover, as new information becomes available, the disease processes implemented in these models can be represented more precisely and parameters can be more accurately defined. Impact of selective culling of most susceptible calves or future super-shedders can be studied when information on genetic susceptibility markers is available. However, complex models are not necessarily able to represent a biological system better than simpler ones. In summary, based on current knowledge, *Map* transmission could be more precisely modelled by including the recently described calf-to-calf transmission pathway, a more detailed contact structure between animals within a herd, and explicit incorporation of indirect transmission, representing *Map* transmission in the environment. Consequences of this improved precision on model accuracy still would have to be investigated.

The number of compartments represented in the models varies. The compartments should reflect the pathogenesis of the disease and thus susceptible, transiently infectious, latently infected, infectious and resistant states should be represented. Moreover, clinically affected animals are specific infectious animals which all shed a high load of *Map*. *In utero* transmission is of greater risk for them and their probability of being culled higher. Having a specific compartment thus seems essential. Other compartments could be added depending on the aim of modelling. For example, super-shedders have been described and their role in the infection dynamics needs further investigation. Two modelling options could be used: either considering a large variability of shedding within the infectious compartment, with a small proportion of individuals shedding really high amount of *Map* (to represent heterogeneity of shedding in time); or considering an extra compartment for super-shedders, super-shedders being considered as specific animals.

It is now acknowledged that calf-to-calf transmission occurs, albeit at a lower rate than adult-to-calf transmission. Furthermore, this newly acknowledged

route of transmission appears to be necessary for *Map* persistence in the only published model that takes account of calf-to-calf transmission. However, little is known on calf-to-calf transmission rate or on the contribution of this route to total transmission in a herd. A study reported that in one herd, 9.5% of the offspring of test-negative dams, not exposed to other positive cows in the calving pen, became infected (Benedictus, *et al.*, 2008). Overall, a risk of 6% was not explained by exposure to a positive dam, exposure in the calving area, or by being raised with a calf that later in life becomes a high shedder. The difference, 3.5%, can be considered as an estimate of the risk of becoming infected due to calf-to-calf transmission in this herd. Hence, it is important to develop models which evaluate whether calf-to-calf transmission might be an important critical point for controlling *Map* transmission.

In all published models, it is assumed that calves can become infected through several transmission routes during the susceptible period prior to the age of one year. However, the relative importance of each route in *Map* infection dynamics within an infected herd is poorly understood. To assess more precisely the contribution of the different routes of transmission, the level of shedding has to be explicitly modelled. Moreover, to account for indirect milk and colostrum contamination by both faeces of the dam and the global contamination of the environment around birth, an overall infectiousness of the herd has to be considered. Information on the relative importance of each route of transmission is required for defining more cost-effective preventive measures as farmers are unlikely to implement management procedures that affect several mechanisms at the same time, but instead will need to prioritize their actions. Knowing the relative importance of each route of infection would allow balanced decisions in relation to the most cost-effective control measures.

Currently available models differ substantially in relation to their assumptions with respect to herd size, management and structure (Table V). The influence of these assumptions on the infection process is not known. While 3 papers study the impact of herd size on model outputs, none studies the impact of calf-to-calf and adult-to-calf contacts. In order to determine which characteristics of the herd management or herd structure have to be accounted for in future modelling, one would need a model enabling to study one-at-a-time the impact of considering or not one option. Moreover, it is currently difficult to compare or characterize available models in terms of accuracy as baseline scenario (acting as ‘gold standard’) and outputs of interest are missing or not reported. It is indeed at present not possible to perform a model comparison in achieving maintenance of infection, given available data on published models. Future model simulation should produce outputs on *Map* persistence in a herd so that comparisons could be performed on critical features. The impact of contact structure on *Map* transmission has never or partially been addressed. This would necessitate developing a new model. A definite conclusion on the need to complicate model structure is not currently possible, but neglecting the contact structure and ignoring transmission via the environment are likely to highly influence the model outputs. Direct contact

Table V: Summary of assumptions incorporated in 8 Mycobacterium avium paratuberculosis *(*Map*) transmission models in cattle herds (for assumptions on transmission, see Table IV)*

Reference		Susceptibility to infection[1]		Stages of infection	Shedding		Survival of *Map* in the environment influences transmission	Contact structure influences transmission
		In young animals	In adults		Exists in calves	Varies between statuses and influence risk of infection		
N°1	(Collins and Morgan, 1991a)	Constant		2				
N°2	(Groenendaal, *et al.*, 2002)[2]	Decreases with age		4		Yes		Yes
N°3	(Van Roermund, *et al.*, 2002)	Decreases with age		4		Yes		
N°4	(Pouillot, *et al.*, 2004)	Constant		3		Yes		
N°5	(Van Roermund, *et al.*, 2005)	Decreases with age		4		Yes		
N°7	(Kudahl, *et al.*, 2007a)	Decreases with age		4		Yes		
N°6	(Humphry, *et al.*, 2006)	Decreases with age	Yes	4		Yes	Yes	
N°8	(Mitchell, *et al.*, 2008)	Constant		4	Yes	Yes		Yes

[1] empty cell: No

between calves or sharing the same environment depends on the sub-grouping of animals of the same age and on their housing. When modelling calf-to-calf transmission via the environment, one should not forget to consider that calves shed less and produce fewer faeces than adults.

Selecting a density or frequency-dependent function of transmission can have an impact on the outputs (Hoch, *et al.*, 2008). The choice of the transmission function must be based on the biological knowledge on modelled pathogen and host and/or an experimental data. It is difficult to select a priori one or the other function for paratuberculosis where indirect transmission is considered. However, the frequency-dependent function assumes that the occupied area is constant whatever the population size (Begon, *et al.*, 2002), which is usually not the case for the size of pens and farm facilities when the size of the herd increases. Therefore, a density dependent function seems to better reflect exposure of susceptible animals.

Simplifications have been performed in all models in terms of detection of the different stages of infection. Test sensitivities applied for each model compartment are specified as constant in a time period, ignoring that intervals between tests are often long (and up to one year). Using a constant sensitivity during one year is questionable as there is no data in the literature on test sensitivity when applied to up to one year before the true status is known. It is thus probable that average sensitivities are over-estimated in such models.

Adequate parameterization of models such as the ones reviewed here is often difficult, due to lack of data. Consequently, several parameters are highly uncertain, particularly those related to pathogen transmission and between-group rates of contact. Uncertainties can be evaluated through sensitivity analyses that determine which parameters have a strong influence on the results and thus should be studied in priority in future field research. The reviewed models have mainly been evaluated through a sensitivity analysis but only for a small number of parameters, and only one at a time. Only model N°4 performed an analysis for several parameters simultaneously. A more systematic sensitivity analysis taking account of interactions between parameters and of all the unknown or uncertain parameters should be performed in order to determine where uncertainty must be reduced and to evaluate whether a model can be used as a predictive tool or to improve our understanding (Saltelli, *et al.*, 2000).

There is a need for new models which consider indirect transmission via the environment taking account of *Map* survival and contact structure between animals in a herd. Such models should provide outputs on persistence and relative importance of the routes of transmission in order to be able to provide critical features on the construction of future *Map* transmission models in terms of herd management and herd structure characteristics.

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References

Anderson, J. L., Meece, J. K., Koziczkowski, J. J., Clark, D. L., Radcliff, R. P., Nolden, C. A., Samuel, M. D. and Ellingson, J. L. E., *Mycobacterium avium* subsp. *paratuberculosis* in scavenging mammals in Wisconsin, J. Wildl. Dis. (2007) 43:302-308.

Antognoli, M. C., Hirst, H. L., Garry, F. B. and Salman, M. D., Immune response to and faecal shedding of *Mycobacterium avium* ssp. *paratuberculosis* in young dairy calves, and the association between test results in the calves and the infection status of their dams, Zoonoses Public Health. (2007) 54:152-159.

Beard, P. M., Daniels, M. J., Henderson, D., Pirie, A., Rudge, K., Buxton, D., Rhind, S., Greig, A., Hutchings, M. R., McKendrick, I., Stevenson, K. and Sharp, J. M., Paratuberculosis infection of nonruminant wildlife in Scotland, J. Clin. Microbiol. (2001) 39:1517-1521.

Begg, D. J. and Whittington, R. J., Experimental animal infection models for Johne's disease, an infectious enteropathy caused by *Mycobacterium avium* subsp. *paratuberculosis*, Vet. J. (2008) 176:129-145.

Begon, M., Bennett, M., Bowers, R. G., French, N. P., Hazel, S. M. and Turner, J., A clarification of transmission terms in host-microparasite models : numbers, densities and areas, Epidemiol. Infect. (2002) 129:147-153.

Benedictus, A., Mitchell, R. M., Linde-Widmann, M., Sweeney, R., Fyock, T., Schukken, Y. H. and Whitlock, R. H., Transmission parameters of *Mycobacterium avium* subspecies *paratuberculosis* infections in a dairy herd going through a control program, Prev. Vet. Med. (2008) 83:215-227.

Benedictus, G., Dijkhuizen, A. A. and Stelwagen, J., Economic losses due to paratuberculosis in dairy cattle, Vet. Rec. (1987) 121:142-146.

Bolton, M. W., Grooms, D. L. and Kaneene, J. B., Fecal shedding of *Mycobacterium avium* subsp. *paratuberculosis* in calves: implications for disease control and management, Proceedings of the 8th International Colloquium on Paratuberculosis, Copenhagen, Denmark, (2005), pp. 596-600.

Chiodini, R. J., Van Kruiningen, H. J. and Merkal, R. S., Ruminant paratuberculosis (Johne's disease): the current status and future prospects, Cornell Vet. (1984) 74:218-262.

Clarke, C. J., The pathology and pathogenesis of paratuberculosis in ruminants and other species, J. Comp. Path. (1997) 116:217-261.

Collins, M. T. and Morgan, I. R., Epidemiological model of paratuberculosis in dairy cattle, Prev. Vet. Med. (1991a) 11:131-146.

Collins, M. T. and Morgan, I. R., Economic decision analysis model of a paratuberculosis test and cull program, J. Am. Vet. Med. Assoc. (1991b) 199:1724-1729.

Collins, M. T. and Morgan, I. R., Simulation model of paratuberculosis

control in a dairy herd, Prev. Vet. Med. (1992) 14:21-32.

European Commission, Possible links between Crohn's disease and Paratuberculosis. Report of the Scientific Committee on Animal Health and Animal Welfare (2000) http://ec.europa.eu/food/fs/sc/scah/out38_en.pdf, [consulted 5 November 2008], pp. 76.

Crossley, B. M., Zagmutt-Vergara, F. J., Fyock, T. L., Whitlock, R. H. and Gardner, I. A., Fecal shedding of *Mycobacterium avium* subsp. *paratuberculosis* by dairy cows, Vet. Microbiol. (2005) 107:257-263.

Daniels, M. J., Hutchings, M. R., Beard, P. M., Henderson, D., Greig, A., Stevenson, K. and Sharp, J. M., Do non-ruminant wildlife pose a risk of paratuberculosis to domestic livestock and vice versa in Scotland?, J. Wildl. Dis. (2003) 39:10-15.

Dieguez, F. J., Arnaiz, I., Sanjuan, M. L., Vilar, M. J. and Yus, E., Management practices associated with *Mycobacterium avium* subspecies *paratuberculosis* infection and the effects of the infection on dairy herds, Vet. Rec. (2008) 162:614-617.

Dorshorst, N. C., Collins, M. T. and Lombard, J. E., Decision analysis model for paratuberculosis control in commercial dairy herds, Prev. Vet. Med. (2006) 75:92-122.

Doyle, T. M., Susceptibility to Johne's disease in relation to age, Vet. Rec. (1953) 65:363-364.

Dufour, B., Pouillot, R. and Durand, B., A cost/benefit study of paratuberculosis certification in French cattle herds, Vet. Res. (2004) 35:69-81.

Ezanno, P., van Schaik, G., Weber, M. F. and Heesterbeek, J. A., A modeling study on the sustainability of a certification-and-monitoring program for paratuberculosis in cattle, Vet. Res. (2005) 36:811-26.

Ezanno, P., Fourichon, C. and Seegers, H., Influence of herd structure and type of virus introduction on the spread of bovine viral diarrhoea virus (BVDV) on the spread of bovine viral diarrhoea virus (BVDV) within a dairy herd, Vet. Res. (2008) 39:39.

Frank, D. N., *Mycobacterium avium* subspecies *paratuberculosis* and Crohn's disease, Lancet Infect. Dis. (2008) 8:345-345.

Gay, J. M. and Sherman, D. M., Factors in the epidemiology and control of ruminant paratuberculosis, Vet. Med. (1992) 87:1133-1139.

Gonda, M. G., Chang, Y. M., Shook, G. E., Collins, M. T. and Kirkpatrick, B. W., Genetic variation of *Mycobacterium avium* ssp. *paratuberculosis* infection in US Holsteins, J. Dairy Sci. (2006) 89:1804-1812.

Gonda, M. G., Kirkpatrick, B. W., Shook, G. E. and Collins, M. T., Identification of a QTL on BTA20 affecting susceptibility to *Mycobacterium avium* ssp. *paratuberculosis* infection in US Holsteins, Anim. Genet. (2007) 38:389-396.

Groenendaal, H., Nielen, M., Jalvingh, A. W., Horst, S. H., Galligan, D. T. and Hesselink, J. W., A simulation of Johne's disease control, Prev. Vet. Med. (2002) 54:225-245.

Groenendaal, H. and Galligan, D. T., Economic consequences of control programs for paratuberculosis in midsize dairy farms in the United States, J. Am. Vet. Med. Assoc. (2003) 223:1757-1768.

Groenendaal, H., Nielen, M. and Hesselink, J. W., Development of the Dutch Johne's disease control program supported by a simulation model, Prev. Vet. Med. (2003) 60:69-90.

Groenendaal, H. and Zagmutt, F. J., Scenario analysis of changes in consumption of dairy products caused by a hypothetical causal link between *Mycobacterium avium* subspecies *paratuberculosis* and Crohn's disease, J. Dairy Sci. (2008) 91:3245-3258.

Hagan, W. A., Age as a factor in susceptibility to Johne's disease, Cornell Vet. (1938) 28:34-40.

Hoch, T., Fourichon, C., Viet, A. F. and Seegers, H., Influence of the transmission function on a simulated pathogen spread within a population, Epidemiol. Infect. (2008) 136:1374-1382.

Humphry, R. W., Stott, A. W., Adams, C. and Gunn, G. J., A model of the relationship between the epidemiology of Johne's disease and the environment in suckler-beef herds, Vet. J. (2006) 172:432-445.

Jakobsen, M. B., Alban, L. and Nielsen, S. S., A cross-sectional study of paratuberculosis in 1155 Danish dairy cows, Prev. Vet. Med. (2000) 46:15-27.

Johnson-Ifearulundu, Y. J. and Kaneene, J. B., Epidemiology and economic impact of subclinical Johne's disease: a review, Vet. Bull. (1997) 67:437-447.

Kalis, C. H. J., Hesselink, J. W., Barkema, H. W. and Collins, M. T., Use of long-term vaccination with a killed vaccine to prevent fecal shedding of Mycobacterium avium subsp paratuberculosis in dairy herds, Am. J. Vet. Res. (2001) 62:270-274.

Kalis, C. H. J., Collins, M. T., Barkema, H. W. and Hesselink, J. W., Certification of herds as free of Mycobacterium paratuberculosis infection : actual pooled faecal results versus certification model predictions, Prev. Vet. Med. (2004) 65:189-204.

Koets, A. P., Adugna, G., Janss, L. L. G., Van, W. H. J., Kalis, C. H. J., Wentink, G. H., Rutten, V. P. M. G. and Schukken, Y. H., Genetic variation of susceptibility to *Mycobacterium avium* subsp. *paratuberculosis* infection in dairy cattle, J. Dairy Sci. (2000) 83:2702-2708.

Kormendy, B., The effect of vaccination on the prevalence of paratuberculosis in large dairy herds, Vet. Microbiol. (1994) 41:117-125.

Kostoulas, P., Nielsen, S. S., Browne, W. J. and Leontides, L., A Bayesian Weibull survival model for time to infection data measured with delay, Prev. Vet. Med. (2010) 94:191-201.

Kruip, T. A. M., Muskens, J., Van, R. H. J. W., Bakker, D. and Stockhofe-Zurwieden, N., Lack of association of *Mycobacterium avium* subsp. *paratuberculosis* with oocytes and embryos from moderate shedders of the pathogen, Theriogenology. (2003) 59:1651-1660.

Kudahl, A. B., Ostergaard, S., Sorensen, J. T. and Nielsen, S. S., A stochastic model simulating paratuberculosis in a dairy herd, Prev. Vet. Med. (2007a) 78:97-117.

Kudahl, A. B., Sorensen, J. T., Nielsen, S. S. and Ostergaard, S., Simulated economic effects of improving the sensitivity of a diagnostic test in paratuberculosis control, Prev. Vet. Med. (2007b) 78:118-129.

Kudahl, A. B., Nielsen, S. S. and Ostergaard, S., Economy, efficacy, and feasibility of a risk-based control program against paratuberculosis, J. Dairy Sci. (2008) 91:4599-4609.

Larsen, A. B., Merkal, R. S. and Vardaman, T. H., Survival time of *Mycobacterium paratuberculosis*, Am. J. Vet. Res. (1956) 17:549-551.

Larsen, A. B., Merkal, R. S. and Cutlip, R. C., Age of cattle as related to resistance to infection with *Mycobacterium paratuberculosis*, Am. J. Vet. Res. (1975) 36:255-257.

Lovell, R., Levi, M. and Francis, J., Studies on the survival of Johne's bacilli, J. Comp. Pathol. (1994) 54:120-129.

Lu, Z., Mitchell, R. M., Smith, R. L., Van Kessel, J. S., Chapagain, P. P., Schukken, Y. H. and Grohn, Y. T., The importance of culling in Johne's disease control, J. Theor. Biol. (2008) DOI:10.1016/j.jtbi.2008.05.008.

Manning, E. J., *Mycobacterium avium* subspecies *paratuberculosis*: a review of current knowledge, J. Zoo Wildl. Med. (2001) 32:293-304.

McKenna, S. L. B., Keefe, G. P., Tiwari, A., VanLeeuwen, J. and Barkema, H. W., Johne's disease in Canada Part II: Disease impacts, risk factors, and control programs for dairy producers, Can. Vet. J. (2006) 47:1089-1099.

Mitchell, R. M., Whitlock, R. H., Stehman, S. M., Benedictus, A., Chapagain, P. P., Grohn, Y. T. and Schukken, Y. H., Simulation modeling to evaluate the persistence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) on commercial dairy farms in the United States, Prev. Vet. Med. (2008) 83:360-380.

Mortensen, H., Nielsen, S. S. and Berg, P., Genetic variation and heritability of the antibody response to *Mycobacterium avium* subsp. *paratuberculosis* in Danish Holstein cows, J. Dairy Sci. (2004) 87:2108-2113.

Muskens, J., Elbers, A. R. W., Van, W. H. J. and Noordhuizen, J. P. T. M., Herd management practices associated with paratuberculosis seroprevalence in Dutch dairy herds, J. Vet. Med. B. (2003) 50:372-377.

Nauta, M. J. and van der Giessen, J. W. B., Human exposure to Mycobacterium paratuberculosis via pasteurised milk: A modelling approach, Vet. Rec. (1998) 143:293-296.

Nielsen, S. S. and Ersboll, A. K., Age at occurrence of *Mycobacterium avium* subspecies *paratuberculosis* in naturally infected dairy cows, J. Dairy Sci. (2006) 89:4557-4566.

Obasanjo, I. O., Gröhn, Y. T. and Mohammed, H. O., Farm factors associated with the presence of *Mycobacterium paratuberculosis* infection in dairy herds on the New York State paratuberculosis control program, Prev. Vet. Med. (1997) 32:243-251.

Oreskes, N., Shrader-Frechette, K. and Belitz, K., Verification, Validation, and Confirmation of Numerical Models in the Earth Sciences, Science. (1994) 263:641-646.

Pouillot, R., Dufour, B. and Durand, B., A deterministic and stochastic simulation model for intra-herd paratuberculosis transmission, Vet. Res. (2004) 35:53-68.

Rankin, J. D., The experimental infection of cattle with *Mycobacterium johnei*. III. Calves maintained in an infectious environment, J. Comp. Pathol. (1961) 71:10-15.

Rossiter, C. A. and Burhans, W. S., Farm-specific approach to paratuberculosis (Johne's disease) control, Vet. Clin. North Am. Food Anim. Pract. (1996) 12:383-415.

Saltelli, A., Chan, K. A. and Scott, E. M., Sensitivity analysis, John Wiley & Sons, LTD, Chichester, England, (2000), pp. 475.

Shafran, I. and Burgunder, P., Potential pathogenic role of *Mycobacterium avium* subspecies *paratuberculosis* in Crohn's disease, Inflamm. Bowel Dis. (2008) 10.1002/ibd.20513.

Smythe, R. H., Some observations on Johne's disease, Vet. Rec. (1950) 62:429-441.

Sockett, D. C., Carr, D. J., Richards, W. D. and Collins, M. T., A repository of specimens for comparison of diagnostic testing procedures for bovine paratuberculosis, J. Vet. Diagn. Invest. (1992) 4:188-191.

Streeter, R. N., Hoffsis, G. F., Bech-Nielsen, S., Shulaw, W. P. and Rings, M., Isolation of *Mycobacterium paratuberculosis* from colostrum and milk of subclinically infected cows, Am. J. Vet. Res. (1995) 56:1322-1324.

Sweeney, R. W., Whitlock, R. H. and Rosenberger, A. E., *Mycobacterium paratuberculosis* isolated from fetuses of infected cows not manifesting signs of the disease, Am. J. Vet. Res. (1992a) 53:477-480.

Sweeney, R. W., Whitlock, R. H. and Rosenberger, A. E., *Mycobacterium paratuberculosis* cultured from milk and supramammary lymph nodes of infected asymptomatic cows, J. Clin. Microbiol. (1992b) 30:166-171.

Tavornpanich, S., Munoz-Zanzi, C. A., Wells, S. J., Raizman, E. A., Carpenter, T. E., Johnson, W. O. and Gardner, I. A., Simulation model for evaluation of testing strategies for detection of paratuberculosis in Midwestern US dairy herds, Prev. Vet. Med. (2008) 83:65-82.

Taylor, A. W., Experimental Johne's disease in cattle, J. Comp. Pathol. (1953) 63:355-373.

Taylor, T. K., Wilks, C. R. and McQueen, D. S., Isolation of *Mycobacterium paratuberculosis* from the milk of a cow with Johne's disease, Vet. Rec. (1981) 109:532-533.

van Roermund, H. J. W. and de Jong, M. C. M., Within-herd transmission of paratuberculosis and the possible role of infectious calves, Proceedings of the 7th International Colloquium on Paratuberculosis, Bilbao, Spain, (2002), pp. 92.

Van Roermund, H. J. W., Weber, M. F., Graat, E. A. M. and De Jong, M. C. M., Monitoring programmes for paratuberculosis-unsuspected cattle herds, based on quantification of between-herd transmission, Proceedings of the 7th International Colloquium on Paratuberculosis, Bilbao, Spain, (2002), pp. 371-375.

Van Roermund, H. J. W., Weber, M. F., de Koeijer, A. A., Velthuis, A. G. J. and de Jong, M. C. M., Development of a milk quality assurance program for paratuberculosis: from within- and between-herd dynamics to economic decision analysis, Proceedings of the 8th International Colloquium on Paratuberculosis, Copenhagen, Denmark, (2005), pp. 51-59.

Van Roermund, H. J. W., Bakker, D., Willemsen, P. T. J. and De Jong, M. C. M., Horizontal transmission of *Mycobacterium avium* subsp. *paratuberculosis* in cattle in an experimental setting: Calves can transmit the infection to other calves, Vet. Microbiol. (2007) 122:270-279.

Van Schaik, G., Kalis, C. H. J., Benedictus, G., Dijkhuizen, A. A. and Huirne, R. B. M., Cost-benefit analysis of vaccination against paratuberculosis in dairy cattle, Vet. Rec. (1996) 139:624-627.

Weber, M. F., Groenendaal, H., Van, R. H. J. W. and Nielen, M., Simulation of alternatives for the Dutch Johne's disease certification-and-monitoring program, Prev. Vet. Med. (2004) 62:1-17.

Weber, M. F., Kogut, J., de Bree, J., van Schaik, G. and Nielen, M., Survival analysis of age at onset of shedding of *Mycobacterium avium* subsp. *paratuberculosis*, Proceedings of the 11th International Society for Veterinary Epidemiology and Economics, (2006), pp. 444.

Weber, M. F., Nielen, M., Velthuis, A. G. J. and van Roermund, H. J. W., Milk quality assurance for paratuberculosis: simulation of within-herd infection dynamics and economics, Vet. Res. (2008) 39:12.

Weber, M. F., Verhoeff, J., van Schaik, G. and van Maanen, C., Evaluation of Ziehl-Neelsen stained faecal smear and ELISA as tools for surveillance of clinical paratuberculosis in cattle in the Netherlands, Prev. Vet. Med. (2009) 92:265-266.

Wells, S. J. and Wagner, B. A., Herd-level risk factors for infection with mycobacterium paratuberculosis in US dairies and association between familiarity of the herd manager with the disease or prior diagnosis of the

disease in that herd and use of preventive measures, J. Am. Vet. Med. Ass. (2000) 216:1450-1457.

Wentink, G. H., Bongers, J. H., Zeeuwen, A. A. and Jaartsveld, F. H., Incidence of paratuberculosis after vaccination against *M. paratuberculosis* in two infected dairy herds, Zentrabl. Veterinärmed - B. (1994) 41:517:522.

Whitlock, R. H. and Buergelt, C., Preclinical and clinical manifestations of paratuberculosis (including pathology), Vet. Clin. North Am. Food Anim. Pract. (1996) 12:345-356.

Whitlock, R. H., Wells, S. J., Sweeney, R. W. and Van Tiem, J., ELISA and fecal culture for paratuberculosis (Johne's disease): sensitivity and specificity of each method, Vet. Microbiol. (2000) 77:378-398.

Whittington, R. J., Marshall, D. J., Nicholls, P. J., Marsh, I. B. and Reddacliff, L. A., Survival and dormancy of *Mycobacterium avium* subsp. *paratuberculosis* in the environment, Appl. Environ. Microbiol. (2004) 70:2989-3004.

Whittington, R. J., Marsh, I. B. and Reddacliff, L. A., Survival of *Mycobacterium avium* subsp. *paratuberculosis* in dam water and sediment, Appl. Environ. Microbiol. (2005) 71:5304-5308.

Whittington, R. J. and Windsor, P. A., In utero infection of cattle with *Mycobacterium avium* subsp. *paratuberculosis*: A critical review and meta-analysis, Vet. J. (2009) 179:60-69.

Windsor, P. A. and Whittington, R. J., Evidence for age susceptibility of cattle to Johne's disease, Vet. J. (2010) 184:37-44.

Chapter 2

Spontaneous fadeout versus persistence of *Mycobacterium avium* subsp. *paratuberculosis* infection in a dairy herd: a modelling study

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Abstract

Epidemiological models enable to better understand the dynamics of infectious diseases and to assess *ex-ante* control strategies. For *Mycobacterium avium* subsp. *paratuberculosis* (*Map*), possible transmission routes have been described, but *Map* spread in a herd and the relative importance of the routes are currently insufficiently understood to prioritize control measures. We aim to predict early after *Map* introduction in a dairy cattle herd whether infection is likely to fade out or persist, when no control measures are implemented, using a modelling approach. Both vertical transmission and horizontal transmission via the ingestion of colostrum, milk, or faeces present in the contaminated environment were modelled. Calf-to-calf indirect transmission was possible. Six health states were represented: susceptible, transiently infectious, latently infected, subclinically infected, clinically affected, and resistant. The model was partially validated by comparing the simulated prevalence with field data. Housing facilities and contacts between animals were specifically considered for calves and heifers. After the introduction of one infected animal in a naive herd, fadeout occurred in 66% of the runs. When *Map* persisted, the prevalence of infected animals increased to 88% in 25 years. The two main transmission routes were via the farm's environment and *in utero* transmission. Calf-to-calf transmission was minor. Fadeout *versus Map* persistence could be differentiated with the number of clinically affected animals, which was rarely above one when fadeout occurred. Therefore, early detection of affected animals is crucial in preventing *Map* persistence in dairy herds.

Keywords: stochastic model, paratuberculosis, fadeout, transmission route, persistent infection

1 Introduction

In dairy herds, paratuberculosis, a worldwide disease, provokes decreases in milk production, drops in carcass slaughter value, and premature culling. However, due to the long incubation period (Weber, *et al.*, 2009) and the low sensitivity of available diagnostic tests (Nielsen, 2008), studying the infection dynamics in the field is nearly impossible. Therefore, modelling is used to better understand the spread of *Mycobacterium avium* subspecies *paratuberculosis* (*Map*) within a herd.

Our objective is to predict as early as possible after *Map* first introduction in a dairy cattle herd whether infection is likely to fade out or to persist using a modelling approach. The results could then be used to inform the implementation of control methods. Indeed, we will determine the point of no return after which *Map* will persist and spread in the herd without control, i.e. when control actions must ideally be implemented. Therefore, we assume that no further infected animals are introduced to avoid the possibility that persistence of *Map* might be due to continuous reintroductions (i.e. no fadeout being possible). Such a situation will be typical for herds with very low yearly purchase rates (e.g. dairy herds in Brittany without any fattening activity; (Ezanno, *et al.*, 2006)) or in the context of certification, when only certified animals are purchased (with a very low risk of being infected; (Ezanno, *et al.*, 2005)). In Europe, control of *Map* introduction into cattle herds has indeed priority over control of within-herd *Map* spread.

There is no published model on *Map* spread within a dairy herd that takes account of *Map* survival in the environment (Marcé, *et al.*, 2010a). Yet, the survival of *Map* in the environment can result in a delay between shedding by infectious animals and infection of susceptible animals. As a result of contamination of the farm environment, infection of susceptible animals can occur in the absence of infectious animals (Jorgensen, 1977; Whittington, *et al.*, 2004). Here, we explicitly model transmission via the environment. Furthermore, calf-to-calf transmission recently has been demonstrated (Van Roermund, *et al.*, 2007). Hence, transmission routes are: vertical, horizontal via the ingestion of contaminated colostrum or milk, or horizontal via the ingestion of adult or calf faeces. We propose a new model that accounts for all of these transmission routes, thus rendering it possible to identify which routes contribute the most to *Map* spread in the modelled dairy herd.

2 Materials and methods

We develop a model of *Map* spread within a dairy cattle herd initially naive towards *Map* infection, following the introduction of a single infected cow. We use this model to predict *Map* spontaneous fadeout or persistence as early as possible after *Map* introduction, before any control measure is implemented.

2.1 Model description

A discrete time compartmental model is developed to represent *Map* spread in a dairy cattle herd. We couple a model that simulates the population dynamics within a dairy herd and explicitly represents animal housing facilities with an epidemiological model of *Map* transmission. A time step of 1 week is chosen as the longest possible to allow the different transmission routes and calf exposure in housing facilities to be represented. A stochastic model is used in order to study the chance of fadeout of the disease *versus* persistence probability. Because of the slow progression of paratuberculosis, we choose to study the infection over a 25-year period. The model is implemented with Scilab 5.1[1].

2.1.1 Population dynamics

The population dynamics only considers characteristics related to *Map* transmission. Contacts between susceptible animals and any environment contaminated by shedding animals depends on the time spent by animals on farm, the time spent in individual and collective pens, and possible shared environments. An ageing process occurs before the infection process at each time step. An exit rate for mortality, sale, and culling is defined per age class (Table I).

In Europe, dairy herds generally are structured in groups, the younger animals being separated from the older ones (Marcé, *et al.*, 2010b). Here, group definition accounts for animal housing and management, and the maximal age (*u*, Table II) at which an animal is susceptible (Figure 1). Therefore, contacts between susceptible animals and contaminated environments can be assessed. Calves below 1 year of age are either in individual pens (from birth to *m*), in collective pens before weaning (from *m* to *w*), or in collective pens after weaning (from *w* to *y*). Calves in individual pens have limited contacts with the faeces of calves from contiguous pens (*nb*). Such a calf housing facility management follows

[1] Available on line: http://www.scilab.org [consulted 11 February 2010]

Table I: Parameters for herd management and population dynamics used in a Mycobacterium avium paratuberculosis *infection dynamics model within a structured dairy herd*

Notation	Value	Definition	Source
σ_B	0.07	Mortality rate of calves at birth	a[1]
σ_m	0.206	Exit rate of male calves, weeks 2 to 4 (per week)	
σ_{C1}	0.015	Death rate of female calves, weeks 1 and 2 (individual housing facilities) (per week)	[2]
σ_{C2}	0.0035	Death rate of female calves, weeks 3 to weaning (collective housing facilities) (per week)	[2]
σ_{C3}	0.00019	Death rate of heifers from weaning to first calving (per week)	b
σ_h	0.11	Sale rate of bred heifers 10 weeks before 1st calving	b
σ_{Ai}	27, 25, 31, 31, 62	Yearly culling rate of cows in parity 1, 2, 3, 4 and above 5 respectively (%)	[3],a
m	2	Maximal age in individual pen (weeks)	Marcé et al., 2010b
w	10	Weaning age (weeks)	Marcé et al., 2010b
y	52	Age when entering the young heifer group (weeks)	
nb	2	Number of neighbours for a calf in an individual pen	b
h	91	Age at first artificial insemination (weeks)	a
cal	130	Age at first calving (weeks)	a,b
cci	56.3	Calving-to-calving interval (weeks)	a,b
b	5	Quantity of colostrum fed to calves (L/day for 3 days)	b
d	7	Quantity of milk fed to calves after 3 days (L/day/calf)	b
$prop$	0.85	Proportion of lactating cows	a
ε	25	Quantity of milk or colostrum produced (L/day/cow)	a
f_1	0.5	Quantity of faeces produced by a non-weaned calf (kg/day)	b
f_2	5.5	Quantity of faeces produced by a weaned calf (kg/day)	b
f_Y	10	Quantity of faeces produced by a heifer (kg/day)	b
f_A	30	Quantity of faeces produced by a cow (kg/day)	b
$Graz$	[14-46]	Grazing period (1 being the first week of the year)	b
K_c	110	Number of cows above which the heifer selling rate increases	-

[a] Agricultural statistics

[b] Expert opinions

[1] Rio O., Frequency and risks of mortality and health disorders of calves in dairy cattle herds [in French], Doctorate in Veterinary Medicine Thesis, Nantes, France, 1999.

[2] Jégou V., Porhiel J.Y., Brunschwig P., Risk management factors affecting mortality among dairy calves herds in 80 herds in Brittany [in French], in: Proc. Journées Bovines Nantaises, Nantes, France, 2006, pp. 6.

[3] Beaudeau F., Review of culling and replacement practices in dairy cattle herds [in French], Institut Supérieur des Productions Animales, Master Thesis, Rennes, France, 1991.

Table II: Parameters for infection and transmission used in a Mycobacterium avium *subsp.* paratuberculosis (Map) *infection dynamics model within a structured dairy herd**

Notation	Value	Definition	Source
p_X		Probability of *in utero* transmission for cow in health state X	Benedictus *et al.*, 2008; Whittington & Windsor, 2009
	p_L=0.149	X = latently infected (L)	
	p_{Is}=0.149	X = subclinically infected (I_S)	
	p_{Ic}=0.65	X = clinically affected (I_C)	
u	52	Maximal age in the susceptible compartment (weeks)	Hagan, 1938; Whitlock & Buergelt, 1995
h	0.1	Susceptibility follows an exponential decrease exp(-h(age-1)))	Windsor & Whittington, 2010
v_X		Mean time spent in health state X (weeks)	
	v_T =25	X = transiently infectious (T)	Van Roermund *et al.*, 2007
	v_L=52	X = latently infected (L)	Nielsen & Ersboll, 2006; Nielsen, 2008
	v_{Is} =104	X = subclinically infected (I_S)	Matthews, 1947
	v_{Ic} =26	X = clinically affected (I_C)	a
sh_X		Probability of shedding in colostrum or milk for a cow in health state X	Streeter et al., 1995; Sweeney *et al.*, 1992
	sh_L=0	X = latently infected (L)	
	sh_{Is}=0.4	X = subclinically infected (I_S)	
	sh_{Ic}=0.9	X = clinically affected (I_C)	
α	10^6	*Map* infectious dose	Begg et al., 2008
β_l	$5\times10^{-4}\times7$	Transmission rate if ingestion of an infectious dose (per week)	b
β_c	$5\times10^{-5}\times7$	Transmission rate if one infectious dose is present in the local environment (per week)	Van Roermund *et al.*, 2007
β_g	$9.5\times10^{-7}\times7$	Transmission rate if one infectious dose is present in the global environment (per week)	Van Roermund *et al.*, 2007
β_o	$5\times10^{-6}\times7$	Transmission rate if one infectious dose is present on pasture (per week)	b
g_X		Decrease in milk production for cattle in health state X (per week)	[4]
	g_{Is} = 2.5×7	X = subclinically infected (I_S)	
	g_{Ic} = 4×7	X = clinically affected (I_C)	
μ_k		Removal rate of *Map* from environment k	Jorgensen, 1977; Whittington *et al.*, 2004
	μ_g = 0.4	all the environments (per week)	
	μ_{ip} = 0.67	individual pens (when empty)	
	μ_{cp} = 0.17	collective pens (when empty)	

[*] The values of the parameters in the epidemiological model (Tab. II) are estimates based on experimental data reported in the literature.

[a] Expert opinions

[b] Parameters' values are assumed

[4] Nielsen S.S., Enevoldsen C., Toft N., Milk production losses associated with bovine paratuberculosis diagnosed from repeated testing, in: Proc. 11th International Symposia of Veterinary Epidemiology and Economics, Cairns, Australia, 2006. pp. 619.

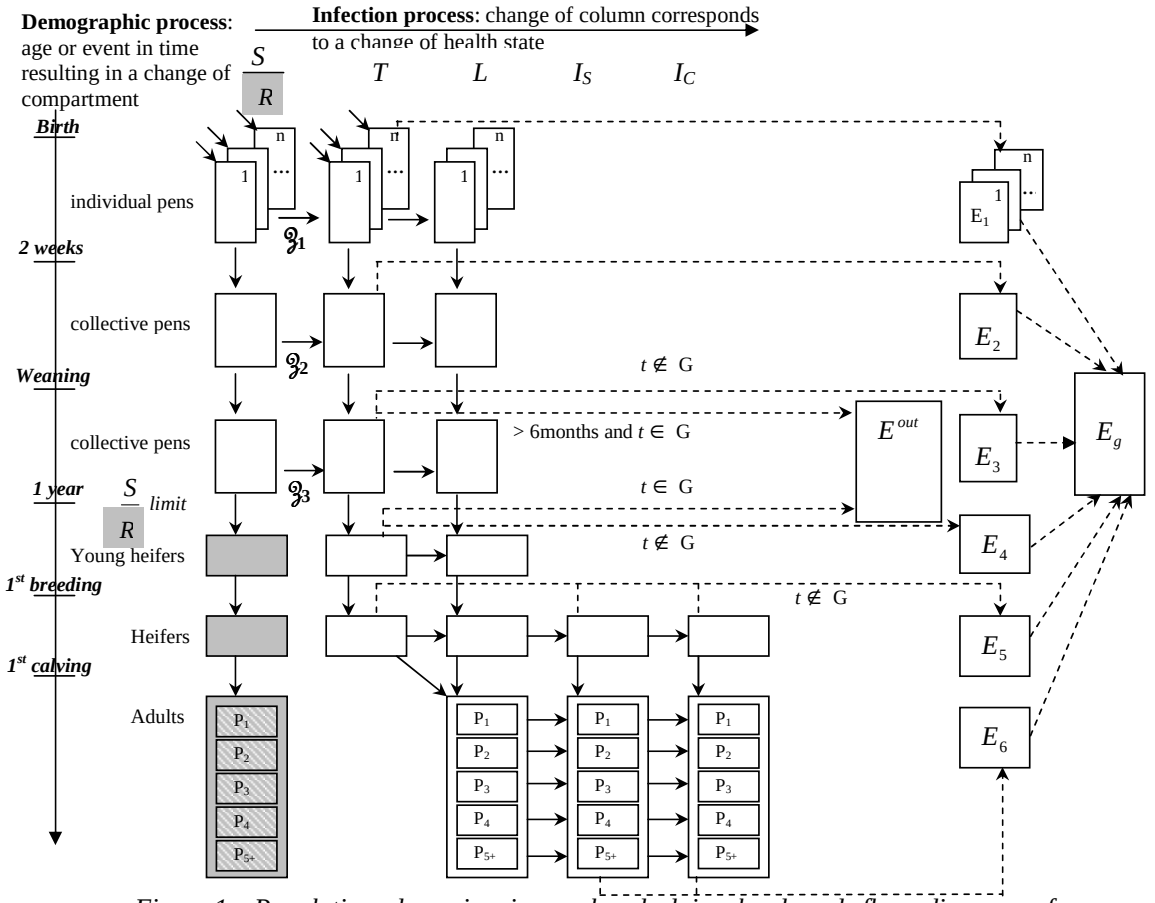

Figure 1: Population dynamics in a closed dairy herd and flow diagram of Mycobacterium avium *subsp.* paratuberculosis (Map) *infection dynamics model, representing infection states, transitions between states, and origin of contamination of the local and whole farm environments*

Legend:
Health states: S = susceptible, R = resistant; T= transiently infectious; L= latently infected; I_S= subclinically infected; I_C= clinically affected

E_l : indoor environment in housing l, with l = 1 to 6 (1 for calves in individual pens, 2 for calves in collective pens before weaning, 3 for calves in collective pens after weaning before 6 months of age or during winter season, 4 for young heifers during winter season, 5 for heifers during winter season, and 6 for adults during winter season), E_g : environment of the whole farm, E^{out} : outdoor environment of calves when they are grazing; n: number of individual pens; the population dynamics has to be read vertically; β_1 to β_3: transmission functions for horizontal infection; t: time; G: grazing season; P_i cows in parity i; dotted arrows: contribution to the environment contamination. Exit rates of each compartment are not represented.

European recommendations concerning animal welfare and social contacts[2] and reflects the most common calf management in Europe (Marcé, *et al.*, 2010b). After 1 year of age, the heifers are divided into 2 groups: from 1 year of age to 1st artificial insemination (AI) at age *h*, and from 1st AI to 1st calving at age *cal*. Cows are all gathered in the same batch assuming they are not susceptible. Parities are considered as the culling rate is higher for older cows and to account for age in the progress of *Map* infection.

$X(a,t)$ represents the number of animals in health state X and age a at time t. Age is given in weeks until first calving (*cal*) and in parities (*cal*+1 to *cal*+5) after calving. An individual-based model is used until age *m*, when calves move to collective pens. Then, a compartmental model is used. If $a \leq m$, an index k indicates in which individual pen the calf is: $X(a,t,k) = 0$ or 1 depending on the occupancy of pen k. The total number of calves of age a at time t is:

$X(a,t) = \sum_{k=1}^{n} X(a,t,k)$, with n the number of individual pens.

The herd model is calibrated by integrating knowledge from various sources, from published data to experts' knowledge, to realistically represent a French dairy cattle herd (Table I). All male calves (half the calves) exit the herd during the 2nd to 4th week after birth (rate σ_m). Closed herds are modelled: there is no purchase of heifers for replacement. All female calves are thus kept to give flexibility to regulate the number of cows. Herd size is assumed to be stable over time. Heifers can be sold but only 10 weeks before the first calving (rate σ_h). Above a given number of cows (K_c), the heifer sale rate increases. Under this threshold, the sale rate decreases. An all-year round calving is modelled with a mean calving-to-calving interval *cci*. Animals older than 6 months of age graze from April to November (*Graz*).

2.1.2 Infection process and *Map* transmission

The progression of individual animals through different *Map* infection states is a complex continuous process which is converted into discrete phases to enable the model parameterization based on current knowledge. Animals are classified into mutually exclusive health states: susceptible (*S*), resistant (*R*), transiently infectious (*T*) (infectious only for a limited period of time), latently infected (*L*) (infected not infectious), subclinically infected (*Is*) (infected and infectious but not affected), and clinically affected (*Ic*) (infected, infectious, and affected) (Nielsen, 2008). Parameters are displayed in Tables I (herd dynamics), II (infection

[2] Council Directive 97/2/EC of 20 January 1997 amending Directive 91/629/EEC laying down minimum standards for the protection of calves

process), and III (shedding characteristics). Assumptions are based on current knowledge on *Map*.

Vertical transmission occurs with probability p_X (*T* calf born to an infected cow). Horizontal transmission occurs by ingestion of colostrum, milk, or faeces. It depends on animal susceptibility, varying with age (maximal the first week of age and decreasing exponentially (*h*) until 1 year of age (*u*)). Under field conditions, animals older than one year of age have a low susceptibility to *Map* infection (Hagan, 1938; Windsor and Whittington, 2010) and in the current model are therefore assumed to be resistant to infection. If infected, there is no possible recovery. We assume an exponential distribution of the durations in infection states *T*, *L*, *Is*, and *Ic*. A transiently infectious state is assumed as infected calves have been reported to shed *Map* (Van Roermund, *et al.*, 2007). The transition from *T* to *L* either is modelled using a binomial distribution of probability $1/v_T$, v_T being the mean duration of the transiently infectious period, or occurs at the latest when the age at first calving (*cal*) is reached. A latent state is assumed because, if the absence of shedding has not been proven, the detection of infectious adults and heifers is hardly possible before animals reach one to two years of age, indicating at least quite a low level of shedding (Whitlock, *et al.*, 2000; Nielsen and Ersboll, 2006; Weber *et al.*, 2010). Latent animals are assumed not to shed *Map*, since shedding can be considered to be negligible compared with that of other infected adults. The transition from *L* to *Is* is possible only after the 1st AI (at age *h*). Subclinical animals are assumed to shed sufficient quantities of *Map* to be detectable and to contribute to *Map* spread within the herd, without having any obvious clinical signs. The transitions from *L* to *Is*, *Is* to *Ic*, and *Ic* to exit of the herd are modelled using binomial distributions of probabilities $1/v_X$ ($X = L$, *Is*, or *Ic*), v_X being the mean time spent in state *X*. There is no additional mortality for *Is* and *Ic* cattle, but v_{Ic} accounts for additional culling.

Depending on their age, *S* calves are not all exposed to the same transmission routes. Calves born to infected dams can be infected via colostrum ingestion in the first week of age. During the first 2 weeks, calves are housed in individual pens. They can be infected via milk ingestion, exposure to the environment of the whole farm (global environment), or indirect transmission from infected calves of neighbouring pens. Before weaning, calves housed collectively can be infected via milk ingestion, exposure to the local environment of their pens, or exposure to the global environment. Inside (during winter), weaned calves can be infected via exposure to the local or to the global environment. On pasture, they can only be infected via exposure to the local environment shared with young heifers.

Colostrum and milk contamination occurs because of direct shedding or indirect faecal contamination. A calf ingests the colostrum of its dam. A

Table III: Summary of published data and modelled distributions of the quantities of Mycobacterium avium *subsp.* paratuberculosis (Map) *shed, depending on the health state (X) and the route of transmission (r) in a* Map *infection dynamics model within a structured dairy herd*

Route of transmission (r)	Health state (*X*)	Literature				Model
		Minimal value	Maximal value	Mean value	Source	$\mathcal{F}(X,r)$
Map direct shedding in milk and colostrum (*Map*/L)	Subclinically infected	2.2×10^4	8.8×10^4	5×10^4	Sweeney *et al.*, 1992	$10^5\times$beta(8,8)
	Clinically affected	-	-	5×10^4	Giese & Ahrens, 2000	$10^5\times$beta(8,8)
Map indirect shedding in milk and colostrum (faecal contamination) (*Map*/L)	Subclinically infected	0	2×10^{10}	40	Magnusson *et al.*, 2006; Vissers *et al.*, 2006	$1+10^3\times$beta(1,25)
	Clinically affected	700	2×10^{10}	14×10^4	Magnusson *et al.*, 2006; Vissers *et al.*,2006	$10^{(3+10\times\text{beta}(50,200))}$
Map shedding in faeces (*Map*/kg)	Transiently infectious	6×10^4	6.3×10^5	3×10^5	Van Roermund *et al.*, 2007	$10^6\times$beta(8.8,19)
	Subclinically infected	10^4	10^{15}	2.6×10^6	Rossiter & Burhans, 1996	$10^{(4+10\times\text{beta}(2.65,17))}$
	Clinically affected	10^8	10^{15}	10^{10}	Jorgensen, 1982; Whittington *et al.*, 2000	$10^{(8+10\times\text{beta}(2,17))}$

*Table IV: Proportion (%) of runs having 0 to more than 3 clinically affected and/or subclinically infected animals (*Is*) detected (sensitivity of 0.5 and specificity of 1 for the tests used for* Is *animals detection) after 1 to 5 years of simulation in herds with spontaneous fadeout or persistent infection*

	Cumulated number of animals (*nr*)	% of runs with *nr* clinically affected animals					% of runs with *nr* clinically affected & detected subclinically infected animals				
		Time (in years)					Time (in years)				
		1	2	3	4	5	1	2	3	4	5
Proportion among herds with fadeout (282 runs)	0	75	67	64	62	62	40	37	37	35	35
	1	25	33	36	36	35	50	48	45	45	44
	2	0	0	0	1	2	10	14	17	17	17
	≥3	0	0	0	1	1	0	0	1	3	4
Proportion among persistently infected herds (118 runs)	0	48	23	9	5	2	24	8	2	1	1
	1	52	75	67	40	18	46	51	30	14	3
	2	0	2	15	15	19	60	34	25	22	8
	≥3	0	0	9	40	61	0	7	43	63	88

calf *k* born to a cow in state $X \in \{Is, Ic\}$ ingests at time *t* the following amount of bacteria:

$$q_c^k = Bernouilli\ (sh_X)\left[f(X, indirect) + f(X, direct)\right]b \qquad \text{(Eq. 1)}$$

with $f(X,r)$ the quantity of bacteria per litre shed by an animal in state *X* through route *r* ($f(X,r) \sim \mathcal{F}(X,r)$), sh_X the probability of shedding in colostrum for cows in state *X*, and b the quantity of colostrum fed to calf. The number of calves infected via colostrum ingestion is then:

$$inf(c,t) = \sum_{k=1}^{k=n}\left[S(1,k,t)\,Bernouilli\left(1-\exp(-\frac{\beta_l q_c^k}{\alpha})\right)\right] \qquad \text{(Eq. 2)}$$

with $S(1,k,t) = 1$ if there is a susceptible calf of one week of age in pen *k* at time *t* and 0 otherwise, β_l the transmission rate if ingestion of an infectious dose, and α the infectious dose. Similarly, the number of calves infected via milk ingestion is:

$$inf(m,t) = \sum_{a=1}^{a=w}\left[Bin\left(S(a,t), 1-\exp(-\exp[-h(a-1)]\frac{\beta_l q_l}{\alpha})\right)\right] \qquad \text{(Eq. 3)}$$

with $S(a,t)$ the number of susceptible calves of age *a* at time *t*, q_l the quantity of bacteria ingested per calf via milk ingestion. q_l depends on the quantity of milk drank per calf (*d*) and the quantity of bacteria in the tank, which depends on the proportion of *Ic* and *Is* lactating (*prop*) and shedding (sh_X) cows, these cows either directly shedding in milk ($f(X,direct)$) or because of faecal contamination of the milk ($f(X,indirect)$), and the quantity of milk they produce ($\varepsilon - g_X$).

Faecal-oral transmission is indirect, occurring by ingestion of bacteria present in the environment. Two types of environment are modelled to differentiate indirect adult-to-calf from indirect calf-to-calf transmissions (Figure 1). E_g is the quantity of *Map* in the global environment, contaminated by all of the shedding animals. It is assumed that all calves are equally exposed to the farm's environment, not accounting for possible variation in distribution of *Map*. E_1 to E_3 are the quantities of *Map* in the calves' local environments, exclusively contaminated by *T* animals housed in the associated facilities (Figure 1). We assume a homogeneous distribution of calves' faeces in a local environment or that all calves in a contaminated pen have the same probability of ingesting *Map* during a week. Susceptible animals are exposed to *Map* in the global and their local environments. The global environment is the sum of the local environments for calves and adults. All infectious cattle shed *Map* in their faeces. We assume shedding varies with the infection state, but also over time for a given infectious animal. We assume *T* animals shed on average almost as much bacteria per kg of faeces as *Is* animals but with a lower

variability, *Ic* animals shedding much more (Figure 2). To model such a heterogeneity in shedding between animals and states, we fit distribution laws $\mathcal{F}(X,\text{faeces})$ (Figure 2) of *Map* quantities shed at time *t* per kilogramme of faeces by a given animal of state *X* to published observed data (Table III). At time *t*, the quantity of *Map* per environment is updated, according to the removal rate μ (mortality of *Map*, cleaning of the barn, straw management) and *Map* shed by infectious animals. We assume no bacterium survives on pasture during winter; pastures are free of *Map* at next turn-out. In individual pen *k*, a susceptible calf of age *a* is infected at time *t* because of *Map* residuals in the pen with probability:

$$P_{\text{inf}}^{k}(a,t) = 1 - \exp(-\exp(-h(a-1))\frac{\beta_c E_l(k,t)}{\alpha}) \quad \text{(Eq. 4)}$$

with β_c the indirect calf-to-calf transmission rate.

Calves also can be infected because of their infectious neighbours (randomly sampled among calves). In collective pen *i*, susceptible calves of age *a* are infected at time *t* via calf-to-calf indirect transmission with probability:

$$P_{\text{inf}}^{i}(a,t) = 1 - \exp(-\exp(-h(a-1))\frac{\beta_c E_i(t)}{\alpha N_i(t)}) \quad \text{(Eq. 5)}$$

with $N_i(t)$ the number of animals in local environment *i* at time *t*. Susceptible calves of age *a* are infected at time *t* via the global environment with probability:

$$P_{\text{inf}}^{g}(a,t) = 1 - \exp(-\exp(-h(a-1))\frac{\beta_g E_g(t)}{\alpha N(t)}) \quad \text{(Eq. 6)}$$

with β_g the indirect transmission rate from this environment and $N(t)$ the herd size.

2.1.3 Initial conditions

All animals younger than *u* are initially susceptible, other animals being resistant to infection. A subclinically infected parity 1 cow is introduced once in the herd with no further introduction. For each run, the date of introduction corresponds to the first week of January, i.e. 3 months before grazing starts. No specific measure is implemented in the herd to prevent or control *Map* infection. No change in herd management is implemented over time. Studied herds have on average of 277 animals (118 calves and young heifers, 45 bred heifers, and 114 cows).

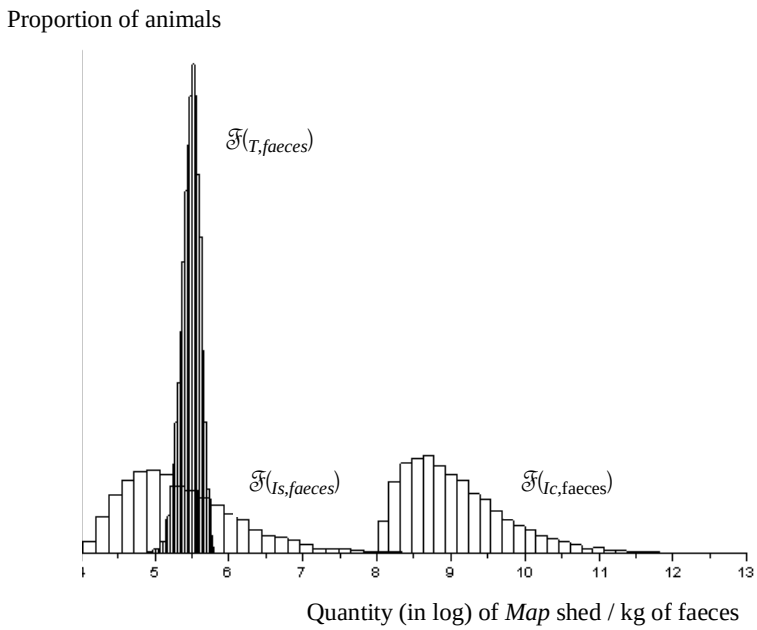

Figure 2: Distribution of the amount of Mycobacterium avium *subsp.* paratuberculosis (Map) *shed per kg of faeces of transiently infectious ($\mathcal{F}_T$), subclinically infected ($\mathcal{F}_{Is}$) and clinically affected ($\mathcal{F}_{Ic}$) animals used in the* Map *spread model within a dairy herd*[*]

[*]Distributions are here in log(*Map)*/kg of faeces (and not in *Map*/animal/day). Transiently infectious animals produce from 0.5 to 10 kg of faeces per day during 25 weeks on average (f_1, f_2, f_Y), whereas *Is* and *Ic* animals are cows producing 30 kg of faeces per day (f_A) for a longer period of time (Tab. I and II). Adults' contribution to total *Map* shed is thus more important than the one of transiently infectious animals.

2.1.4 Model outputs

Results are obtained from 400 runs over 25 years. We monitored the stability of means and variances of model outputs with increasing number of runs and stopped simulations when further only resulted in small changes of these estimates. Therefore, runs are numerous enough to obtain stable simulated results. The first output is the infection persistence over time, i.e. the percentage of runs with the infection still present. We can deduce from this output the proportion of runs ending with fadeout. Other outputs then are studied separately for runs with persistent infection or runs with fadeout. The second output is the prevalence of infected ($T+L+I_S+I_C$), infectious ($T+I_S+I_C$) and affected animals (I_C) over time, these categories being defined by Nielsen & Toft (Nielsen and Toft, 2008). For runs with persistent infection, the pseudo-equilibrium of the prevalence is estimated. Among the two types of runs, the proportion of animals that become I_C or detected with a systematic test (sensitivity of 0.5 and specificity of 1) during the early infection dynamics is studied. The third output is the relative contribution of the transmission routes to the number of newly infected animals.

2.2 Model evaluation

First, model outputs are compared with published data and field data from infected herds (Guicharnaud, 2009; Nielsen and Toft, 2009). The simulated proportion of infected adults is compared to the estimated baseline prevalence of infected adults on farms that voluntarily participated in a control program based in Brittany (France) (Taisne, 2009). Data corresponds to 59 herds enrolled in the program between 2002 and 2005 and in which more than 20 adults per herd were tested in the year of enrolment. All adults older than 24 months of age were tested annually using both ELISA and either PCR or faecal culture until 2007, and systematic ELISA and PCR in faeces of ELISA positive animals in 2008. Ziehl-Neelsen tests were performed when suspect clinical signs were observed. Individual statuses of adults during the first year of the program implementation (i.e. before any control measure was introduced) are retrospectively attributed based on a maximum of three successive annual results. These statuses are defined as: clinically affected (Ziehl-Neelsen positive test in the first year), subclinically infected (PCR or faecal culture positive in the first year but Ziehl-Neelsen negative if performed), latently infected (seropositive in the first year but PCR or faecal culture negative or negative in all tests in the first year with a positive test later, whatever the test), and resistant (testing negative in all tests). For animals always testing negative but with only one or two tests (instead of three), we assume that they are either resistant (optimistic option which may under-estimate infection) or latently infected (pessimistic option which may over-estimate infection). Based on these optimistic and pessimistic distributions, we

estimate the distribution of animals per infection state at the start of the program and the within-herd prevalence at enrolment. To compare model outputs with field data, we assume farmers usually detect the disease from 5 to 9 years after *Map* introduction (time needed for clinical cases to occur). We calculate the distribution of the mean simulated prevalence in infected adults in infected herds over this time period.

Second, a hypothesis-testing approach is used to validate the model, assuming a constant herd structure. We verify that either our conclusions are robust to variation in model parameters, or that parameter variation induces unrealistic within-herd prevalence and therefore cannot be retained. A one-at-a-time sensitivity analysis is performed for uncertain parameters (ν_T, u, h, p_x, sh_x, β_l, β_c, β_g, β_o, $\mathcal{F}(T,\text{faeces})$). Variations of ±50% from reference values are tested where applicable (ν_T, u, h, p_x, sh_x, β_l, β_c, β_g, β_o). For $\mathcal{F}(T,\text{faeces})$, the worst plausible case is tested, T animals shedding (per kilogram of faeces) as much as *Is* animals, with the same variability.

Third, to evaluate how the conclusions change with herd management, a model evaluation is performed as regards to variations of parameters managed on farm (μ_k, K_c, ν_{Ic}, *Graz*). Variations of ±50% from nominal values are tested for μ_k, and ν_{Ic}. For K_c (closely related to herd size), limits of 50 vs. 500 cows are tested. Lastly, a delay in the start of grazing (same duration but starts in the week *Map* is introduced vs. ends in the week before *Map* is introduced) and a variation in its duration (same start but duration of 28 vs. 37 weeks) are tested.

3 Results

3.1 Spontaneous fadeout of *Map* infection without control measure

Spontaneous fadeout occurred in 66% of the runs (Figure 3). In 43% of the runs, it occurred within the first 2 years (early extinction), while it occurred less quickly in the remaining 23%. Herds still infected 7 years after *Map* introduction had thereafter a fadeout probability below 6%. When shedding animals were no longer present on the farm, new infection of cattle from residual *Map* in the environment was rare, with a mean weekly probability of 3%.

The probability of fadeout only slightly varied with uncertain parameters (from 62 to 71%). It decreased to 51% when the mean time spent in state *Ic* increased by 50%, and to 58% when *Map* removal from the global environment decreased by 50%. Other parameters relating to herd management only had little influence on the fadeout probability.

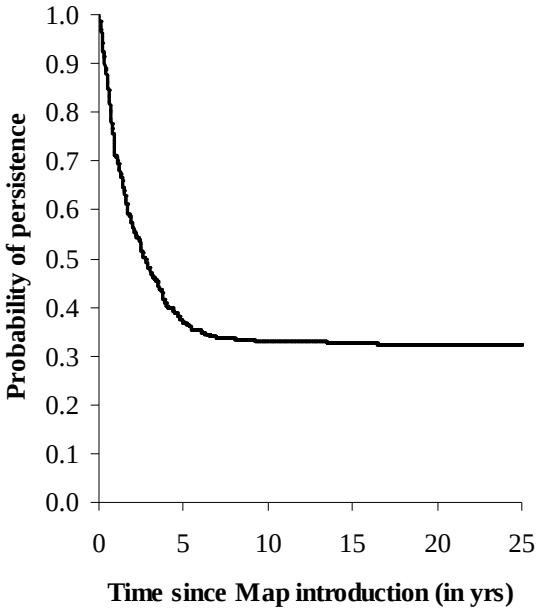

Figure 3: Probability of persistence over time (proportion of runs where an infected animal is still present) of Mycobacterium avium *subsp.* paratuberculosis (Map) *infection in a dairy cattle herd after a single* Map *introduction (*t*=0) in the herd*

It needs to be emphasized that yearly single introduction of *Map* would lead to a decrease in the cumulative probability of spontaneous fadeout, which can be calculated for n years using 0.66^n (e.g. 66% the first year as in the present study, 44% the second year, 29% the third year, etc.).

3.2 *Map* spread within persistently infected herds

Prevalence of infection reached a pseudo-equilibrium (when accounting only for runs in which infection persisted) 23 years after *Map* introduction when no control measure was implemented (Figure 4). At the end of the simulation period, the prevalence of infected, infectious, and affected animals reached 88%, 44%, and 6%, respectively. In adults, prevalence of infected, infectious, and affected animals was 87%, 67%, and 15%, respectively. Annual incidence reached 15% (Figure 4).

Comparing the simulated and the observed distributions of prevalence in infected herds indicated that the model over-estimated the cases when infected herds had a low prevalence (more than 40% of the infected runs had a prevalence in infected adults less than 5%; Figure 5). For other levels of prevalence, simulated and observed distributions were similar.

Varying uncertain parameters produced in most cases (u, ν_T, p_{Ic}, sh_X, β_c, β_l, β_o, $\mathfrak{F}(T,faeces)$) prevalence distributions similar to the reference scenario and therefore these parameters cannot be more precisely estimated from the sensitivity analysis. For others (h, p_L, β_g), a variation of ±50% resulted in a simulated prevalence not consistent with the observed prevalence, thus indicating that the true value of these uncertain parameters is likely to be within a smaller interval than ±50% of their reference value.

Among infected adults, the model provided mean proportions of L, I_S, and I_C animals 25 years after *Map* introduction of 60, 32, and 8%, respectively (Figure 6A). These proportions slightly varied over time, except in the transient period when prevalence was very low. In field data (Figure 6B), the proportion of animals per infection state depended on the option: the pessimistic option resulted as expected in a large proportion of latently infected animals. The mean proportion of subclinically infected animals varied from 17 to 40% in the optimistic option, and from 3 to 22% in the pessimistic option. Simulation values were in between the 2 assumptions (Figure 6).

At the herd level, the main transmission routes were indirect transmission via the contaminated global environment, then *in utero* transmission. Transmission via colostrum or milk ingestion and calf-to-calf indirect transmission appeared to be minor routes (Figure 7). For high within-herd prevalence, the two main transmission routes equally contributed to new infections (Figure 7B). For parameter variations resulting in plausible

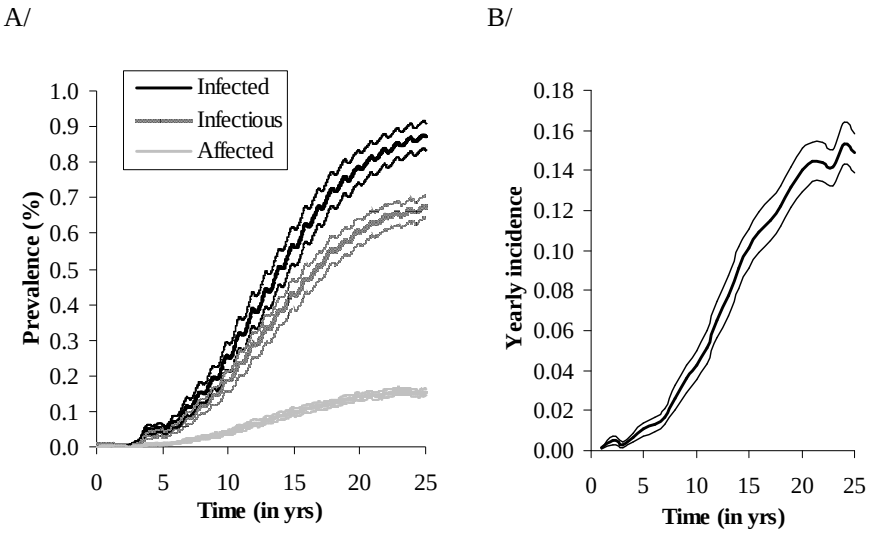

Figure 4: Mycobacterium avium *subsp.* paratuberculosis (Map) *spread in a persistently infected dairy cattle herd since* Map *introduction (t=0). A/ Mean prevalence over time of infected (black), infectious (dark grey), and affected (light grey) adults (> 30 months) and related confidence intervals. B/ Mean annual incidence and related confidence interval.*

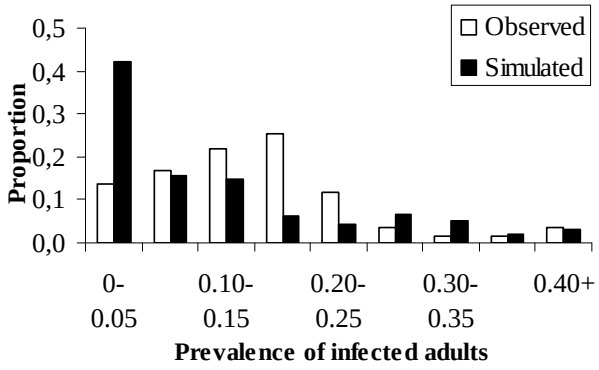

Figure 5: Comparison of the simulated and the observed distributions of the prevalence in Mycobacterium avium *subsp.* paratuberculosis (Map) *infected adults in infected dairy cattle herds. The simulated distribution corresponds to runs of a* Map *spread model within a dairy cattle herd, the mean prevalence from year 5 to year 9 since* Map *introduction in the herd (t=0) being calculated for each run still infected. The observed distribution is based on individual life long determined statuses in 59 dairy herds at enrolment in a paratuberculosis control program in France, before any control measure is implemented.*

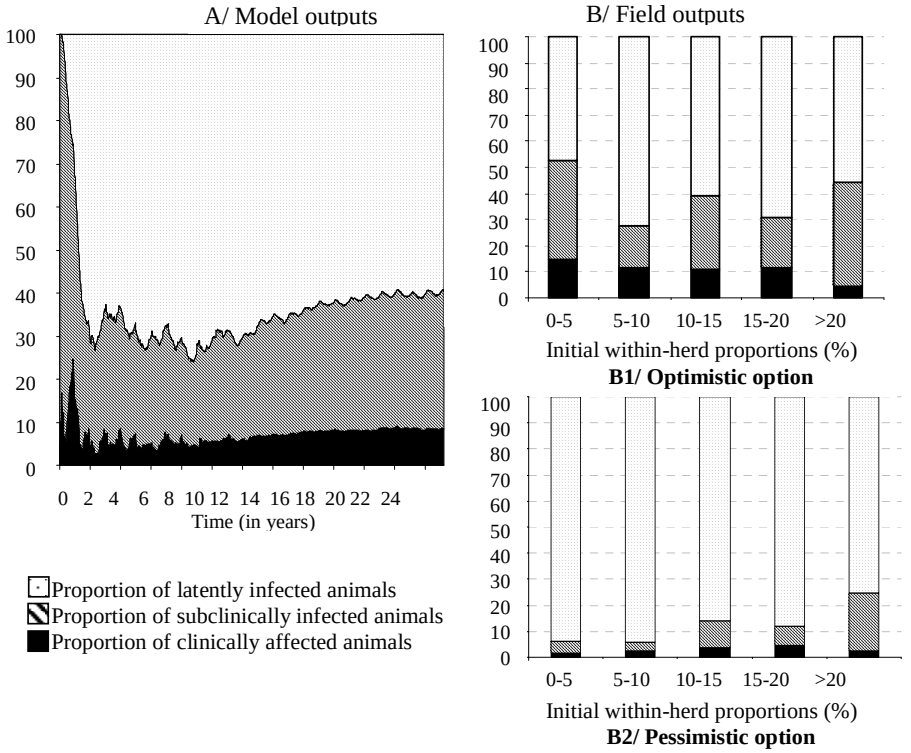

Figure 6: Comparison of the simulated and observed distributions of Mycobacterium avium *subsp.* paratuberculosis (Map) *infected adults per infection state in infected dairy cattle herds. A/ Simulated mean distribution over time in persistently infected herds as predicted by a* Map *spread model within a dairy cattle herd; B/ Mean percentage of tested adults per infection states based on a life long determined status in 59 herds at enrolment in a paratuberculosis control program in France, before any control measure is implemented, according to the range of the initial within-herd prevalence. Animals tested twice or less and having negative results assumed to be either resistant (state not shown) (B1: optimistic option) or latently infected (B2: Pessimistic option).*

A/ B/

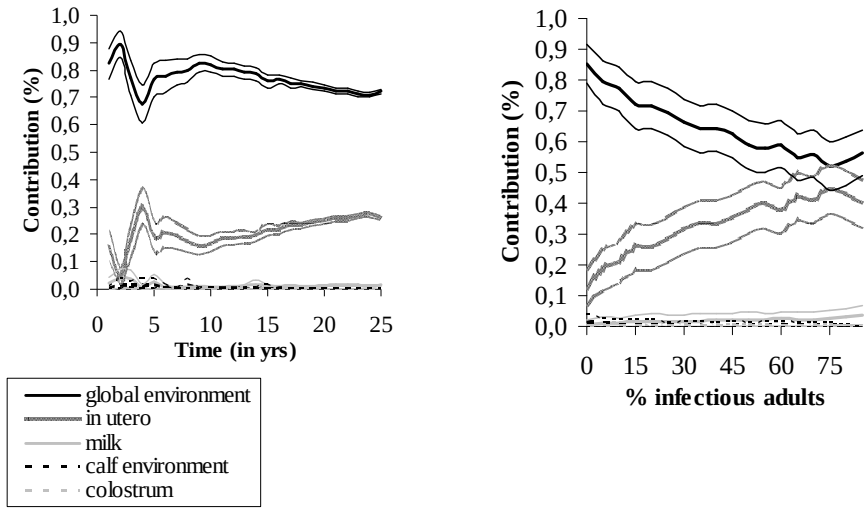

Figure 7: Mean relative contributions of the 5 transmission routes of Mycobacterium avium *subsp.* paratuberculosis (Map) *infection in persistently infected dairy cattle herds (118 runs out of 400). A/ over time since* Map *introduction in the herd; B/ over prevalence of infectious adults.* Map *is introduced only once (t=0).*

results, these conclusions remained unchanged. Even an increase of one log (*10) of the indirect transmission rate in the calf environment barely changed the contribution of calf-to-calf indirect transmission, which slightly increased for a low within-herd prevalence. Assuming *T* animals shed as much as *Is* animals (per kg of faeces) resulted in calf-to-calf indirect transmission contributing as much as *in utero* transmission for a very low within-herd prevalence, the contribution decreasing for a prevalence higher than 5%.

3.3 Characteristics of the runs ending in fadeout *vs*. persistent infection

No secondary infection (on top of the first introduced case) was observed in 75% of the runs with fadeout, contrary to herds persistently infected. Only 3% of the runs ending with fadeout had at least 2 clinically affected animals (simultaneously or successively) over 5 years, compared to 80% of the persistently infected runs (Table IV). When combining clinical surveillance and systematic testing of cows, more than 2 animals were detectable after 3 years in 18% of the runs with fadeout and in 68% of the runs with persistent infection (21% and 96%, respectively after 5 years).

Based on the model outputs in Table IV, we can predict at the herd level the probability of *Map* persistence when below a given detection threshold *versus* the probability of spontaneous fadeout when over this threshold. If a control programme based on clinical surveillance is implemented when at least one affected animal is observed in 5 years, the programme is unnecessarily implemented (fadeout would have spontaneously occurred) in 48% of the cases (i.e. the number of runs over the threshold ending with fadeout over the total number of runs over the threshold). If no control programme is implemented (no affected animals in 5 years after *Map* introduction), a persistent infection occurs in 1% of the cases. For a threshold of 2 affected animals, these proportions are 9% and 8%, respectively. For a threshold of 3, they are 4% and 14%, respectively. However, only 24% of the persistently infected herds had at least 2 affected animals within 3 years after *Map* introduction, 80% within 5 years. If the control programme is based on both clinical surveillance and imperfect tests (assuming a sensitivity of 0.5 and a specificity of 1) targeting adults, the proportions become 61% and 2% for at least 1 detected animal in 3 years after *Map* introduction, 39% and 14% for a threshold of 2, and 5% and 19% for a threshold of 3. 68% of the persistently infected herds had at least 2 detected animals within 3 years after *Map* introduction, 96% within 5 years.

*Table IV: Proportion (%) of runs having 0 to more than 3 clinically affected and/or subclinically infected animals (*Is*) detected (sensitivity of 0.5 and specificity of 1 for the tests used for* Is *animals detection) after 1 to 5 years of simulation in herds with spontaneous fadeout or persistent infection*

	Cumulated number of animals (*nr*)	% of runs with *nr* clinically affected animals					% of runs with *nr* clinically affected & detected subclinically infected animals				
		Time (in years)					Time (in years)				
		1	2	3	4	5	1	2	3	4	5
Proportion among herds with fadeout (282 runs)	0	75	67	64	62	62	40	37	37	35	35
	1	25	33	36	36	35	50	48	45	45	44
	2	0	0	0	1	2	10	14	17	17	17
	≥3	0	0	0	1	1	0	0	1	3	4
Proportion among persistently infected herds (118 runs)	0	48	23	9	5	2	24	8	2	1	1
	1	52	75	67	40	18	46	51	30	14	3
	2	0	2	15	15	19	60	34	25	22	8
	≥3	0	0	9	40	61	0	7	43	63	88

4 Discussion

The results from model experimentation have improved the understanding of *Map* spread within a dairy herd. Fadeout could occur even without implementation of control measures in an infected herd. This demonstrates the usefulness of a modelling approach, since such fadeout cannot be observed in the field given the low prevalence of infection and low likelihood of detection using available diagnostic methods.

The cumulated number of clinically affected animals appears to be a good indicator of the progression of *Map* infection dynamics towards persistence. Furthermore, it is very easy to use in the field. A threshold of 2 affected cows seems adequate to trigger control measures in a herd. However, a farmer may miss the 1st clinical case and be unaware that there already have been 2 cases in his herd. An earlier indicator would be useful. Combining clinical surveillance with an imperfect test implemented on all potentially infected adults could reduce the time needed for detection. In that case, a threshold of 3 detected animals seems adequate. To assess the economic advantage of such surveillance, both the costs and benefits of early detection need to be analyzed.

In the absence of control measures, the simulated mean prevalence in infected cattle increased to 90% after 25 years in the model, as previously published models also have shown (Groenendaal, *et al.*, 2002; Pouillot, *et al.*, 2004; Kudahl, *et al.*, 2007; Mitchell, *et al.*, 2008). These levels of prevalence are not expected with field data as control measures will be implemented long before such levels are reached. However, herds with high apparent prevalence are found, which corresponds to these levels of true prevalence. Moreover, simulated prevalence between 5 and 9 years after *Map* introduction was lower than levels observed on farms prior to enrolment in a control programme. This suggests that the range of observed prevalence at control programme enrolment typically corresponds to a more advanced stage of within-herd *Map* dynamics, when without any control measure fadeout would rarely occur.

With this new model, it was possible to assess the relative importance of transmission routes on *Map* spread in a dairy herd. This model accounts not only for vertical transmission and horizontal transmission via the ingestion of *Map* in milk and colostrum, as has been done in previously published models (Marcé, *et al.*, 2010a), but also for indirect contacts between animals of different ages raised in different groups, and horizontal transmission via the ingestion of faeces present in the contaminated environment. Possible exposure of calves to adults or to other calves is modelled and the level of exposure varies depending on calf age and calf housing facilities. In persistently infected herds, contamination of the environment by adults was the main transmission route, *in utero* transmission being the second. Calf-to-calf transmission appeared to be a

minor route of transmission. However, in this model, milk and colostrum routes of transmission correspond to liquid contamination by the dam (direct shedding or faecal contamination), not contamination through the environment. On the other hand, possible faecal contamination of buckets used to give milk to calves is considered to be an element of global environment contamination, not the milk route of transmission. As a priority, exposure of calves to any environment contaminated by adult faeces should be reduced, particularly at and just after birth when calves are the most susceptible.

The model has been evaluated and provides qualitative predictions such as ranking routes and the description of possible dynamics. . The model validation has been performed by comparing model outputs with field data. A hypothesis-testing approach has been used allowing us to conclude that our findings are robust to variation in uncertain model parameters. However, only a partial validation has been possible because the introduction date of *Map* in a herd was not known in the observed data. Furthermore, we assumed here herds are closed (a single *Map* introduction), whereas data may concern open herds with multiple introduction of potentially infected cattle. Finally, in practice, when paratuberculosis is diagnosed, farmers are likely to change their routines to ensure their animals' welfare and protect their economic interests. It would be unethical to recommend that they do nothing. In contrast, we can model herds in which no control measures are implemented.

In the model, we neglected some processes and factors that may interfere with *Map* spread but that are not yet sufficiently described. First, we did not represent passive or intermittent shedding in the model. The intermittent shedding sometimes noticed (Whittington, *et al.*, 2000) indeed could be explained by the low sensitivity of diagnostic tests or by heterogeneity of faeces or milk samplings (Pradhan, *et al.*, 2009) which lead to an intermittent detection of infectious animals. If such intermittent shedding were to be shown, a different modelling approach would have to be used, where a given probability of shedding in the latent state according to age or to the physiological status (in gestation, in lactation, etc) would have to be assumed. However, given the current knowledge such a model cannot be parameterized. Moreover, such intermittent shedders would not be directly in contact with susceptible calves but be raised with other adults, their contribution to the environment contamination thus being diluted by the one of subclinical and clinical animals. Second, super-shedders have been described (Hovingh, *et al.*, 2006; Whitlock *et al.*, 2005) but it is unknown whether they are specific animals or if shedding of all infectious animals varies highly over time. Therefore, we assumed here any animal can shed a high amount of *Map* at random time. Third, experimental animal models suggest there could be genetic factors responsible for resistance or susceptibility to *Map* infection (Koets, *et al.*, 2000). Several genes have been identified to date. However, current

knowledge is insufficient to include such genetic factors in modelling. Lastly, the incubation period is inversely related to the challenge dose, clinical signs occurring sooner under experimental than natural conditions (Whittington and Sergeant, 2001). However, the mechanism of the dose-response effect, the potential cumulative exposure, and the minimum infection dose are still uncertain. Therefore, this has not been included in the model.

The model could be adapted to open dairy herds and used to evaluate control measures in both open and closed herds. Furthermore, this model could be used for herds of different sizes having similar herd structure and management. Herd management is driven by a number of parameters which gives flexibility to the model. However, the model would need to be modified if the structure of the herd is markedly different as exposure to the contaminated environment would differ.

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References

Begg D.J., Whittington R.J., Experimental animal infection models for Johne's disease, an infectious enteropathy caused by *Mycobacterium avium* subsp. *paratuberculosis*, Vet. J. (2008) 176:129-145.

Benedictus A., Mitchell R.M., Linde-Widmann M., Sweeney R., Fyock T., Schukken Y.H., Whitlock R.H., Transmission parameters of *Mycobacterium avium* subspecies *paratuberculosis* infections in a dairy herd going through a control program, Prev. Vet. Med. (2008) 83:215-227.

Ezanno, P., van Schaik, G., Weber, M. F. and Heesterbeek, J. A., A modeling study on the sustainability of a certification-and-monitoring program for paratuberculosis in cattle, Vet. Res. (2005) 36:811-26.

Ezanno, P., Fourichon, C., Beaudeau, F. and Seegers, H., Between-herd movements of cattle as a tool for evaluating the risk of introducing infected animals, Anim. Res. (2006) 55:189-208.

Groenendaal, H., Nielen, M., Jalvingh, A. W., Horst, S. H., Galligan, D. T. and Hesselink, J. W., A simulation of Johne's disease control, Preventive Veterinary Medicine. (2002) 54:225-245.

Guicharnaud, M., Prevalence of paratuberculosis in dairy cattle herd worldwide: review and analysis [in French], Doctorate in Veterinary Medicine Thesis, Nantes, France, (2009).

Hagan, W. A., Age as a factor in susceptibility to Johne's disease, Cornell Vet. (1938) 28:34-40.

Hovingh, E., Whitlock, R., Sweeney, R., Fyock, T., Wolfgang, D., Smith, J. and Schukken, Y., Identification and implications of *Map* supershedders, Joint meeting of the ADSA, AMSA, ASAS and PSA, Minneapolis, MN, (2006), pp. abstract, 1 page.

Jorgensen, J. B., Survival of *Mycobacterium paratuberculosis* in slurry, Nord. Vet. Med. (1977) 29:267-270.

Koets, A. P., Adugna, G., Janss, L. L. G., Van, W. H. J., Kalis, C. H. J., Wentink, G. H., Rutten, V. P. M. G. and Schukken, Y. H., Genetic variation of susceptibility to *Mycobacterium avium* subsp. *paratuberculosis* infection in dairy cattle, J. Dairy Sci. (2000) 83:2702-2708.

Kudahl, A. B., Ostergaard, S., Sorensen, J. T. and Nielsen, S. S., A stochastic model simulating paratuberculosis in a dairy herd, Prev. Vet. Med. (2007) 78:97-117.

Marcé, C., Ezanno, P., Seegers, H., Pfeiffer, D. U. and Fourichon, C., Modeling within-herd transmission of *Mycobacterium avium*

paratuberculosis in dairy cattle: a review, J. Dairy Sci. (2010a) 93:4455-4470.

Marcé, C., Guatteo, R., Bareille, N. and Fourichon, C., Dairy calf housing systems across Europe and risk for calf infectious diseases, Animal. (2010b) 4:1588-1596.

Matthews H.T., On Johne's disease, Vet. Rec. (1947) 59:397-401.

Mitchell, R. M., Whitlock, R. H., Stehman, S. M., Benedictus, A., Chapagain, P. P., Grohn, Y. T. and Schukken, Y. H., Simulation modeling to evaluate the persistence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) on commercial dairy farms in the United States, Prev. Vet. Med. (2008) 83:360-380.

Nielsen, S. S., Transitions in diagnostic tests used for detection of *Mycobacterium avium* subsp. *paratuberculosis* infections in cattle, Vet. Microbiol. (2008) 132:274-282.

Nielsen S.S., Ersboll A.K., Age at occurrence of *Mycobacterium avium* subspecies *paratuberculosis* in naturally infected dairy cows, J. Dairy Sci. (2006) 89:4557-4566.

Nielsen, S. S. and Toft, N., Ante mortem diagnosis of paratuberculosis: A review of accuracies of ELISA, interferon-γ assay and faecal culture techniques, Vet. Microbiol. (2008) 129:217-235.

Nielsen, S. S. and Toft, N., A review of prevalences of paratuberculosis in farmed animals in Europe, Prev. Vet. Med. (2009) 88:1-14.

Pouillot, R., Dufour, B. and Durand, B., A deterministic and stochastic simulation model for intra-herd paratuberculosis transmission, Vet. Res. (2004) 35:53-68.

Pradhan, A. K., Kramer, A. J., Mitchell, R. M., Whitlock, R. H., Smith, J. M., Hovingh, E., Van Kessel, J. S., Karns, J. S. and Schukken, Y. H., Multilocus short sequence repeat analysis of *Mycobacterium avium* subsp. *paratuberculosis* isolates from dairy herds in Northeastern United States of a longitudinal study indicates low shedders are truly infected, Proceedings of the 10th International Colloquium on Paratuberculosis, Minneapolis, Minnesota, USA, (2009), pp. 30-33.

Streeter R.N., Hoffsis G.F., Bech-Nielsen S., Shulaw W.P., Rings M., Isolation of *Mycobacterium paratuberculosis* from colostrum and milk of subclinically infected cows, Am. J. Vet. Res. (1995) 56:1322-1324.

Sweeney R.W., Whitlock R.H., Rosenberger A.E., *Mycobacterium paratuberculosis* cultured from milk and supramammary lymph nodes of infected asymptomatic cows, J. Clin. Microbiol. (1992) 30:166-171.

Taisne, D., A control programme for paratuberculosis in infected dairy herds in the Ille-et-Vilaine county [in French], Doctorate in Veterinary Medicine Thesis, Nantes, France, (2009).

Van Roermund, H. J. W., Bakker, D., Willemsen, P. T. J. and De Jong, M. C. M., Horizontal transmission of *Mycobacterium avium* subsp. *paratuberculosis* in cattle in an experimental setting: Calves can transmit the infection to other calves, Vet. Microbiol. (2007) 122:270-279.

Weber M.F., Kogut J., de Bree J., van Schaik G., Nielen M., Age at which dairy cattle become *Mycobacterium avium* subsp. *paratuberculosis* faecal culture positive, Prev. Vet. Med. (2010) 97:29-36.

Weber, M.F., Verhoeff, J., van Schaik, G. and van Maanen, C., Evaluation of Ziehl-Neelsen stained faecal smear and ELISA as tools for surveillance of clinical paratuberculosis in cattle in the Netherlands, Prev. Vet. Med. (2009) 92:265-266.

Whitlock R.H., Buergelt C., Preclinical and clinical manifestations of paratuberculosis (including pathology), Vet. Clin. North Am. Food Anim. Pract. (1996) 12:345-356.

Whitlock R.H., Sweeney R.W., Fyock T.L., et al., MAP Super-Shedders: Another factor in the control of Johne's disease, in: Proc. of the 8th International Colloquium on Paratuberculosis., Copenhagen, Denmark, 2005. pp. 42.

Whitlock, R. H., Wells, S. J., Sweeney, R. W. and Van Tiem, J., ELISA and fecal culture for paratuberculosis (Johne's disease): sensitivity and specificity of each method, Vet. Microbiol. (2000) 77:378-398.

Whittington, R. J., Reddacliff, L. A., Marsh, I. B., McAllistair, S. and Saunders, V., Temporal patterns and quantification of excretion of *Mycobacterium avium* subsp *paratuberculosis* in sheep with Johne's disease, Aust. Vet. J. (2000) 78:34-37.

Whittington, R. J. and Sergeant, E. S. G., Progress towards understanding the spread, detection and control of *Mycobacterium avium* subsp *paratuberculosis* in animal populations, Aust. Vet. J. (2001) 79:267-278.

Whittington, R. J., Marshall, D. J., Nicholls, P. J., Marsh, I. B. and Reddacliff, L. A., Survival and dormancy of *Mycobacterium avium* subsp. *paratuberculosis* in the environment, Appl. Environ. Microbiol. (2004) 70:2989-3004.

Whittington R.J., Windsor P.A., In utero infection of cattle with *Mycobacterium avium* subsp. *paratuberculosis*: A critical review and meta-analysis, Vet. J. (2009) 179:60-69.

Windsor, P. A. and Whittington, R. J., Evidence for age susceptibility of cattle to Johne's disease, Vet. J. (2010) 184:37-44.

Chapter 3

Dairy calf housing systems across Europe and risk for calf infectious diseases

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Abstract

Enteric and respiratory diseases are the most frequent health disorders of calves. They are associated with death or lower growth rate and induce treatment costs. Enteric and respiratory pathogens can be transmitted via contacts between calves which depend on calf housing systems and management. This study aimed at describing the main calf housing systems across Europe and at assessing the consequences of such housing facilities in terms of risk for calf infectious diseases. This was done through the use of a questionnaire distributed to experts in epidemiology and cattle farming systems in each European country. A literature review was performed on the risk factors associated with disease transmission and targeted in the questionnaire. Answers from 14 countries were obtained. A wide range of housing systems were described. However, four main systems could be identified and ranked in ascending order of risk for neonatal diarrhoea and respiratory infectious diseases: individual pen until weaning, individual pen for four weeks, individual pen for two weeks, and collective pen from the separation of the calf with its dam. Although the housing systems are known to play a role in disease transmission, they are currently not fully described in literature concerning risk factors for calf diseases. In a given farm, the risk assessment for calf infectious diseases should consider classical risk factors such as hygiene, feeding practices and air conditioning, on top of a precise description of the housing system.

***Keywords*:** Calf diseases, calf housing, dairy herd, Europe, qualitative risk assessment

Implications

Calf diseases and mortality have short-term and long-term detrimental effects on performance of a dairy farm. Risk factors for calf infectious diseases associated with design and management of housing facilities have been demonstrated in different surveys. In order to assess if calf health can been improved in Europe, it is necessary to understand how calves are currently raised and to identify consequences in terms of risk factors of transmissible calf diseases. Differences between countries in terms of calf management can result in different control measures used in each country.

1 Introduction

Infectious diseases such as diarrhoea and respiratory diseases are the most frequent health disorders of calves during their three first months of life (Olsson, *et al.*, 1993, Virtala, *et al.*, 1996, Svensson, *et al.*, 2006a, Gulliksen, *et al.*, 2009d). They impair both growth rate and replacement capacity of the herd. For other diseases such as paratuberculosis, only young animals are susceptible to the pathogen; therefore their exposure to the pathogen at a young age is critical (Doyle, 1953). Lastly for some zoonotic pathogens such as high shiga toxin-producing *Escherichia coli*, a high prevalence can be found more specifically in calves (Garber, *et al.*, 1995). In all these examples, direct contacts between calves or their exposure to a contaminated environment enable the transmission of the pathogens. Calf housing systems result in variable risk for direct contacts and risk for transmission via the equipment or the environment.

Dairy calves are raised under a wide variety of housing systems, defined by the facilities (number of calf pens, group size) and their use at different ages. Calf management takes into account several factors such as practicality, animal welfare, regulations, and existing facilities on a farm. Nowadays, the European Union (EU) regulations set a maximal age limit (eight weeks) for raising calves in individual pens and the necessity for calves to have social contact with other animals (Council Directive 91/629/EEC and Council Directive 97/2/EC). Individual pens for calves must not have solid walls (except those for isolating sick animals), but perforated walls which allow the calves to have direct visual and tactile contact. In this general framework, very different housing systems can be chosen, while complying with EU regulations. When comparing calf disease incidence in different areas, or when summarising known risk factors of calf diseases to define control plans, one should be aware of the differences in calf housing systems.

The objective of this study was to describe the main dairy calf housing systems across Europe, to identify differences influencing the exposure of calves to pathogens and to assess the risk for calf infectious diseases for each reported type of calf housing system.

2 Material and method

The study was conducted through a country-level questionnaire on dominant housing systems and through a qualitative risk assessment for the transmission of the most frequent pathogens involved in diarrhoea and respiratory diseases of dairy calves.

2.1 Questionnaire conception and distribution

Thirty seven questionnaires were distributed to persons with experience in the field of calf raising and infectious diseases. These persons were collected from or through the participants of the Society of Veterinary Epidemiology and Preventive Medicine (SVEPM) conference, held in London from 1 to 3 April, 2009, which is attended by epidemiologists who are aware of disease transmission and risk assessment. Answers were expected within a month by mail, fax, or email. The questionnaire had been previously tested on veterinarians of the National veterinary school of Nantes, France, and on professionals or people working regularly in contact with dairy herds in France, the Netherlands and the United Kingdom (UK).

2.2 Study population

European countries holding more than 300,000 dairy cows were targeted in this study. It was however not possible to collect questionnaires from Bulgaria, Czech Republic, Hungary, Lithuania, Poland, Portugal and Romania, in absence of relevant contacts. Greece was added and at the end, 14 countries were included holding 73% of the dairy cows in the EU for 84% of the milk delivery (EUROSTAT, 2009).

2.3 Questionnaire design

The questionnaire aimed at identifying the most common practice, or two main practices in terms of dairy calf housing facilities (and in terms of dairy herd size).

It was subdivided into five parts. In the first part, information on the country, on the person's background to check for experience in dairy farming systems and the mean number of cows on dairy farm were asked. Name and email address were facultative. The following four parts asked for housing facilities and management from birth to weaning, after weaning, before first calving, and on pastures. Finally, additional information or comments or remarks could be added at the end of the document.

The questionnaire was composed of 25 closed questions and two open questions to specify the answer of a closed question.

Vocabulary linked to housing facilities was used such as individual or collective pens. When really specific, a brief definition was added after the word. For example igloos and huts were defined as individual pens with complete separation from other pens in contrast with individual pens where a calf can have at least one neighbour with which it shares the wall of its pen.

The questionnaire can be available from the first author on request.

2.4 Assessment of the relevance of the answers

On top of the fact that only experienced persons were asked to answer, experience was assessed via a question on the number of dairy farms they visited during the last 12 months. When results were obtained from more than one expert for a country, similar answers were aggregated. When answers differed, all the information on specific areas has been kept.

2.5 Review of risk factors and qualitative risk assessment

A literature review of the main risk factors for neonatal diarrhoea and calf respiratory diseases complex (BRD: Bovine Respiratory Disease) linked to housing facilities was performed (mainly on Pubmed and CAB abstract databases). Only studies from 1990 to present were considered as being relevant. The search terms were: bovine respiratory disorders, bovine respiratory diseases, bovine respiratory disease complex and/or neonatal diarrhoea, enteric infectious diseases and dairy calf/calves combined with risk factors. Information relative to hygiene practices (removing litter, disinfection, straw quantity) and air conditioning while being recognized as main risk factors for calf diseases (Svensson, *et al.*, 2003, Lago, *et al.*, 2006, Svensson, *et al.*, 2006b) were not investigated through the questionnaire because these practices and data are very different between farms even within the same calf housing system.

Finally, a qualitative risk assessment of the different reported calf housing systems for neonatal diarrhoea and BRD complex was performed. A level of risk was assigned to each system based on identified risk factors for calf-to-calf pathogen transmission from birth to weaning and age of occurrence of these diseases. Therefore, for neonatal diarrhoea, viral, bacterial and parasitic diseases were distinguished. Five risk factors were considered. Three were directly linked to housing facilities as contacts between calves are likely to increase the transmission of the pathogen (individual pens, hutches and pen size). Two were linked to the management performed and were factors enhancing pathogen transmission (automatic milk feeding, variable age of calves in a same pen).

3 Results

3.1 Global analysis of the answers

Among the 37 questionnaires distributed, 24 were completed either by the person contacted (10) or by a contact of that person (14). If no answer was received within two months, a reminder email was sent. At the end, answers

from Austria (6), Belgium (1), Denmark (1), Finland (1), France (1), Germany (2), Greece (1), Ireland (1), Italy (1), Spain (2), Sweden (1), Switzerland (3), The Netherlands (1), and UK (South East and South West England, Wales) (2) were collected. When it was not possible to consider one most common practice in terms of calf housing system, several practices were kept for a country, one for each region using certain housing system.

Apart from the numeric answers, there were generally few comments added in the questionnaire. One of the main comments was that there was huge variation between farms in terms of calf rearing practices (France, Ireland, Switzerland, Spain, Sweden); the answer given was sometimes an interval instead of a number.

3.2 Description of housing systems

Reported results include description of housing systems and figures describing the typical system they rely on.

3.2.1 Assessment of the relevance of the answers

Answers were given generally by vets and / or researchers working in a university (herd health management department), or clinicians, or vets working with large animals. Their answers were based on their own observations in 2008 with more than 50 farm observations for 8 out of 24 questionnaires, between 10 and 50 observations for 12 questionnaires and on 10 observations (but around 400 in the last 10 years) for one questionnaire. One questionnaire was based on a previous survey done on 96 dairy farms (Austria), one was based on statistics (Denmark), and one was based on a report of the Swiss federal office of agriculture.

3.2.2 Mean size of the dairy herds

Mean size of dairy herds was reported to vary from around 10 to around 120 cows that had calved at least once (Table 1). In terms of mean size, the smallest farms were found in some regions of Switzerland (Alpine and central regions) and Austria (Alps). The biggest farms were found in Denmark and South East and South West England. A rapid increase of the average herd size was reported in the Netherlands.

3.2.3 Housing system from birth to weaning

All countries but Belgium, Germany and Sweden described having both types of systems with calves either housed in individual pens before being moved to collective pens or directly housed in collective pens after birth (Table 2).

Table I: Main characteristics of dairy calf housing systems in the European countries (number of animals, number of pens and change of pen)

	Mean number of cows	Most frequent number of individual pens	Mean number of calves in a collective pen before weaning	Most frequent number of collective pens before weaning	Change of pen around weaning	Mean number of calves in a collective pen after weaning	Change of pen between weaning and 1st calving (reason)††	Mean number of calves in a collective pen if change of pen
Austria (Alps)	10	3	2-5	2-5	Yes	2-5	Yes (pasturing)	2-5
Austria (non Alps)	12	5	3-8	2	Yes	3-8	Yes (AI)	5-10
Belgium (Flanders)	50	10	5-10	15	Yes	10-20	Yes (AI)	10-20
Denmark	110	15-20	2	10-20	Yes	-	No	10-20
Finland	24	28	5-10	2	Yes	5-10	Yes	5-10
France	40	8	2-5	3	Yes	5-10	Yes (space availability)	5-10
Germany	80	6	12	2	Yes	10-20	Yes (between 4 and 8 months)	5-20
Greece	50	6	2-5	2	Yes	10-20	Yes (AI and space availability)	10-20
Ireland	70	10	4-6		Yes	2-30	Yes	10
Italy	75	10-15	5-10	1-3	Yes	10-20	Yes	5-10
Netherlands	70	10	2-10	3	Yes	5-20	Yes (at 10 and 20 weeks)	10-20
Spain‡	25 (70)	6-7▪ (10)	2-5 (5-10)	1-2	Yes	5-10	Yes (every 4-6 months until pregnancy)	10-20 (5-10)
Sweden	48	†	15-20	3-6	Yes	20-25	Yes	6-40*
Switzerland (Central & West)	16	3	5-10	2	Yes	10-20	Yes (around 3 weeks before calving)	10-20
Switzerland (East)	25	3-5	5-10	1-2	No	-	Yes	5-10
Switzerland (Alpine region)	15	5	5-10	1	Yes	5-10	Yes (at 10-12 months)	5-10
United Kingdom (Wales)	120	10-20	5-10	3	Yes	5-10	Yes (natural mating, pasturing, before calving notably)	Varies (depends on management)
United-Kingdom	112	20	5-10	5	Yes	10-20	Yes, (natural mating at 15 months)	20-40

Legend: - = not applicable; ‡ Numbers for Basque country (North of Spain) are in brackets if different; ▪ 20% of cows in milk at farm; † large variation, but not enough pens when excessive calvings; * dependent on herd size and type of pen (individual or collective); †† Artificial Insemination

Table II: Repartition of herds according to dairy calf housing systems after the calf is separated from its dam in the European countries

	% of herds where newborn calves are housed in individual pens	**% of herds where newborn calves are directly housed in collective pens**
Austria (Alps)	50	50
Austria (non Alps)	70	30
Belgium (Flanders)	100	0
Denmark	80	20
Finland	80	20
France	85	15
Germany	100	0
Greece	35	65
Ireland	10	90
Italy	90	10
Netherlands	80	20
Spain	40	60
Sweden	100 for 2 first weeks 60-65 after first two weeks	0
Switzerland (Central)	100	0
Switzerland (East)	60	40
Switzerland (Alpine region)	25	75
United Kingdom (Wales)	75	25
United-Kingdom	60	40

Depending on the country, the variability in housing systems differed (Figure 1), as well as the percentage of each type of practice. In Germany and Belgium, calves were described to always be housed in individual pens before moving to collective pens. In Sweden, all calves were described to be housed in individual housing for two weeks before either staying in individual pens (60-65% of the calves) or being moved to collective pens. Most common maximum age in individual pens varied from 2 to 14 weeks with a median at eight weeks (Figure 2). During this period, calves could be housed either in huts (individual covered pens with complete separation from other pens, generally outside) or in individual pens with possible contact with neighbouring calves. In order to comply with the EU-regulation on social contact, Denmark kept calves in pairs with a low fence between single pens.

The use of individual pens after birth was reported to be the most frequent system in 11 out of 14 countries (Table 2). In individual pens, possible contacts with neighbours were reported to be predominant. The proportion of each individual housing facility varied depending on the country. One region of Austria (Alps) with small holdings reported using huts only. On the contrary, huts were reported being rare in Sweden (less than 1-3% of herds using huts). The most frequent number of individual pens (whatever the type) varied with average herd size, but not proportionally as one individual calf pen was available for 1 to 13 cows (calculated from Table 1). This number was reported to vary largely with herd size in Sweden, with a general lack of pens. During period with numerous calving, farmers generally have to use other solutions such as group pens in premises otherwise not used for calves.

Before weaning, three main types of collective pens could be identified: pens with less than five calves (five countries), pens with less than 10 calves (eight countries), and pens with more than 10 calves (12 to 20 calves) in two countries (Table 1, Figure 3). The different group sizes of the collective pens were observed in herds of different size (Table 1). In most of the countries, several collective pens (two or more) were used. However, in three countries, only one collective pen was available per herd. Italy with a large herd size was one of these three countries; the two other countries having a small mean herd size. Mean size of collective pens has been reported to decrease over time in Sweden, decreasing from 20-25 calves 10 years ago, if automatic feeders were used, to mainly groups of 15 nowadays. However, the number of collective pens was stated not being sufficient implying too large group sizes notably before weaning. Still in Sweden, fixed groups were reported, at least in larger herds (>160 cows) for the period before weaning; calves being generally mixed together with other calves after weaning.

3.2.4 Housing system after weaning

From the questionnaires it was found that the change of housing system could occur either at weaning or 1 week to 10 days after weaning in order to limit the stress already caused by the change of feed. In typical dairy herds within

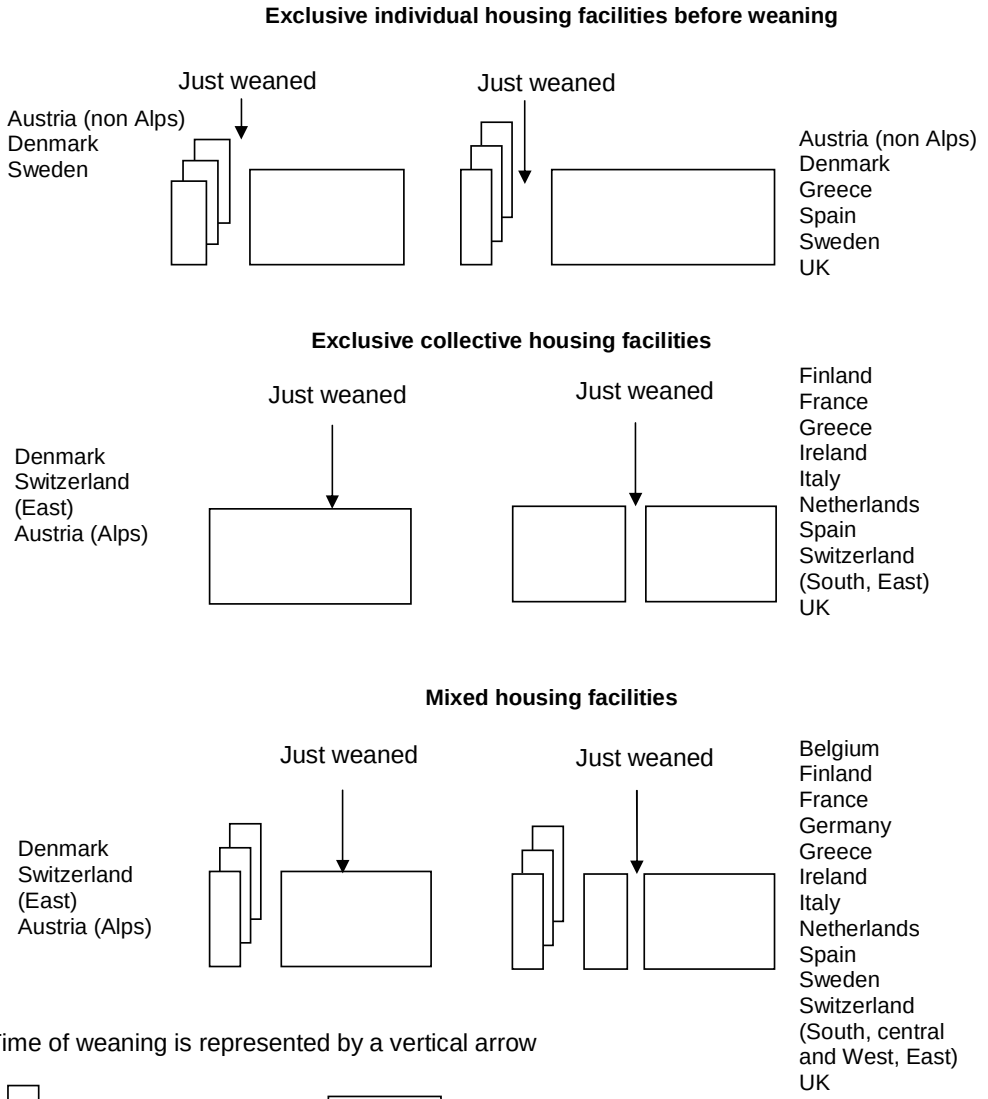
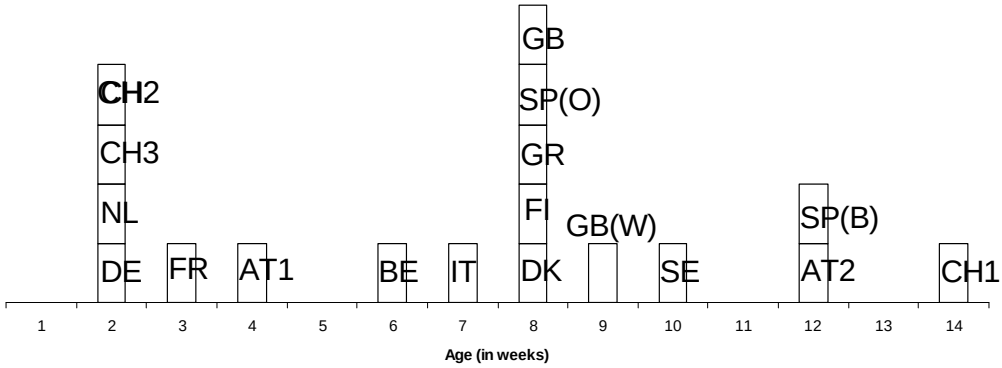


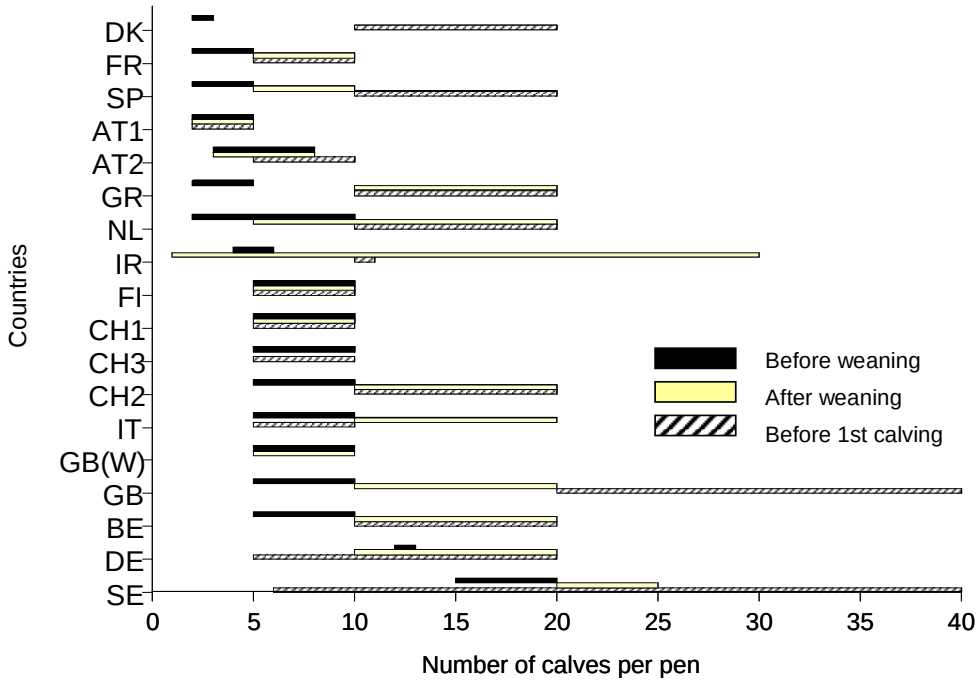
Figure 1: Types of dairy calf housing systems in the European countries, before and after weaning

AT1: Austria (Alps); AT2: Austria (non Alps); BE: Belgium; CH1: Switzerland (Alpine region); CH2: Switzerland (Central, West); CH3: Switzerland (East); DE: Germany; DK: Denmark; FI: Finland; FR: France; GB: Great Britain (South East, South West); GB(W): Wales; GR: Greece; IT: Italy; NL: The Netherlands; SE: Sweden; SP(B): Basque country; SP(O): Spain (other than Basque country)



† Maximal age is represented when information on most common age is not provided in questionnaires (Spain, and Sweden). No information available for Ireland

Figure 2: Most common or maximum age† of dairy calves in individual pen in the European countries



AT1: Austria (Alps); AT2: Austria (non Alps); BE: Belgium; CH1: Switzerland (Alpine region); CH2: Switzerland (Central, West); CH3: Switzerland (East); DE: Germany; DK: Denmark; FI: Finland; FR: France; GB: Great Britain (South East, South West); GB(W): Wales; GR: Greece; IR: Ireland; IT: Italy; NL: The Netherlands; SE: Sweden; SP: Spain
Countries are ordered depending on the number of calves per pen before weaning (increasing number). Different areas of the same countries have been grouped together.

Figure 3: Number of calves per collective pen before weaning, after weaning, and before 1st calving for typical dairy herds in the European countries

Europe, the most frequent age at weaning varied between 6 and 12 weeks of age, with a mean and a median at nine weeks (Figure 4).

Calves could be changed from one collective pen to another one at weaning (Figure 1). Additionally, during this change of pen, calves could either be mainly maintained and raised as a group (Alpine region of Austria, Italy, Spain, Alpine region of Switzerland, UK) or mainly shifted and mixed with another group (Belgium, Denmark, France, Greece, Ireland, Eastern, central and Western Sweden, Switzerland), or a mix of both (Austria, Finland, Germany, Sweden). The size of the new collective pen varied from 2 to 30 calves depending on available space; the most frequent case being either between 5 and 10 for five countries or between 10 and 20 for six countries (Figure 3). Only one region out of the 14 countries studied declared never moving their calves around weaning (East Switzerland).

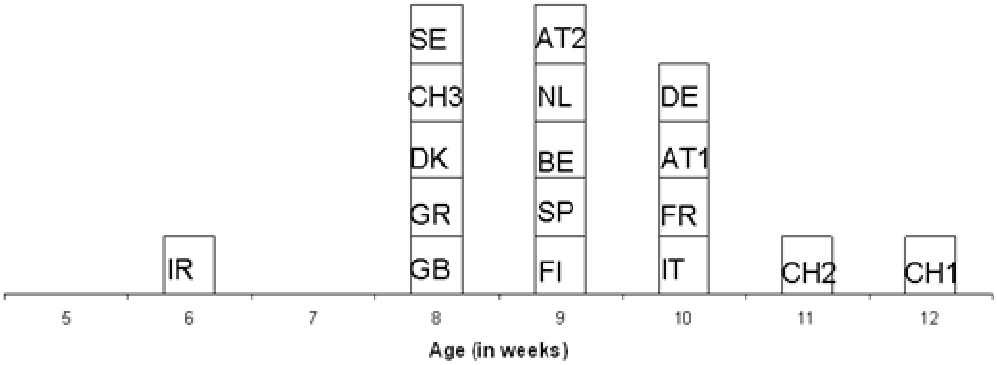
3.2.5 Housing system before first calving

Except for Denmark, young animals were grouped in a new pen between weaning and first calving. This could occur early after weaning, around artificial insemination or natural mating, close to calving (around three weeks before), during summer for pasturing, after high mountain pasturing (around 10-12 months of age), or depending on space availability. At that relocation, permanent groups were generally maintained and raised as a group (Finland, France, Italy, East and Alpine region of Switzerland, UK).

The size of the new pen (in terms of number of animals) varied between 2 and 40, the most frequent case being between 5 and 20 (Figure 2). Size was reported to depend on the type of pen in Sweden: generally 6-8 animals in slatted floor pen, 10-20 animals in litter pens, and 20-40 animals if housed in loose house systems with cubicles.

3.2.6 Grazing of calves

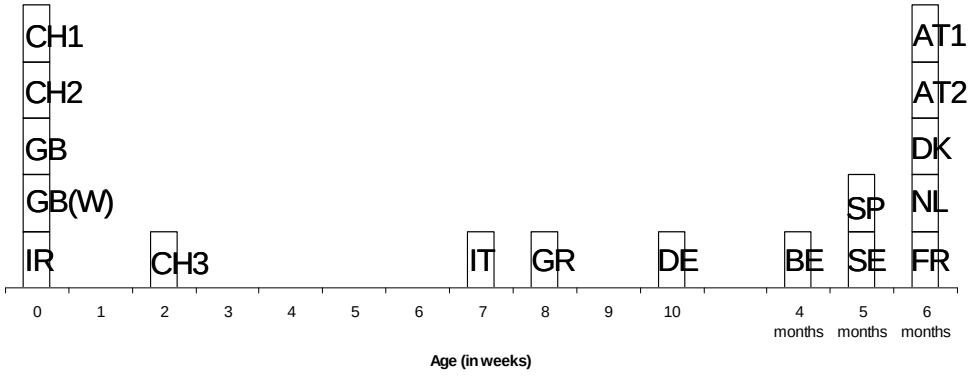
Reported age at first possible grazing varied from birth to six months (Figure 5.1). Age limit varied in Denmark depending on whether herds were organic (one day as a minimum age for grazing for 10-15% of organic herds) or non-organic (six months as a minimum). Most common age at grazing could be very early (less than three months) to quite late (more than 10 months) (Figure 5.2). Frequency of grazing varied with age of animals. Calves and heifers above six months of age were almost always allowed to go outside; grazing depending on season, with the exception of the Alpine region of Switzerland. In Sweden, it is mandatory that young stock and dairy cows should be kept on pasture during summer time. Calves were almost never outside between 0 and 6 weeks of age; however it was sometimes possible in Ireland, Central, Eastern and Western Switzerland. Weaning could be the period of time at which grazing was allowed (UK, Finland, Germany). In the Netherlands, an



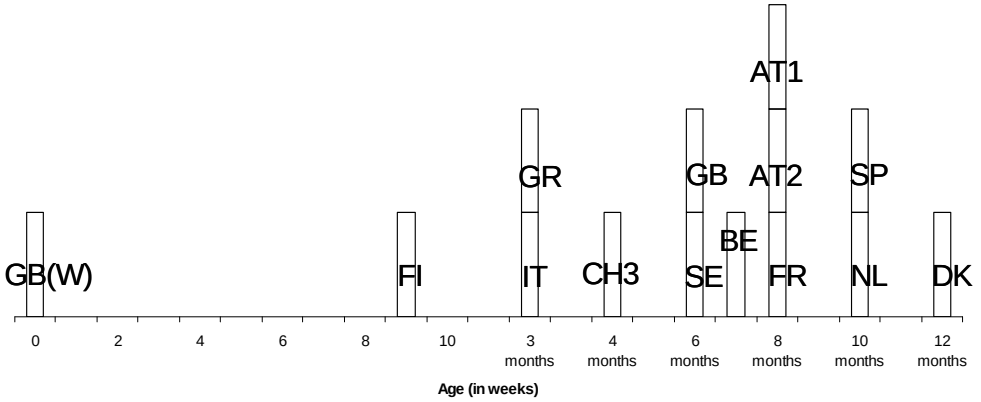
AT1: Austria (Alps); AT2: Austria (non Alps); BE: Belgium; CH1: Switzerland (Alpine region); CH2: Switzerland (Central, West); CH3: Switzerland (East); DE: Germany; DK: Denmark; FI: Finland; FR: France; GB: Great Britain (South East, South West); GB(W): Wales; GR: Greece; IR: Ireland; IT: Italy; NL: The Netherlands; SE: Sweden; SP: Spain. No information available for Wales

Figure 4: Most frequent age at weaning of dairy calves in the European countries

(1)



(2)



AT1: Austria (Alps); AT2: Austria (non Alps); BE: Belgium; CH1: Switzerland (Alpine region); CH2: Switzerland (Central, West); CH3: Switzerland (East); DE: Germany; DK: Denmark; FI: Finland; FR: France; GB: Great Britain (South East, South West); GB(W): Wales; GR: Greece; IR: Ireland; IT: Italy; NL: The Netherlands; SE: Sweden; SP: Spain. No information available for (1) Finland, (2) Ireland, Germany and Switzerland (Alpine, Central and West regions)

Figure 5: Minimum (1) and most common (2) age at first grazing outside for dairy calves in the European countries

increasing proportion of farmers (including most of herds with automated milking system) were reported not grazing any cattle at all anymore. Grazing was also not common in Greece and Spain (except North Spain).

3.3 Qualitative risk assessment

Reported risk factors related to housing facilities for respiratory and enteric infectious diseases of calves have been listed in Table 3. Risk factors are likely not to be independent because the number of calves within a pen, the age differences within a pen, the herd size and the calving season pattern interact. The pens of calves located near their dams are also at risk for pathogen transmission such as *Cryptosporidium parvum* (Silverlås, *et al.*, 2009).

Individual housing was reported to be at lower risk than collective housing both for enteric and respiratory diseases. The risk for both type of diseases decreased if hutches were used. Collective housing facilities were associated with a higher risk for enteric and respiratory diseases. The risk was increased when the size of the collective pen increased and with age variability within a pen if automated milk feeders were used. The resulting overall estimated risk for enteric and respiratory diseases in the most common housing systems described is displayed in Figure 6.

4 Discussion

This study aimed at describing the most common housing system for calves used in the main dairy production countries of Europe. It confirms that a wide range of practices exists with variations both between- and within-countries. It appeared that many risk factors for calf morbidity reported in literature are linked to the housing systems described in this study. Housing systems have also been reported to have an effect on mortality (Svensson, *et al.*, 2006b, Gulliksen, *et al.*, 2009c). Risk factors are associated to characteristics of housing systems that vary a lot in our study, particularly when it comes to collective rearing at young age. Moreover, we can assume that for a given herd size, larger groups are associated with higher age differences which also increase the risk. Control measure selection should be done after a specific risk assessment for each farm.

Expert data collected in this study fit with the system known by the experts and thus cannot be considered as mean data. Before using the expert's reported data, we checked that they had sufficient knowledge on the epidemiology of calf diseases and on cattle farming systems. However, it has to be reminded that experts usually are persons that are aware of the systems of the main production areas of their country. In some countries, experts spontaneously made mention of variability depending on the areas. Other experts only captured the most frequent system. Such a system can sometimes not be representative of the whole country, notably in mountainous areas.

Table III: Reported risk factors related to housing systems associated with occurrence of neonatal diarrhoea and respiratory disorders in non-weaned dairy calves

Risk factors	References for Neonatal diarrhoea	References for Bovine Respiratory Disease complex
Increased herd size (>50-70 cow-year)	(Frank and Kaneene, 1993, Gulliksen, *et al.*, 2009a)a	(Norström, *et al.*, 2000, Gulliksen, *et al.*, 2009b)b
Group housing *vs* individual housing	(Barrington, *et al.*, 2002, Svensson and Linberg, 2006, Svensson, *et al.*, 2006b, Gulliksen, *et al.*, 2009a)a	(Svensson, *et al.*, 2003, Lago, *et al.*, 2006, Svensson and Linberg, 2006)
Individual pen *vs* hutches	-	(Waltner-Toews, *et al.*, 1986)
Housing in presence of adult cattle *vs* hutches	-	(Virtala, *et al.*, 1999)
Large group size (>10-12 calves)	-	(Maatje, *et al.*, 1993, Losinger and Heinrichs, 1996, Svensson, *et al.*, 2003, Svensson and Linberg, 2006)
Large age variability within a batch	(Svensson, *et al.*, 2006b)	(Maddox-Hyttel, *et al.*, 2006, Gulliksen, *et al.*, 2009b)b
Sharing housing with dams during the first week of life	(Svensson, *et al.*, 2003, Svensson, *et al.*, 2006a, Svensson, *et al.*, 2006b, Silverlås, *et al.*, 2009)	(Svensson, *et al.*, 2003, Svensson, *et al.*, 2006b, Gulliksen, *et al.*, 2009b)b
Placing the calf pens along an exit or outer wall	(Svensson, *et al.*, 2003, Lundborg, *et al.*, 2005)	-
Placing young stock in proximity to calves and cows	(Silverlås, *et al.*, 2009)	-
Automatic milk feeding system	-	(Maatje, *et al.*, 1993, Svensson, *et al.*, 2003)

* >6 calves prior to weaning

Calf housing system (birth to weaning)					Risk of neonatal diarrhoea			Risk of respiratory disease	Additional remarks
Calving	**2 weeks**	**4 weeks**	**6 weeks**	**Weaning**	E. coli	Viruses	Parasites		
Individual housing					-	-	-	-	↓ risk if hutches
Individual housing			Collective housing		-	-	+	- / +	↓ risk if hutches
Individual housing		Collective housing			-	++	+++	++	↓ risk if hutches
Collective housing					+++	+++	+++	+++	↑ risk if automated milk feeder, with increased pen size, and with large age variability
					Calves < 1 month of age			All calves (whatever their age)	

-: low; +: moderate; ++:high; +++: very high
↓: decrease; ↑: increase

Figure 6: Qualitative risk assessment of housing system for the main infectious diseases of dairy calves

Four different types of calf housing systems could be identified (Figure 6) on the basis of reported calf management before weaning. First of all, calves can be raised in individual housing until weaning, with a minimum of eight weeks. This housing system is predominant in Austria, Denmark, Finland, Sweden, and UK and frequently used in Greece and Spain. Such a housing system minimises the risk for pathogen transmission between young animals. Furthermore, raising calves in individual pens facilitates the surveillance for the farmer. However, the work load is increased (notably when it comes to feed distribution and cleaning). This system also requires a sufficient number of individual pens in the farm, condition that can be costly notably when calvings are grouped. Calves should be able to have social contacts with their herdmates according to the European regulations.

While individual pens until weaning seem to be the most relevant system to decrease the risk for pathogen transmission, the EU regulation does not allow such a system after eight weeks of age. To circumvent this rule, some countries such as Denmark gather calves in pairs in such "individual pens". Furthermore, the decrease of the risk for pathogen transmission in individual pen can be limited if difficulties are encountered in the cleaning of individual pens between successive calves. Lastly, sanitary risk is postponed to the period after weaning when calves enter a collective pen. In this housing system where calves can be housed in individual housing until weaning, with a minimum of eight weeks, respiratory disorders are the main problem and risk assessment needs to be concentrated on the period of changes around weaning when calves enter collective pens.

The second housing system that can be identified is the one in which calves can be housed in individual pen for a minimum of four weeks. This system is used in Austria (Alps), Belgium and Italy. The number of pens needed on a farm is more flexible than for the first described housing system. The risk in terms of disease transmission is linked to the fact that calves are mixed into collective pens 3 to 6 weeks before weaning. The first collective pen can be used either solely until weaning or also after weaning, meaning that calves of different ages can be mixed. Large age differences increase the risk for disease transmission, notably for respiratory disorders which are more important in such housing systems (Svensson, *et al.*, 2003). The age of occurrence of neonatal diarrhoea due to the main bacterial and viral pathogens (*E. coli*, rotavirus and coronavirus) being comprised within the first three weeks of life (Bartels, *et al.*, 2010), we can therefore assume that gathering within a same pen calves aged of more than one month is at a lower risk for occurrence of diarrhoea (except for cryptosporidiosis and coccidiosis for which shedding occurs in later ages) compared to gathering calves of two weeks of age for which shedding of enteric pathogens is frequent.

The third housing system consists in housing calves in an individual pen for a short period of 2 to 3 weeks after separation from the dam before changing them to a collective pen. This system is predominant in France, Germany, the Netherlands and Switzerland. It is also frequent in Sweden. The number of individual pens is reduced, decreasing the work load of the farmer. Risk for

disease transmission is mainly linked to the mixing of calves with different ages in the collective pens. If weaning occurs late, the difference of age between the non-weaned calves becomes important, with really young calves being housed with older ones.

Finally, calves can be housed just after birth in collective pens. This system is predominant in Greece, Ireland, Spain, and the Alpine region of Switzerland. It is also frequent in the Alpine region of Austria, Denmark, Finland, France, the Netherlands, and UK. This system is particularly convenient when automatic milk feeders are used. However, it is reported to be associated with an increased risk of both neonatal diarrhoea and respiratory diseases (Maatje, *et al.*, 1993, Svensson, *et al.*, 2003). The size of the collective pens varies depending of the country, from small pens of 2 to 5 calves (two calves for Denmark) to larger pens. In Sweden, pen size tends to decrease nowadays. In order to decrease the high risk for infectious diseases in this kind of system, supplementary preventive measures such as vaccination of cows can be advised.

After weaning, the risk for disease transmission comes from the number of calves per pen (pen size), which has been reported to vary largely in our study. Calves of different age can be mixed either after first service (limited risk) or really early, depending on the country.

Age at first grazing varies a lot depending on the country. No data are available on the sanitary risk linked to age at grazing, except for parasites, for which exposure of calves also depends on the way pastures are used.

5 Conclusion

This study provides a first description of major differences in calf housing systems across the main dairy countries of Europe. Four different types of calves housing facilities were identified. The least risky in terms of enteric and respiratory infectious diseases is housing calves in individual pens until weaning or at least eight weeks before moving them to collective pens. Housing calves in collective pens directly from the separation from the dam after birth is the most risky housing system. Housing calves in individual pens prevents contact between calves and cleaning occurs more often as individual pens are more often empty. However, collective housing facilities still exist as they are more convenient and necessitate less work from the farmer. Differences in housing systems have to be acknowledged when using thresholds for analysis of disease incidence and when designing calf health control plans. For a specific herd, one should thus adapt the risk assessment to the housing system used, while considering in the mean time other factors not studied here such as hygiene, feeding practices, and air conditioning.

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References

Barrington, G. M., Gay, J. M. and Evermann, J. F., Biosecurity for neonatal gastrointestinal diseases, Vet. Clin. North Am. Food Anim. Pract. (2002) 18:7-34.

Bartels, C. J. M., Holzhauer, M., Jorritsma, R., Swart, W. A. J. M. and Lam, T. J. G. M., Prevalence, prediction and risk factors of enteropathogens in normal and non-normal faeces of young Dutch dairy calves, Prev. Vet. Med. (2010) 93:162-169.

Doyle, T. M., Susceptibility to Johne's disease in relation to age, Vet. Rec. (1953) 65:363-364.

European Commission, Agricultural statistics - Main results -- 2007-08 (2009): pp. 131.

Frank, N. A. and Kaneene, J. B., Management risk factors associated with calf diarrhea in Michigan dairy herds, J. Dairy Sci. (1993) 76:1313-1323.

Garber, L. P., Wells, S. J., Hancock, D. D., Doyle, M. P., Tuttle, J., Shere, J. A. and Zhao, T., Risk factors for fecal shedding of *Escherichia coli* 0157:H7 in dairy calves, J. Am. Vet. Med. Assoc. (1995) 207:46-49.

Gulliksen, S. M., Jor, E., Lie, K. I., Hamnes, I. S., Loken, T., Akerstedt, J. and Osteras, O., Enteropathogens and risk factors for diarrhea in Norwegian dairy calves, J. Dairy Sci. (2009a) 92:5057-5066.

Gulliksen, S. M., Jor, E., Lie, K. I., Loken, T., Akerstedt, J. and Osteras, O., Respiratory infections in Norwegian dairy calves, J. Dairy Sci. (2009b) 92:5139-5146.

Gulliksen, S. M., Lie, K. I., Loken, T. and Osteras, O., Calf mortality in Norwegian dairy herds, J. Dairy Sci. (2009c) 92:2782-95.

Gulliksen, S. M., Lie, K. I. and Osteras, O., Calf health monitoring in Norwegian dairy herds, J. Dairy Sci. (2009d) 92:1660-9.

Lago, A., McGuirk, S. M., Bennett, T. B., Cook, N. B. and Nordlund, K. V., Calf respiratory disease and pen microenvironments in naturally ventilated calf barns in winter, J. Dairy Sci. (2006) 89:4014-4025.

Losinger, W. C. and Heinrichs, A. J., Management variables associated with high mortality rates attributable to respiratory tract problems in female calves prior to weaning, J. Am. Vet. Med. Assoc. (1996) 209:1756-1759.

Lundborg, G. K., Svensson, E. C. and Oltenacu, P. A., Herd-level risk factors for infectious diseases in Swedish dairy calves aged 0-90 days, Prev. Vet. Med. (2005) 68:123-143.

Maatje, K., Verhoeff, J., Kremer, W. D., Cruijsen, A. L. and Van den Ingh, T. S., Automated feeding of milk replacer and health control of group-housed veal calves, Vet. Rec. (1993) 133:266-270.

Maddox-Hyttel, C., Langkjaer, R. B., Enemark, H. L. and Vigre, H.,

Cryptosporidium and *Giardia* in different age groups of Danish cattle and pigs -- occurrence and management associated risk factors, Vet. Parasitol. (2006) 141:48-59.

Norström, M., Skjerve, E. and Jarp, J., Risk factors for epidemic respiratory disease in Norwegian cattle herds, Prev. Vet. Med. (2000) 44:87-96.

Olsson, S. O., Viring, S., Emanuelsson, U. and Jacobsson, S. O., Calf diseases and mortality in Swedish Dairy Herds, Acta Vet. Scand. (1993) 34:263-269.

Silverlås, C., Emanuelson, U., de Verdier, K. and Björkman, C., Prevalence and associated management factors of *Cryptosporidium* shedding in 50 Swedish dairy herds, Prev. Vet. Med. (2009) In Press, Corrected Proof.

Svensson, C., Lundborg, K., Emanuelson, U. and Olsson, S. O., Morbidity in Swedish dairy calves from birth to 90 days of age and individual calf-level risk factors for infectious diseases, Prev. Vet. Med. (2003) 58:179-197.

Svensson, C., Hultgren, J. and Oltenacu, P. A., Morbidity in 3-7-month-old dairy calves in south-western Sweden, and risk factors for diarrhoea and respiratory disease, Prev. Vet. Med. (2006a) 74:162-179.

Svensson, C. and Linberg, P., The effect of group size on health and growth rate of Swedish dairy calves housed in pens with automatic milk feeders, Prev. Vet. Med. (2006) 73:43-53.

Svensson, C., Linder, A. and Olsson, S. O., Mortality in Swedish dairy calves and replacement heifers, J. Dairy Sci. (2006b) 89:4769-4777.

Virtala, A. M. K., Mechor, G. D., Gröhn, Y. T. and Erb, H. N., Morbidity from nonrespiratory diseases and mortality in dairy heifers during the first three months of life, J. Am. Vet. Med. Assoc. (1996) 208:2043-2046.

Virtala, A. M. K., Gröhn, Y. T., Mechor, G. D. and Erb, H. N., The effect of maternally derived immunoglobulin G on the risk of respiratory disease in heifers during the first 3 months of life, Prev. Vet. Med. (1999) 39:25-37.

Waltner-Toews, D., Martin, S. W. and Meek, A. H., Dairy calf management, morbidity and mortality in Ontario Holstein herds. III. Association of management with morbidity, Prev. Vet. Med. (1986) 4:137-158.

Chapter 4

Within-herd contact structure and transmission of *Mycobacterium avium* subspecies *paratuberculosis* in a persistently infected dairy cattle herd

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Abstract

Within-herd transmission of pathogens occurs either by direct or indirect contact between susceptible and infected animals. In dairy herds that are structured into groups, the way in which animals encounter each other and share an environment can affect pathogen transmission. Dairy cattle are heterogeneous in terms of susceptibility and infectivity with respect to *Mycobacterium avium* subspecies *paratuberculosis* (*Map*) transmission. It is mainly young animals that are susceptible and adults that are infectious. Both vertical and horizontal transmission through the ingestion of *Map* shed into the environment by adults and transiently infected calves can occur. Our objective was to assess the effect of contact structure on *Map* transmission in persistently infected dairy herds and to examine the effect of isolating calves from other calves or from adults before weaning. We developed a stochastic compartmental model of *Map* transmission in a closed dairy herd. The model reflects the *Map* infection process and herd management characteristics. Indirect transmission via the environment was modelled explicitly. Six infection states (susceptible, resistant, transiently infectious, latently infected, subclinically infected, and clinically affected) and two contaminated farm area environments (whole farm and calf area) were modelled. Calves were housed in hutches, individual indoor pens, or group indoor pens. Two different levels of exposure of calves to a farm environment contaminated by adults were possible: no exposure and indirect exposure through fomites. Three herd sizes were studied. We found that contacts between calves before weaning did not influence *Map* transmission in a herd, whereas the level of exposure of calves to an environment contaminated by adults and the starting age of exposure of calves to adults were pivotal. Early culling of clinically affected adults led to a lower prevalence of infectious adults over time. The results were independent of herd size. Despite the many transmission routes that are known, the best control approach is to limit the exposure of calves to adult faeces through the systematic separation of adults and calves in combination with hygiene measures. Reducing contact between calves does not appear effective.

***Keywords*:** Cattle housing, dairy cattle, herd-level, Mycobacterium avium subspecies paratuberculosis, modelling, transmission

1 Introduction

Paratuberculosis has a significant economic impact on dairy cattle producers around the world (Kennedy and Benedictus, 2001; Kennedy and Nielsen, 2007). Eradication programmes have been implemented in many countries for >20 years, but their success has been limited (Nielsen, 2009). Programmes aiming to reduce transmission, rather than eradicate the disease, consequently have been organized. They are based on control measures such as test-and-cull, hygiene and feed management. To date, few impact assessments of these control measures have been carried out (Nielsen, 2009).

Mycobacterium avium subspecies *paratuberculosis* (*Map*) is transmitted indirectly through the ingestion of contaminated faeces (faecal-oral transmission), contaminated milk, and colostrum (Taylor et al., 1981; Streeter et al., 1995). Mainly calves are susceptible. Although it was once thought that only adults could shed the bacteria in their faeces (Chiodini *et al.*, 1984), *Map* faecal shedding now has been described in young stock as well (Bolton et al., 2005; Antognoli et al., 2007). Calf-to-calf transmission has also been shown (Van Roermund *et al.*, 2007). The importance of this transmission route on within-herd infection dynamics has not yet been evaluated. The impact of reducing calf exposure either to *Map* shed by other calves or to *Map* present in the farm environment, by the setting and the hygiene of the housing facilities is not known.

Dairy herds are typically organised into groups structured by age. Therefore, housing and management influence how animals interact, referred to as the herd contact structure. Calf-to-calf transmission can occur via the contamination of a shared pen environment, whereas adult-to-calf transmission is contingent on calves being exposed to fomites contaminated by adult faeces.

The influence of herd structure on disease transmission can be assessed through a modelling approach because changes in herd structure can be tested with everything else being held constant (Turner et al., 2003; Ezanno et al., 2008). In contrast, field studies investigating *Map* transmission are difficult and expensive because paratuberculosis infection occurs mainly in young stock and is characterised by a long incubation period (1 to $\geq$ 15 years) before any clinical signs arise. Field studies are further complicated by the low and varying sensitivity of diagnostic tests. Several *Map* transmission models already exist but they do not consider *Map* persistence in the environment or its indirect transmission (Marcé et al., 2010a). Furthermore, the effect of dairy herds being managed as multiple sub-groups has never been studied. The objective of this study was to assess the effect of within-herd contact structure on *Map* transmission in persistently infected dairy herds. Specifically, we aimed to assess the effect of preventing contact between calves and between calves and adults before weaning, while explicitly modelling the persistence of *Map* in the environment.

We focused on the impact of decreasing the exposure of calves to different contaminated environments on the prevalence of infectious adults, specifically through the use of different housing facilities, cleaning and culling clinically affected adults.

2 Materials and methods

2.1 Model of within-herd *Map* transmission

2.1.1 General characteristics of the model

We developed a new mathematical compartmental model that explicitly represents indirect calf-to-calf and adult-to-calf transmission to study *Map* transmission in a dairy herd (detailed description of the full model in (Marcé et al., Submitted). We used the model here to simulate variations in the contact structure. Unlike other published models involving dairy herds, which consider faecal-oral transmission as being linked directly to the presence of infectious animals (Marcé et al., 2010a), the presence and persistence of *Map* in the environment was considered in our study by explicitly modelling environmental contamination. Both the local contamination of the environment (i.e. calf housing facilities, E_i) and the global contamination of the environment of the whole farm (E_g) specifically were represented (Figure 1). In our model, both adults and calves were considered to be potentially infectious, i.e. could shed *Map* in their faeces and thus contaminate the environment. However, the quantity of *Map* organisms shed varied depending on the age of the animal and its infection status.

This stochastic model was run in discrete time steps with a time interval of 1 week, which made it possible to represent the different routes of transmission and take into account calf housing facilities before weaning. Both herd dynamics and the infection process were modelled.

We decided to model closed dairy herds (no purchase of animals) so that the effect of within-herd contact structure could be understood separately from the effect of *Map* introduction. Closed dairy herds are fairly common in the main dairy areas of Europe (e.g. 50% of specialised dairy herds in western France and northern Netherlands) (van Schaik et al., 2002; Ezanno et al., 2006). The simulation process was based on persistently infected herds, in which no *Map* control measures were implemented. These persistently infected herds were generated by introducing a single infected heifer into a susceptible herd, and by selecting the simulations only with herds still infected after 25 years of simulation. Each simulation was run 400 times. With this number of runs, it was possible to obtain a stable distribution of simulated outputs. In the baseline scenario, 33% of the runs resulted in the herd being persistently infected.

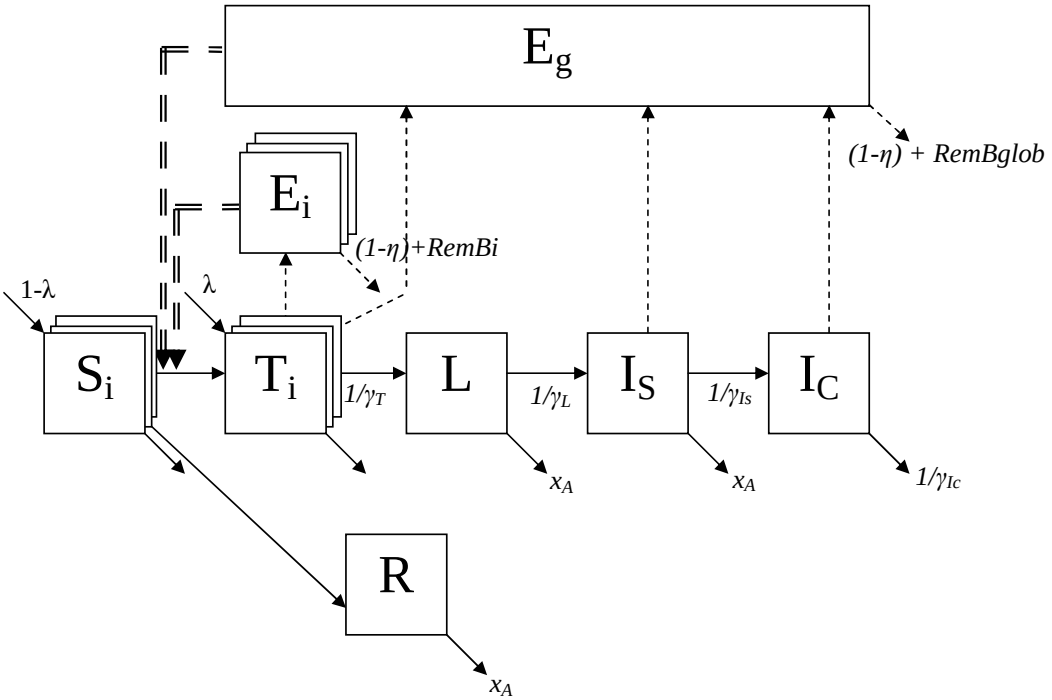

Figure 1: Flow diagram of the *Mycobacterium avium subsp. paratuberculosis* (*Map*) transmission model in a dairy cattle herd, representing infection states (S_i: susceptible; T_i: transiently infectious; L: latently infected; Is: subclinically infected; Ic: clinically affected; R: resistant), transitions between states (solid lines), entry and exit of *Map* in the environment (dashed lines), and exposure of calves to *Map* (double line). Calves are separated in groups (i), each having a specific local environment E_i. E_g represents the global environment of the farm (whole environmental contamination resulting from *Map* shedding of T, Is and Ic cattle).[2]

[2] η: survival rate of *Map* in the environment; *RemBInd*: % of *Map* removed in hutch or individual pen (per week); *RemBCol*: % of *Map* removed in group pen (per week); *RemBglob*: % of *Map* removed in the farm (per week); λ: probability of *Map* vertical transmission; γ_T, γ_L, γ_{Is}, γ_{Ic}: mean time spent in compartment T, L, Is, and Ic, respectively; x_A: mean culling rate of adults *RemBi* = *RemBind* or *RemBcol* depending on the pen type. *RemBi* is applied when the pen is emptied.

To assess whether the conclusions would be the same regardless of herd size, three different herd sizes were studied: the first (small herd) with around 35 adults; the second a large herd with around 110 adults; the third an even larger herd with around 500 adults. In the model, we assumed that the larger the herd, the larger the size of the group pen, and thus the larger the number of possible indirect contacts via the same environment.

The model was implemented with Scilab 5.1[1].

2.1.2 Population dynamics

In the model, only the characteristics required to accurately represent *Map* transmission were considered in the population dynamics. The demographics and management of a typical Western European herd were depicted. Calving occurred year round; all male calves were sold between the 2nd and 4th week after birth while all female calves remained in the herd. Animals were organized into five age-based groups: unweaned calves (female cattle <10 weeks of age), weaned calves (female cattle from weaning to 1 year of age), young heifers (female cattle from 1 year to first service), heifers (female cattle from first service to first calving), and cows (adult females). Each group could shed *Map* in their faeces and contaminate associated environments.

Following separation from the dam, individual calves were housed alone for 2 weeks before being moved to group pens. They remained in the same group pen until weaning at 10 weeks of age, when they were moved to a different group pen. Cattle <1 year of age could not be housed in the same pen as adults.

Heifers could be sold before their first calving to regulate herd size. The mean culling rate of adults was 35.5% (x_A) irrespective of the reason for culling, and varied between parities (the culling rate was chosen based on the opinion of three experts who regularly analyse French national databases on culling). Deaths were included in this culling rate.

Animals >6 months of age grazed from April to November. Outdoors, calves and heifers which have not yet calved were grouped together because they often used the same pasture. Adults grazed separately.

2.1.3 Infection process

Animals were classified into mutually exclusive health states: susceptible (S), resistant (*R*), transiently infectious (T) (infectious only for a limited period of

[1] Available on line: http://www.scilab.org [consulted 11 February 2010], Scilab is trademark of INRIA, France.

time), latently infected (L) (infected not infectious), subclinically infected (Is) (infected and infectious but not affected), and clinically affected (Ic) (infected, infectious, and affected) (Nielsen, 2008) (Figure 1). The probability of culling increased for clinically affected animals because they could be identified directly as infected. Infectious adults were defined here by the sum of both subclinically infected and clinically affected cattle above 30 months of age. The assumptions developed below were based on current knowledge on *Map* transmission and on standard calf housing management.

In the model, transmission occurred vertically (*in utero* transmission) or horizontally (ingestion of *Map*). *Mycobacterium avium paratuberculosis* was shed in the colostrum, milk, and faeces of infectious cattle. Vertical transmission resulted in the birth of transiently infected calves, with a probability λ of 0.149 for latently and subclinically infected cows, and 0.65 for clinically affected cows (Benedictus et al., 2008; Whittington and Windsor, 2009).. We assumed that only animals <1 year of age were susceptible to infection. The susceptibility within that age group was modelled as exponentially decreasing (Windsor and Whittington, 2010). If not infected before 1 year of age, animals were assumed to be resistant to *Map* infection.

A susceptible calf could be infected by ingesting contaminated colostrum (of the dam) or milk (from the tank) or through contact with a contaminated environment. The probability of being infected depended on the quantity of faeces or liquid ingested and the number of *Map* infectious doses present in that quantity. Contamination of colostrum and milk occurred either directly (direct shedding of an infected cow) or indirectly (presence of faeces in the liquid). Indirect horizontal transmission via a contaminated environment either occurred locally (calf-to-calf transmission) by ingestion of contaminated calf faeces, or globally by ingestion of fomites contaminated by the faeces of adults present on the farm (adult-to-calf transmission) (Van Roermund et al., 2007). The mixing of animals within sub-groups was considered to be homogeneous.

Transitions between states were modelled based on exponential distributions. A transiently infectious calf shed *Map* consistently for 25 weeks (γ_T) before becoming latently infected (Van Roermund *et al.*, 2007). A latently infected cattle did not shed *Map* for on average 52 weeks (γ_L) (Nielsen and Ersboll, 2006; Nielsen, 2008) until becoming subclinically infected for on average 104 weeks (γ_{Is}) (Matthews, 1947). There was no additional culling for subclinically infected cows. Unculled animals then became clinically affected, i.e. presented symptoms such as diarrhoea and weight loss. The mean time spent by a clinically affected adult on the farm was 6 months (γ_{Ic}). No recovery from the infection was possible.

All transiently infected calves in a calf group contributed to the contamination of their housing facility (E_i). All infectious cattle (whatever their age) contributed to the contamination of the global environment of the farm (E_g). Each calf group housing facility represented a risk of infection for the calves

present in the same housing facility, whereas the whole farm area represented a risk of infection for all groups of animals. Every week, 60% of the bacteria persisted in the barn (with standard cleaning) and 93% on pasture (η) (expert opinion[2]). Additional cleaning and mulching practices further decreased the number of bacteria in calf housing facilities. These practices were carried out whenever a pen was empty, with an efficacy of 0.67 in individual housing (concrete floor with the possibility of clearing out the pens and washing them with high pressure) and 0.17 in group pens (only the litter removed). Efficacy was defined as a percentage of *Map* removed from the environment.

2.1.4 Model outputs

The model generated data on the mean annual incidence of newly infected animals (Incid25), the mean prevalence of infectious cows (subclinically infected and clinically affected cows >30 months of age) (Prev25), and the mean contribution of calf-to-calf horizontal transmission (CalfContr) and cow-to-calf (AdultContr) horizontal transmission to total transmission (proportion of newly infected cattle per route of transmission), in the last year of the 25-year simulation (Table 1). The prevalence of infectious cows was also studied over the 25 years of simulation.

2.1.5 Model evaluation

The parameters for herd management and infection were based on published data and expert knowledge to obtain a simplified representation of a dairy herd infected with *Map*. To calibrate unknown parameters (transmission parameters), model outputs (prevalence over time) were compared with published field data on apparent prevalence corrected for test characteristics. Two parameters that were very uncertain and able to influence the results linked to the contact structure were the period of time during which a calf could shed *Map* (*PShed*), and the period of time during which cattle are susceptible to *Map* (*PSusc*). The sensitivity of the model to these two parameters therefore was assessed (see below 2.2.1). The infection dynamics described by the proportion of animals in the different infection categories was consistent with published data (excluding published study data used to calibrate the model) and previously published modelling studies.

[2] Two veterinary experts working on dairy cattle: one practitioner in cattle practice, one academic teaching dairy herd health

Table 1: Outputs studied in the sensitivity analysis of the model of *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) transmission within a dairy cattle herd

Name	Definition
Incid25	Mean annual incidence of newly infected animals during the last year of the 25-year simulation
Prev25	Mean prevalence of infectious adults (animals >30 months) during the last year of the 25-year simulation
CalfContr	Mean contribution[1] of calf-to-calf transmission to total transmission during the last year of the 25-year simulation
AdultContr	Mean contribution[2] of adult-to-calf transmission to total transmission during the last year of the 25-year simulation

[1] proportion of newly infected cattle by route of transmission

2.2 Within-herd contact structure scenarios

2.2.1 Sensitivity analysis for contact structure parameters

A sensitivity analysis aiming to identify the parameters related to the contact structure that influence the transmission of *Map* in a dairy herd was carried out. We studied the sensitivity of model outputs to 10 parameters describing calf housing and contacts, exposure of calves to fomites (contaminated by calf and adult faeces), and reduction of environmental contamination through cleaning and through grazing (Table 2). To evaluate the contributions of parameters and their first-order interactions on output variance, we performed a partial factorial design (Saltelli et al., 2000). This sensitivity method suffices when many simulations cannot be performed and when some parameters are qualitative (such as *Graz*: period during which grazing is allowed). This design resulted in 81 scenarios, accounting for three possible values per parameter. The studied values included most likely, minimum, and maximal values which were derived from expert opinions, a review of the literature, and published experimental data. Each scenario was run 400 times.

For each output, a linear regression analysis was fitted using the 10 parameters as factors. A minimum variance criterion was defined and factors accounting for >4% of the variance were retained in the model. The global contribution of factor *i* to the variance in output y is $C_i^y = \left(SS_i^y + 1/2\sum_j SS_{i:j}^y\right)/SS_{tot}^y$, with SS_{tot}^y the total sum of squares of the regression model for output *y*, SS_i^y the sum of squares related to the principal effect of factor *i* for output *y* (nil if factor *i* is not retained in the model), $SS_{i:j}^y$ the sum of squares related to the interaction between factor *i* and factor *j* for output *y* (nil if this interaction is not retained in the model). The sum of the contributions for output *y* equals the coefficient of determination of the regression model R^2.

2.2.2 Scenarios mimicking farm management

The contact structure within a herd has an impact on both the local (calf-to-calf) and the global (adult-to-calf) indirect horizontal transmission. Scenarios were thus defined based on possible calf housing, hygiene, and management, and the implications of these on the contact structure within a herd (Table 3). Good hygiene included preventing calves from being exposed to fomites and weekly cleaning to remove bacteria from the environment.

Our first step was to study the impact of adult-to-calf contact. Several levels of exposure of susceptible calves to an environment contaminated by adults (*Exp*) were studied. Situations from zero exposure (e.g. possible when calves are reared off-site) to an exposure mimicking the situation when adults and

Table 2: Parameters of the model of *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) transmission within a dairy cattle herd used to define the factorial experiment in the sensitivity analysis (in bold, values for the baseline scenario)

Name	Definition	Range of value	Source
TimInd	Time spent in individual housing (in weeks)	[0; **2**; 8]	91/629/EEC and 97/2/EC
Nn	Number of neighbouring calves	[0;**2**;5]	(Marcé et al., 2010b)
Exp	Rate of exposure to fomites contaminated by *Map* shed by adults whatever the housing facility of the calf	[0; 0.5; **1**]	User defined
RemBInd	% of *Map* bacteria removed from individual hutch or pen housing (per week)	[33; **67**; 100]	Expert opinion **
RemBCol	% of removed bacteria in group housing (per week)	[0; **17**; 33]	Expert opinion **
Graz	Period during which animals over 6 months graze	[Never; May-Sept; **April-Nov**]	(Marcé et al., 2010b)
MinGraz	Minimal age for grazing (weeks)	[10; **26**; 35]	(Marcé et al., 2010b)
Size	Herd size (number of adults)	[35; **110**; 500]	(Marcé et al., 2010b)
PShed	Period of time during which a calf can shed *Map* (in weeks)	[10; **25**; 70]	(Rankin, 1961; Van Roermund et al., 2007)
PSusc	Period of time during which cattle are susceptible to *Map** (in weeks)	[13; 26; **52**]	(Windsor and Whittington, 2010)

*Same mean susceptibility of calves to *Map* infection for all scenarios but distributed across different periods of time (same area under the curve of susceptibility according to age).

**Two experts working on dairy cattle

Table 3: Scenarios of calf exposure tested in the model of *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) transmission within a dairy cattle herd

Factors influencing calf exposure	Parameter			
	Name	Definition	Range of value	Source
Exposure of susceptible calves	*Exp*	Overall rate of exposure until 1 year of age to fomites contaminated by *Map* shed by adults	[0; 0.1; 0.2; 0.5; 0.9; 1]	User defined
Calf housing and exposure to adults and to other calves				
Hutch	*Exp*	Reduced rate of exposure while in a hutch to fomites contaminated by *Map* shed by adults	[0]	User defined
	Nn	No exposure to other calves		
Individual pen	*Exp*	Reduced rate of exposure while in an individual pen or until weaning to fomites contaminated by *Map* shed by adults	[0; 0.1; 0.2; 0.5; 0.9; 1]	User defined
	Nn	Possible exposure to 2 neighbour calves (if present)		
Length of the reduced exposure	*TimInd or weaning*	Time spent in individual housing (in weeks) or Weaning age (in weeks)	[0; 2; 8] [10]	91/629/EEC and 97/2/EC; (Marcé et al., 2010b)
Cleaning				
On the whole farm	*RemBglob*	% of *Map* bacteria removed from fomites from the farm (per week)	[20; 40; 60; 100]	Expert opinion *
In individual pens	*RemBInd*	% of *Map* bacteria removed from individual hutch or pen housing (per week)	[33; 67; 100]	Expert opinion *
In group pens	*RemBCol*	% of *Map* bacteria removed from calf group housing (per week)	[0; 17; 33]	Expert opinion *
Elimination of clinically affected cattle	*TIcCull*	Mean time spent by a cow in the clinically affected compartment before being culled (in months)	[3; 6; 9]	Expert opinion **

*Two experts working on dairy cattle
**Three experts having published on culling in observational studies

calves are housed in the same barn but in different pens were modelled. Factors influencing environmental contamination by adults were studied. The impact of cleaning measures was studied through the removal of 20% to 100% of *Map* from the global farm environment at the end of each time step (*RemBglob*).

Our second step was to study the impact of calf housing which could influence both adult-to-calf and calf-to-calf contact structures. In the scenarios studied, the time spent in individual housing (*TimInd*) varied from 0 to 8 weeks to comply with European regulations (Council Directive 91/629/EEC and Council Directive 97/2/EC). Two different types of individual housing could be used: individual hutches and individual pens.

Housing of calves in individual hutches combined three control measures: no exposure of calves to adults until calves are moved to group pens, no contact with neighbouring calves while in individual hutches, and highly effective cleaning of hutches between the occupancy of successive calves.

On the contrary, in individual pens, contacts with fomites contaminated by adults could occur but could be reduced (or not) compared to group pens (particularly through hygiene management practices). Several levels of exposure of calves in individual or group pens to an environment contaminated by adults (*Exp*) were studied. The time during which a calf was less exposed to an environment contaminated by adults varied, either until moving in a group pen at 0, 2, or 8 weeks, or until weaning at 10 weeks of age.

Calf-to-calf indirect contact in individual pens could occur through contact with calves from contiguous pens (with a maximum of 2 neighbours) and imperfect cleaning between two successive calves (*RemBInd*). Calf-to-calf indirect contact in a group pen could occur through contact with other calves in the pen and imperfect cleaning between successive groups of calves (*RemBCol*).

Finally, the effect of different times until the detection and culling of clinically affected animals (*TIcCull*) shedding a high quantity of *Map* was studied (from 3 to 9 months spent by a clinically affected animal on the farm before being culled).

3 Results

3.1 Model parameters contributing to variance in model outputs

Depending on the output, the studied parameters together explained 25 to 67% of output variances (Figure 2). They explained only 25% of the variance in the contribution to infection of the calf-to-calf transmission (CalfContr, in Table

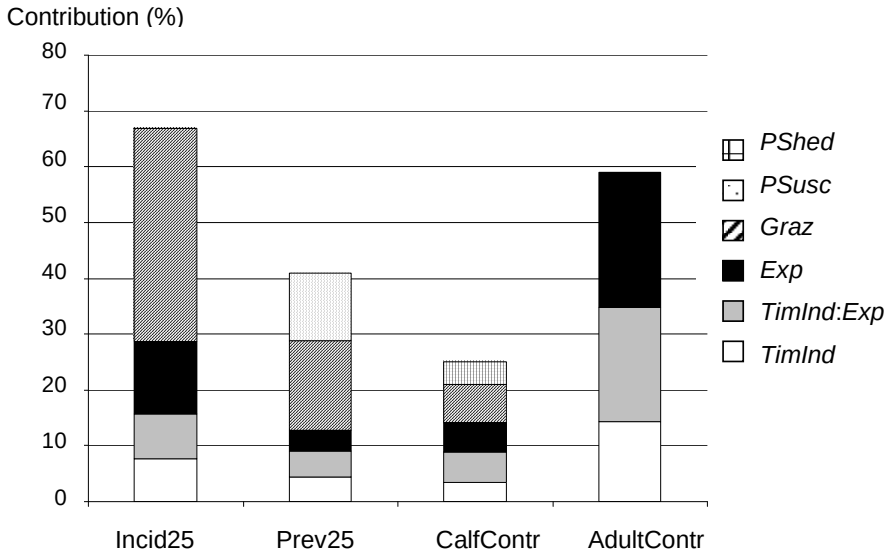

Figure 2: Contributions to the variance of the output (main and interaction effects) of six model input parameters of a *Mycobacterium avium* subsp. *paratuberculosis* transmission model in a dairy cattle herd: period of time during which a calf can shed the bacteria (*PShed*), period of time during which a calf is susceptible (*PSusc*), period during which grazing is allowed (*Graz*), rate of exposure of calf to fomites contaminated by adults (*Exp*), time spent in individual housing (*TimInd*), and herd size (*Size*). The parameter for herd size does not contribute to the variance of the outputs.[3]

[3] Incid25: mean annual incidence of newly infected animals; Prev25: mean prevalence of infectious adults; CalfContr: mean contribution of calf-to-calf transmission; AdultContr: mean contribution of adult-to-calf transmission. All outputs are calculated in the last year of the 25-year simulation in infected herds

1). This output did not appear to be sensitive and remained low whatever the values of the parameters tested (from 0 to 1% for all scenarios).

The parameters contributing the most to model output variance were those related to the exposure of calves to adults (*Exp*, in Table 2), the time spent by calves in individual pens (*TimInd*), and the interaction of these two parameters (Figure 2). The grazing period (*Graz*) also influenced adult-to-calf contact because calves <6 months of age always were kept indoors and therefore were less exposed when adults were grazing. The period of time during which an animal was susceptible to *Map* (*PSusc*) only contributed to variance in the mean prevalence in the 25th year after infection (Prev25). The period of time during which a calf could shed *Map* (*PShed*) only contributed to variance in the mean contribution of the calf-to-calf transmission in the 25th year after infection (CalfContr). For the range of values tested, herd size had no effect on model outputs.

3.2 Adult-to-calf indirect contacts

A reduction in the exposure of susceptible calves to any environment contaminated by adults led to a sharp decrease in *Map* prevalence and could lead to the fadeout of infection if exposure was decreased by >90% (Figure 3). When calves were not exposed to adults until weaning, the prevalence of infectious adults in the 25th year after *Map* introduction was lower (Figure 4B, *Hutch* or individual pen with *Exp* = 0) compared to when exposure was possible prior to weaning (Figure 4B, individual pen with *Exp* > 0).

3.3 Calf-to-calf contacts

The annual prevalence of infectious adults in the 25th year after *Map* introduction was lower when calves stayed longer in individual pens as long as calves were not exposed to adults before they moved into a group pen (Figures 4A, *Hutch* or individual pen with *Exp* = 0). However, even a moderate exposure of calves to adults before calves were moved into a group pen strongly reduced or corrected this effect (Figure 4A, individual pen with *Exp* = 0.2 and 0.5).

When calves were not exposed to adults until weaning, the duration of the time spent in an individual pen no longer influenced prevalence, indicating that preventing calf-to-calf contacts had no additional protective effect even when adult-to-calf contacts are prevented completely (Figure 4B, *Hutch* or individual pen with *Exp* = 0).

The longer the time period that calves spent in individual hutches (from 1 to 8 weeks), the lower was the mean annual prevalence of infectious adults during the last year of simulation (Figure 4A, *Hutch*). Housing calves in hutches added no significant decrease in prevalence compared to housing calves in individual pens when no exposure of calves to adults was possible (Figures 4A and B, *Hutch* & or individual pen with *Exp* = 0).

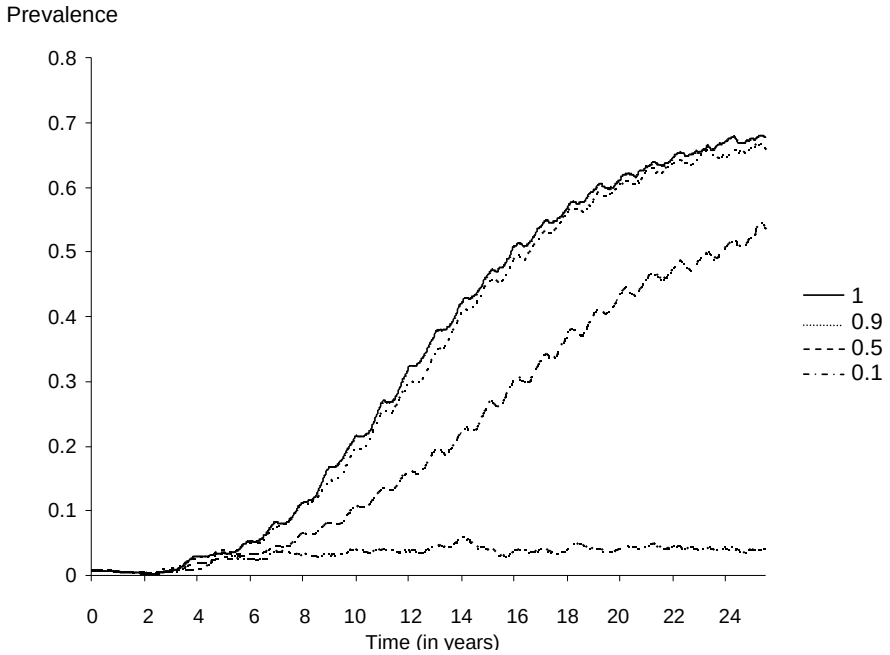

Figure 3: Mean prevalence over time of infectious adults (>30 months) according to the exposure (*Exp*) of calves until 1 year of age to the global environmental contamination with *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) by adults. Outputs of a *Map* transmission model in infected dairy herds of 110 cows. Other parameters are at their baseline value. The baseline scenario is shown as a solid line

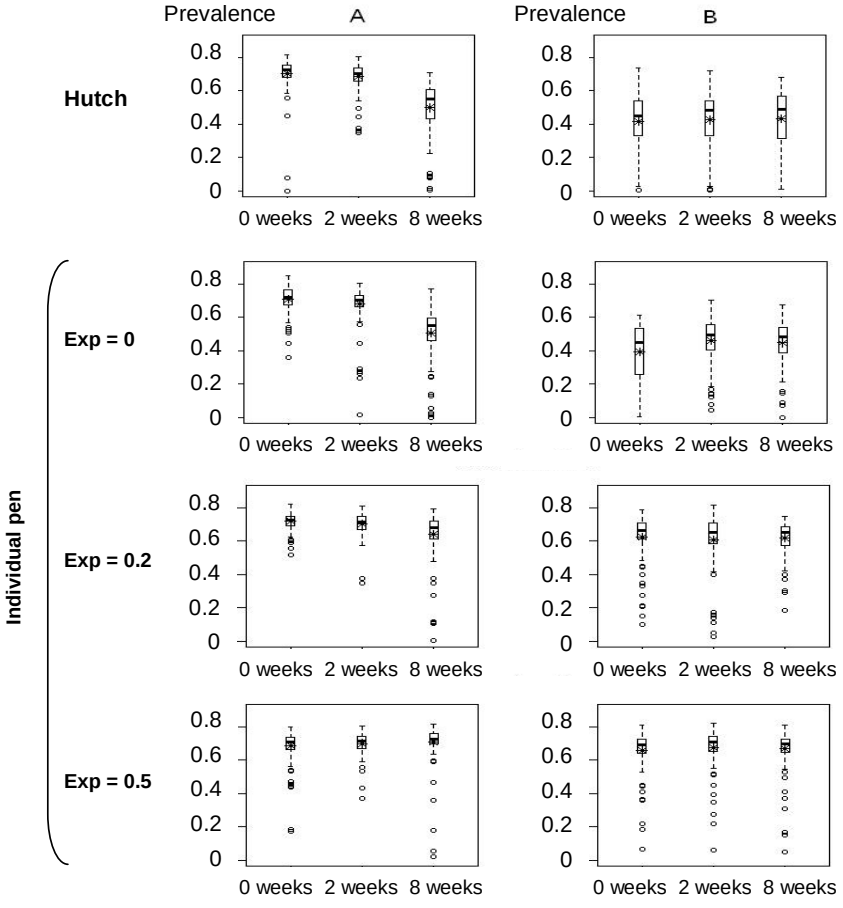

Figure 4: Box plots of the annual prevalence of infectious adults in the 25th year after *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) introduction according to the rate of exposure of calves to adults (*Exp*) and to the time spent by calves in individual pens (*TimInd*). Outputs of a *Map* transmission model in infected dairy herds of 110 cows. A: Adult to calf exposure is only reduced during the individual housing period (from birth to moving to a group pen); B: Adult to calf exposure is reduced from birth to weaning. The quartiles are represented by horizontal lines. The whiskers indicate maximum and minimum values of the simulated distributions that are less than 1.5 IQR lower or higher than the first or the third quartiles, respectively. Simulated values outside the ends of the whiskers are indicated by a dot. Mean value is represented by a star

3.3.1 Reduction of exposure by cleaning

As expected, increasing the percentage of bacteria removed from the farm environment every week led to a decrease in *Map* prevalence (Figure 5A). The lower the quantity of *Map* in the farm environment, the less infection occurred. However, even a cleaning leading to the 100% removal of *Map* every week (*Rembglob* = 100%) had less impact on prevalence than preventing the exposure of calves to fomites contaminated by adult faeces (via housing and management practices).

Increasing the percentage of *Map* removed from individual or group pens had no impact on *Map* prevalence (results not shown).

3.3.2 Reduction of exposure by eliminating clinically affected adults

Decreasing the mean time spent on the farm of clinically affected adults decreased the prevalence of infectious adults 25 years after *Map* introduction in the herd (Figure 5B).

With regards to all of the results presented in paragraphs 3.2 to 3.5, our findings were similar for all herd sizes.

4 Discussion

The mathematical model used here is novel because it allows us to explicitly specify the exposure of calves to contamination in the whole farm environment and in calf housing facilities. It therefore was possible to study the effect of different calf management (individual *versus* group pens) and herd management (hygiene, grazing, herd size) on *Map* transmission.

To develop this model, we integrated the most up-to-date knowledge to define the model compartments, the sojourn time in each compartment, the transmission routes, and the shedding of *Map* according to the infection status. Nevertheless, relevant experimental data to calibrate the transmission parameters linking the exposure in the environment (quantity of bacteria) and the probability of a new infection were scarce (Van Roermund *et al.*, 2007). While it is widely acknowledged that *Map* is indirectly transmitted through the ingestion of contaminated faeces, only a few field studies have quantified the risk of *Map* infection from environmental contamination (Benedictus et al., 2008; Windsor and Whittington, 2010). Furthermore, in these studies, the details of herd management and exposure often were unspecified or were incomplete. Therefore, we calibrated the transmission parameters of our baseline model by comparing model outputs and observed data on within-herd

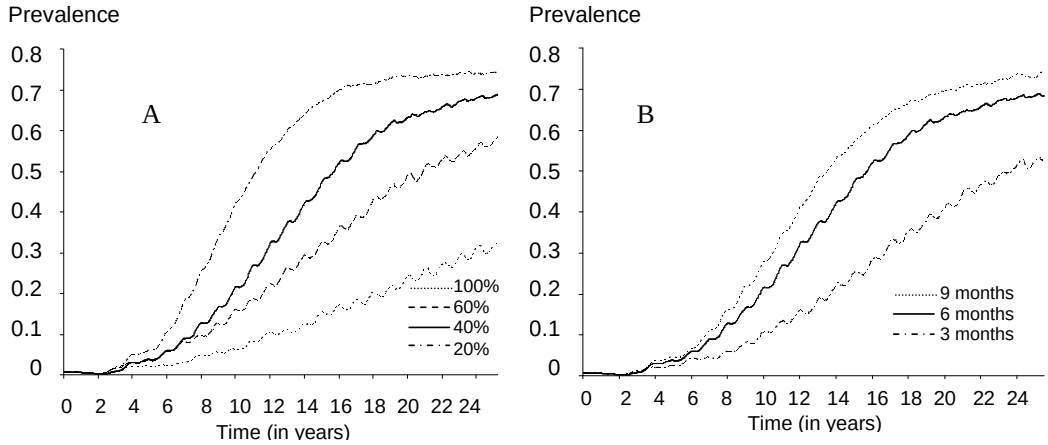

Figure 5: Mean prevalence over time of infectious adults (>30 months) according to A: the percentage of *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) removed by cleaning from the global environment every week (*RemBglob*), B: the mean time spent in the herd by clinically affected adults (*TIcCull*) Outputs of a *Map* transmission model in infected dairy herds of 110 cows. Other parameters are at their baseline value. The baseline scenario is shown as a solid line

prevalence of *Map* infection. In so doing, we assumed that the relative contribution of an infectious animal to the exposure of calves depended on the quantity of bacteria it sheds. We also assumed that all calves in the same housing were exposed in a similar fashion, according to the quantity of *Map* in the environment. Although these assumptions, chosen for parsimony, do not account for heterogeneity of exposure, we believe that they do not influence the major conclusions of this study.

Duration of susceptible state for calves is another parameter with major uncertainty. our assumptions of a long duration of this state (one year), and of an exponential decrease of susceptibility with age, might explain in part the absence of effect of contacts between calves in our results. Nevertheless, the sensitivity analysis explored a range of plausible values that were based on available knowledge, with a very low minimal duration of the susceptible state (13 weeks). The parameter was related neither to the incidence of *Map* infection when the herd was endemically infected (in year 25 after introduction), nor to the relative contribution of calves to *Map* transmission in the herd. We therefore believe that our main conclusions are robust with regards to this uncertainty.

Preventing the exposure of calves to adult faeces was essential for controlling *Map* transmission within a dairy herd. First, the prevalence of infectious cattle was sensitive to parameters involved in transmission via an environment contaminated by adults (rate of exposure of calves in individual pens to fomites contaminated by adults, time spent in individual housing, period during which grazing occurred). Second, the prevalence of infectious cattle decreased when the exposure of calves to adults was delayed. Third, it decreased when there was early detection and culling of clinically affected cattle. In contrast, calf-to-calf contact prior to weaning, assuming no exposure to adults, did not play a significant role in *Map* prevalence within the herd.

With the model simulations, it was possible to investigate a wide range of values describing reduction of calf exposure. Specific scenarios have been chosen here, all of which cannot be achieved by all farmers, particularly full prevention of calf exposure. It effectively can be difficult to strictly separate calves from adults (especially for economic or technical reasons linked to increased workloads or to the design of the barns), or to ensure that calves are not exposed to any environment contaminated by adults. Yet protecting calves from exposure to a contaminated environment appeared to be inefficient if the separation is imperfect. Furthermore, farmers may prefer to keep a calf and dam together for a longer period because they find this easier to manage, they wish to satisfy public concern regarding the practice of immediate separation of cow and calf in commercial milk production (Grondahl et al., 2007), or for the associated health advantages for the calf (Weary and Chua, 2000). A calf also might be raised with its dam to meet organic farming requirements. Our results reinforce the recommendation to strictly separate calves from dams in *Map* infected herds, a practice that often is not followed (Nielsen and Toft, 2010).

Group housing for preweaned calves was associated with *Map* infection in one field study (Wells and Wagner, 2000). For other pathogens, such as Bovine Viral Diarrhoea Virus (BVDV), the influence of the contact structure on virus persistence in the herd was shown, and strictly separating adults from calves was advised (Ezanno *et al.*, 2008). However, in the latter study, the rationale was to prevent the exposure of susceptible adults to infectious calves. The novel feature of our work was that calf housing facilities and different calf management strategies were modelled, and that we modelled both the local pen environment (contaminated by calves) and the whole farm environment (contaminated by all shedding animals). This approach increased our understanding of the respective roles of calf-to-calf and adult-to-calf contacts for *Map* transmission. The basic model structure of herd population dynamics and contacts also could be adapted to study within-herd transmission of other diseases, particularly those to which calves are susceptible.

Practical solutions for farmers to limiting calf exposure to *Map* could be off-site rearing (provided the location is kept biosecure from cow facilities), raising calves on a part of the farm segregated by strict biosecurity measures (as in poultry and pig farms), always moving from cleaner to less clean areas (as applied in the food industry, although this may depend on the pathogen, e.g. BVDV), or using individual calf hutches for 8 weeks in association with strict hygiene measures.

Early culling of clinically affected animals was another key point in reducing *Map* transmission. In practice, detecting such animals can be difficult or take a long time. The delay prior to detection can affect the impact of removing these animals from the herd. Furthermore, culling clinically affected animals has a cost and is not always done by farmers immediately, even when advised, especially when these cows have a high genetic merit, or when the herd milk production objectives are not reached.

The scenarios investigated in this study cover a wide range of calf housing and therefore apply to most major European dairy calf farming systems (Marcé et al, 2010b). Increasing herd size without changing calf contact structure led to the same conclusions. However, in large herds, calves can be raised in several smaller group pens instead of one group pen as modelled here. Nevertheless, because adult-to-calf indirect transmission was by far the most important transmission route, we expect that further modifying calf contact structures would lead to similar conclusions. Although we chose to model closed herds, given the major effect of adult-to-calf contacts found here, we assume that our results also could be applied to open herds to reduce calf exposure. Finally, we assumed that no specific control measure for *Map* was applied (except culling clinically affected adults within a few months). This actually is frequent in many infected herds. Moreover, even when control programmes based on test-and-cull in adult animals are implemented, our conclusions regarding the main effect of adult-to-calf contacts still apply. This is particularly because, despite testing, adult shedders are likely to remain in an infected herd, due to the poor sensitivity of diagnostic tests and to the delay

before a farmer decides to cull infected animals which are not clinically affected (Nielsen and Toft, 2010).

5 Conclusion

In persistently *Map* infected dairy herds, calf-to-calf transmission appeared to be a minor route of transmission, whereas exposure of calves to adults was pivotal. The longer this exposure was delayed, the more *Map* prevalence decreased. Early culling of clinically affected animals resulted in a decrease in the prevalence of infectious adults in persistently infected dairy herds. This conclusion held for each of the three herd sizes studied.

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References

Antognoli, M.C., Hirst, H.L., Garry, F.B., Salman, M.D., 2007. Immune response to and faecal shedding of *Mycobacterium avium* ssp. *paratuberculosis* in young dairy calves, and the association between test results in the calves and the infection status of their dams. Zoonoses Public Health 54, 152-159.

Benedictus, A., Mitchell, R.M., Linde-Widmann, M., Sweeney, R., Fyock, T., Schukken, Y.H., Whitlock, R.H., 2008. Transmission parameters of *Mycobacterium avium* subspecies *paratuberculosis* infections in a dairy herd going through a control program. Prev. Vet. Med. 83, 215-227.

Bolton, M.W., Grooms, D.L., Kaneene, J.B., 2005. Fecal shedding of *Mycobacterium avium* subsp. *paratuberculosis* in calves: implications for disease control and management. In, Proceedings of the 8th International Colloquium on Paratuberculosis, Copenhagen, Denmark, pp. 596-600

Chiodini, R.J., Van Kruiningen, H.J., Merkal, R.S., 1984. Ruminant paratuberculosis (Johne's disease): the current status and future prospects. Cornell Vet. 74, 218-262.

Ezanno, P., Fourichon, C., Beaudeau, F., Seegers, H., 2006. Between-herd movements of cattle as a tool for evaluating the risk of introducing infected animals. Anim. Res. 55, 189-208.

Ezanno, P., Fourichon, C., Seegers, H., 2008. Influence of herd structure and type of virus introduction on the spread of bovine viral diarrhoea virus (BVDV) on the spread of bovine viral diarrhoea virus (BVDV) within a dairy herd. Vet. Res. 39, 39.

Grondahl, A.M., Skancke, E.M., Mejdell, C.M., Jansen, J.H., 2007. Growth rate, health and welfare in a dairy herd with natural suckling until 6-8 weeks of age: a case report. Acta Vet. Scand. 49, 16.

Kennedy, D., Nielsen, D.D., 2007. Report from the first IDF ParaTB forum. Bull. Int. Dairy Fed. 410, 3-7.

Kennedy, D.J., Benedictus, G., 2001. Control of *Mycobacterium avium* subsp. *paratuberculosis* infection in agricultural species. Rev. sci. tech. Off. int. Epi. 20, 151-179.

Marcé, C., Ezanno, P., Seegers, H., Pfeiffer, D.U., Fourichon, C., 2010a. Modeling within-herd transmission of *Mycobacterium avium paratuberculosis* in dairy cattle: a review. J. Dairy Sci. 93, 4455-4470.

Marcé, C., Ezanno, P., Seegers, H., Pfeiffer, D.U., Fourichon, C., Submitted. Can we predict spontaneous fadeout or persistence of *Mycobacterium avium* subsp. *paratuberculosis* infection in a dairy herd: a modelling study. Vet. Res. Under Review.

Marcé, C., Guatteo, R., Bareille, N., Fourichon, C., 2010b. Dairy calf housing systems across Europe and risk for calf infectious diseases. Animal 4, 1588-1596.

Matthews, H.T., 1947. On Johne's disease. Vet. Rec. 59, 397-401.

Nielsen, S.S., 2008. Transitions in diagnostic tests used for detection of *Mycobacterium avium* subsp. *paratuberculosis* infections in cattle. Vet. Microbiol. 132, 274-282.

Nielsen, S.S., 2009. Programmes on Paratuberculosis in Europe. In, Proceedings of the 10th International Colloquium on Paratuberculosis, Minneapolis, Minnesota, USA, pp. 101-108.

Nielsen, S.S., Ersboll, A.K., 2006. Age at occurrence of *Mycobacterium avium* subspecies *paratuberculosis* in naturally infected dairy cows. J. Dairy Sci. 89, 4557-4566.

Nielsen, S.S., Toft, N., 2010. Management practices reducing the test-prevalence of paratuberculosis in Danish dairy herds. In: Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine, Nantes, France, pp. 189-200.

Rankin, J.D., 1961. The experimental infection of cattle with *Mycobacterium johnei*. III. Calves maintained in an infectious environment. J. Comp. Pathol. 71, 10-15.

Saltelli, A., Chan, K.A., Scott, E.M., 2000. Sensitivity analysis. John Wiley & Sons, LTD Chichester, England.

Streeter, R.N., Hoffsis, G.F., Bech-Nielsen, S., Shulaw, W.P., Rings, M., 1995. Isolation of *Mycobacterium paratuberculosis* from colostrum and milk of subclinically infected cows. Am. J. Vet. Res. 56, 1322-1324.

Taylor, T.K., Wilks, C.R., McQueen, D.S., 1981. Isolation of *Mycobacterium paratuberculosis* from the milk of a cow with Johne's disease. Vet. Rec. 109, 532-533.

Turner, J., Begon, M., Bowers, R.G., French, N.P., 2003. A model appropriate to the transmission of a human food-borne pathogen in a multigroup managed herd. Prev. Vet. Med. 57, 175-198.

Van Roermund, H.J.W., Bakker, D., Willemsen, P.T.J., De Jong, M.C.M., 2007. Horizontal transmission of *Mycobacterium avium* subsp. *paratuberculosis* in cattle in an experimental setting: Calves can transmit the infection to other calves. Vet. Microbiol. 122, 270-279.

Van Schaik, G., Schukken, Y.H., Nielen, M., Dijkhuizen, A.A., Barkema, H.W., Benedictus, G., 2002. Probability of and risk factors for introduction of infectious diseases into Dutch SPF dairy farms: a cohort study. Prev. Vet. Med. 54, 279-289.

Weary, D.M., Chua, B., 2000. Effects of early separation on the dairy cow and calf. 1. Separation at 6 h, 1 day and 4 days after birth. Appl. Anim. Behav. Sci. 69, 177-188.

Wells, S.J., Wagner, B.A., 2000. Herd-level risk factors for infection with mycobacterium paratuberculosis in US dairies and association between familiarity of the herd manager with the disease or prior diagnosis of the disease in that herd and use of preventive measures. J. Am. Vet. Med. Ass. 216, 1450-1457.

Whittington, R.J., Windsor, P.A., 2009. In utero infection of cattle with *Mycobacterium avium* subsp. *paratuberculosis*: A critical review and meta-analysis. Vet. J. 179, 60-69.

Windsor, P.A., Whittington, R.J., 2010. Evidence for age susceptibility of cattle to Johne's disease. Vet. J. 184, 37-44.

Chapter 5

Cost-effectiveness of control strategies for *Mycobacterium avium* subspecies *paratuberculosis* in dairy herds based on clinical *versus* active surveillance

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Abstract

Paratuberculosis is terminal disease of ruminants present all over the world that results in a decrease of milk production and of slaughter value. However, on dairy farms with low prevalence, producers do not necessarily see paratuberculosis as a problem although it can turn into an endemic infection of their herd. Control programmes result in slow and limited success. Although it is necessary to decide how to manage paratuberculosis, there is little information on the combined costs of controlling the disease. Limiting the persistence of *Mycobacterium avium* subsp. p*aratuberculosis* infection and preventing for reaching levels of infection that are difficult to control could be a good option. The objective of this study was to compare the cost-effectiveness of implementing in a dairy cattle farm a test-and-cull programme based on systematic testing of the herd or on clinical surveillance where screening is triggered by the occurrence of cases. A dynamic, stochastic bioeconomic model was developed, simulating an initially susceptible herd in which an infected heifer is introduced. Scenarios with an annual ELISA test performed on cows either systematically or based on clinical surveillance were simulated. The action following a positive test and the delay before action varied. Three different levels of hygiene were studied. Herds with paratuberculosis and with either low or high prevalence of other concomitant health disorders were investigated. The epidemiological and economic outputs were both considered. Depending on the targeted objective, there was an advantage to implement a systematic test-and-cull from the introduction of an infected heifer in a susceptible herd compared to a test-and-cull programme triggered by clinical surveillance, or to no control programme. There was no added value of delaying the culling of test-positive cows or culling their two last calves. It was always better to implement a test programme than doing nothing in infected herds from an epidemiological perspective and when looking at the long term perspectives. Return on investment could be expected from minimum 7 years after the start of the programme, depending on the hygiene level in the herd and the scenario studied.

***Keywords*:** *Mycobacterium avium* subspecies *paratuberculosis*, surveillance, control, bioeconomic dynamic model, cost-effectiveness

1 Introduction

Paratuberculosis is of economic importance for dairy producers around the world as it results in a decrease in milk production, reduction in slaughter value, and mortality or premature culling of sick cattle or their offspring in affected dairy herds (Benedictus, *et al.*, 1987; Johnson-Ifearulundu and Kaneene, 1997; Kudahl and Nielsen, 2009; Raizman, *et al.*, 2009). In herds with a low prevalence of *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) infection or with no clinically affected cows, producers usually do not see paratuberculosis as a problem for their herd although some think they have possibly experienced some economic impact of paratuberculosis (Sorge, *et al.*, 2010). There is no treatment available. In Canada, the majority of the farmers enrolled in a voluntary disease control programme because they were concerned that *Map* could be perceived by consumers as a cause for Crohn's disease in humans, which could lead to a decrease of milk consumption (Sorge, *et al.*, 2010). The need for the development of effective and economically viable control programmes against paratuberculosis is thus real.

For more than 20 years eradication and control programmes have been implemented in many countries. Up till now, these programmes have demonstrated only limited success (Sockett, 1996; Jubb and Galvin, 2004; Nielsen, 2009). It is indeed particularly difficult to clear the infection once a farm is infected notably when test-and-cull programmes only are implemented. Modelling studies reported that test-and-cull strategies are not effective in reducing significantly within-herd prevalence, at least under profitable conditions, but that hygienic calf rearing is critical (Groenendaal and Galligan, 1999; Groenendaal and Wolf, 2008; Kudahl, *et al.*, 2008). Another recent modelling study reported that culling only high shedders did not achieve to eradicate *Map* infection within 50 years, although the prevalence was still decreased (Lu, *et al.*, 2008). In practice, the control programmes implemented are rarely only based on test-and-cull strategies (Taisne, 2009; Nielsen and Toft, 2010). Various levels of success in terms of reduction of the prevalence in infected or affected animals have been reported (Jubb and Galvin, 2004; Wells, *et al.*, 2008; Ferrouillet, *et al.*, 2009; Lombard, *et al.*, 2009; Nielsen and Toft, 2010). However, it is suggested that measures associated to test-and-cull actions such as reduction of environmental contamination of heifers up to a year of age, and calf management especially could improve the success of the programmes.

Modelling studies showed that the introduction of a *Map* infected cattle in an initially susceptible herd could be followed either by persistent infection of the herd (state in which the number of animals infected with *Map* is positive over a relatively long term), or by spontaneous fadeout of the infection (prevalence of infected animals equal to zero at a time point) (Lu, *et al.*, 2010; Marcé, *et al.*, 2010). No early difference could be found between the simulations to predict their different evolution, but in the number of clinically affected animals present (simultaneously or successively) in the herd 5 years

after the introduction of a *Map* infected cattle. Indeed, from the moment when two or more clinically affected animals were present (simultaneously or successively), *Map* infection generally persisted over the 25 years of simulation (Marcé, *et al.*, 2010).

In this context, we wonder whether a systematic (early) action would limit the persistence of *Map* infection or maintain low within-herd prevalence at a reasonable cost. The present study is based on a previously developed bioeconomic model ECOMAM/ECOMAST (Seegers, *et al.*, 2004), in which the paratuberculosis components have been added. This model reflects both direct and indirect effects of paratuberculosis related to effects on herd dynamics and herd demographics. It allows evaluating the herd effects of various control strategies against *Map*. The objective of the study is to compare the effectiveness of implementing either a systematic test-and-cull programme or to trigger a test-and-cull programme based on clinical surveillance (start of the programme when the number of clinically affected animals is above or equal to 2), in order to limit the persistence of *Map* infection. Factors likely to influence the effectiveness and the cost of the programme especially with regard to culling are considered. Expected results can depend i/ on transmission: the comparison is thus performed in herds with standard hygiene level, in herds with improved hygiene (lower transmission of *Map*), and in herds with impaired hygiene (higher transmission of *Map*). Several delays between test results and culling are also simulated and the culling can affect different categories of animals; ii/ on the cost of culling which depends on the delay before culling and on the categories of animals culled. In the study, a positive test can result in different actions, such as culling the positive animal only, or culling the positive animal and its last calf; iii/ on the impact of culling on economic outputs notably when other health disorders are concomitant to paratuberculosis infection. A low prevalence of other health disorders is considered in a first step. A scenario with a high prevalence of other health disorders (also resulting in culling) is studied in a second time. The different scenarios are classified based on epidemiological and economic criteria such as persistence of *Map* infection, prevalence of infectious animals, and annual and cumulated gross margins over variable costs.

2 Materials and methods

2.1 General description of the simulation model ECOMAST/ECOMAM

ECOMAST/ECOMAM is a dairy herd-simulation model (simulating a dairy herd including young stock) that enables ex-ante economic assessment of disease control programmes. This simulator was initially built to study mastitis control strategies (Seegers, *et al.*, 2004) and has been further developed to study the spread of paratuberculosis and consecutive production

losses in a closed dairy herd. This individual based model is dynamic, mechanistic and stochastic with a time step of one day.

Each cow and heifer is characterized by age, reproductive status (oestrus, insemination, gestation), parity, days in milk, genetic merit for milk yield, milk yield, fat and protein contents, probability of culling, health status for paratuberculosis and for other simulated diseases such as udder disorders (mastitis, somatic cell counts), reproductive disorders, and ongoing treatments. Herd-level state variables are defined to accumulate the individual simulated events and performances.

ECOMAST/ECOMAM drives reproduction and lactation of the animals in the herd and mimics practices and decision rules of the farmer regarding reproduction, feeding, culling and replacement, and quota management. Daily milk, protein and fat yield are determined by the genetic potential of the cows, their lactation number and lactation stage and can be limited by the feeding plan. The effect of mastitis and other health disorders is then subtracted to calculate the actual yield. More details on the reproductive, milk production and mastitis control strategies can be found in previous publications (Hortet, *et al.*, 1997; Hortet, 1999; Seegers, *et al.*, 2000). Demography and management of a typical French dairy herd is depicted in the model. While events are simulated at the cow level, management decisions are defined at the herd level.

A context with milk quota is simulated. Each month, the annual expected production of the herd during a yearly quota campaign is calculated based on the expected milk yield of lactating cows, pregnant cows and heifers from the beginning of the annual production campaign to its end. The difference between the expected production and the quota available is then calculated. No specific decision regarding quota management is implemented if the difference is small (below a threshold defined by the user). If an over-production is anticipated, earlier drying off, culling or sales of cows or heifers are decided. On the opposite, if an under-production is expected, culling or sales are lowered and no purchases are allowed as we work here on closed dairy herds.

Culling is mainly based on production and reproductive performance of the cows but also takes into account the individual somatic cell count and health status such as paratuberculosis. It is triggered by herd size limits, cumulated milk sales within the quota campaign compared to the milk quota, and forecasted calving and drying-off events. Such decisions occur once a month. In addition, death and involuntary culling randomly occur. Heifers born from the 10% cows with the highest or lowest production level are systematically kept or sold, respectively. Then, a sufficient number of heifers are kept to meet the required replacement rate. All male calves are sold.

2.2 Paratuberculosis simulation process

2.2.1 Disease progression

Each animal has one of the five infection states modelled towards paratuberculosis: susceptible, resistant (if not infected at one year of age), latently infected (not shedding), subclinically infected (shedding but no clinical signs) and clinically affected (shedding).

The risk of getting infected during the first year of life is based on the results of an epidemiological model describing the transmission of *Map* within a dairy herd (Marcé, *et al.*, 2010). The prevalences of infectious subclinically infected adults (prevIs), of infectious clinically affected adults (prevIc), and the incidence of the infection in calves below one year of age are followed over time in this epidemiological model and saved. Five routes of transmission are considered: *in utero* transmission, transmission via colostrum or milk ingestion, and transmission via ingestion of faeces of calves or faeces of adults. A metamodel has been built, aiming at explaining the incidence (horizontal transmission only) of *Map* infection in animals below one year of age by prevIs and prevIc a time t (Figure 1) Three separate models are estimated for 3 age categories: 0 to 4 months, 4 to 8 months, and 8 to 12 months in order to take into account the decrease of susceptibility with age. As a result of the metamodels, the following equations calculated from the epidemiological model are used as inputs in the economic model for herds with standard hygiene:

$Incidence_{[0\text{-}4\ months]}(t) = 2.983\ prevIs(t) + 12.007\ prevIc(t)$ ($r^2 = 0.98$)

$Incidence_{[4\text{-}8\ months]}(t) = 1.926\ prevIs(t) - 4.632\ prevIc(t)$ ($r^2 = 0.58$)

$Incidence_{[8\text{-}12\ months]}(t) = -0.120\ prevIs(t) + 1.860\ prevIc(t)$ ($r^2 = 0.36$)

For herds with improved hygiene, calculated incidence is divided by 2. For herds with impaired hygiene, calculated incidence is multiplied by 2.

In the economic model, every 4 weeks, the true infection state of each animal below one year of age is updated depending on the prevalences prevIs and prevIc at the same time.

Infection can also occur *in utero*. The probabilities for subclinically infected cows and clinically affected cows to give birth to an infected calf are 0.149 and 0.65, respectively (Benedictus, *et al.*, 2008; Whittington and Windsor, 2009).

The time spent in each infection compartment is described in Table I.

In the model, both the true infection status and the diagnosed status (results of tests) of each animal are known.

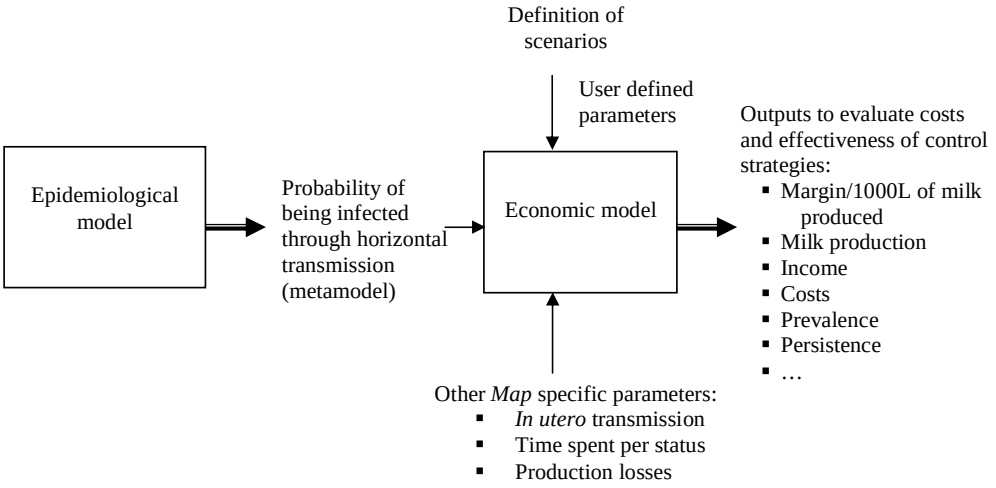

Figure 1: Link between the epidemiological and the economic model for Mycobacterium avium paratuberculosis *- Concept of the metamodel*

Table I: Duration in the Mycobacterium avium *subsp.* paratuberculosis *infection status in the dairy herd economic model*

Infection status	Mean time spent (days)	Standard deviation (days)	Sources
Latent	539	96	(Nielsen and Ersboll, 2006; Van Roermund, *et al.*, 2007; Nielsen, 2008)
Is	728	192	(Matthews, 1947)
Ic	182	28	Variable (Expert opinion on time before culling)

2.2.2 Production effects of paratuberculosis

Estimates of reduced milk yield associated with *Map* infection are abundant (Johnson, *et al.*, 2001; Kudahl, *et al.*, 2004; Hendrick, *et al.*, 2005b; Nielsen, *et al.*, 2006; Beaudeau, *et al.*, 2007; Gonda, *et al.*, 2007; Raizman, *et al.*, 2007; Nielsen, *et al.*, 2009; Raizman, *et al.*, 2009; Smith, *et al.*, 2009; Aly, *et al.*, 2010). The decrease of mean milk yield is estimated between 500 and 1400 kg per cow in the lactation when the infection is detected and between 400 and 800 kg for the lactation preceding the status determination. A decrease is sometimes noticed as early as the first lactation (Kudahl, *et al.*, 2004; Nielsen, *et al.*, 2006). Conversely, there are only few studies analysing the effect of *Map* infection on fertility. They describe effects on different criteria (calving-to-calving or calving-to-conception intervals, calving rate) and their results are contradictory (Abbas, *et al.*, 1983; McNab, *et al.*, 1991; Johnson-Ifearulundu, *et al.*, 2000; Haddad, *et al.*, 2003; Lombard, *et al.*, 2005; Kostoulas, *et al.*, 2006; Gonda, *et al.*, 2007; Raizman, *et al.*, 2007; Marcé, *et al.*, 2009). Slaughter value has also been found to be reduced mainly among clinically affected animals, or shedding animals (Benedictus, *et al.*, 1987; Hutchinson, 1996; Kudahl and Nielsen, 2009).

In ECOMAM/ECOMAST, infected animals have a decrease in milk production, depending on their infection status. Similarly a loss of slaughter value is modelled. No impact of *Map* infection on fertility is considered. Parameters for production losses are described in Table II.

2.2.3 Quality of Map diagnostic tests

The default diagnostic test chosen is a serum ELISA which sensitivity values are described in Table III. These values of commercial kits sensitivity are in accordance with available literature (Nielsen and Toft, 2007) and are here considered credible for our study in which only adults are tested. Infection status influences test-quality. A specificity of 1 is applied as we work in infected herds.

2.3 Herd and initial conditions

We here study herds with a milk quota of 750,000 litres (annual delivery) composed of around 100 cows plus additional young stock. The initial herd is calibrated by simulation to define every state variable for each animal.

The initial herd designed for the simulation presents good reproductive performance and a good level of control for udder health in a first step (Table IV, herd with low prevalence of concomitant health disorders). In a second step, occurrences of mastitis and reproduction disorders are increased (Table IV, herd with high prevalence of concomitant health disorders). These health disorders are indeed frequent in a large number of herds. They influence both

Table II: Production losses associated with Mycobacterium avium *subsp.* paratuberculosis (Map) *infection status: parameters for the dairy herd economic model*

Affected characteristic		Infection state		
		Latent	Subclinically infected	Clinically affected
Milk production[*] (reduction in %)	Min	0	5	15
	Max	6	15	35
	Mode	2	8	20
Slaughter value[#] (reduction in %)	Mean	3	25	41
	Standard deviation	1.5	2.5	5

[*]: beta distribution; [#]: normal distribution

Table III: Sensitivity of serum ELISA in cows in the modelled infection status for Mycobacterium avium *subsp.* paratuberculosis (Map): *default values for the dairy herd* from the introduction of an infected heifer *economic model*

Test-quality	Infection state			
	Latent	Subclinically infectious	Clinically affected	Resistant
Sensitivity	0.1	0.5	1[*]	NA

[*] Test sensitivity is considered to be equal to 1 for clinically affected animals since it is assumed that when a clinical suspicion results in a negative test result, then a second test (Ziehl Neelsen faecal staining) is performed. This second test is used for 15% of the clinically affected animals. Clinical suspicions that occur outside the screening period are all to be confirmed with a Ziehl Neelsen faecal staining. NA: not applicable.

Table IV: Description of the production and reproduction characteristics of the herds studied in the economic model

Production and reproduction herd characteristics	Herds with low prevalence of concomitant health disorders		Herds with high prevalence of concomitant health disorders	
	Mean	Standard Deviation	Mean	Standard Deviation
Days to conception (days)	81	28	104	40
Expected mature milk production (Kg/305-day/cow)	9,000	350	10,800	158
Fat (g/L)	38.3	3.3	45.3	1.8
Protein (g/L)	29.1	2.1	34.5	0.5
Incidence of detected clinical mastitis	13.9	4.8	76.1	27.4
Bulk Milk Somatic Cell Count	94	15.2	421.9	129.1
Culling rate (%)	37.2	7.9	31.7	6.0
Parity	2.7		2.6	

culling decisions and herd production. Such disorders can thus interact with culling rules for paratuberculosis and their consequences. The main characteristics of the initial herd in terms of reproduction, milk production, and culling are described in Table IV.

There is no option to buy or lease extra quota. Also, the closed dairy herd modelled is fairly common (Ezanno, *et al.*, 2006). Calving occurs between July and October for the heifers and between August and December for the cows.

An infected heifer, already in the subclinically infected status since 100 days is introduced once. This heifer is expected to calve one month after the introduction in the herd. Its expected 305-day mature milk production is 9400kg.

A 15-year simulation horizon is studied and 100 replications are run for each scenario.

2.4 Simulated control plans and utility criterion

2.4.1 Studied scenarios

A control plan is characterised by one or more actions, a definition of cows to be acted upon, a time or combination of circumstances at which the actions are to be carried out, and the events consecutive to a positive test. Actions are here categorized into a test-and-cull strategy. While the same test scheme is selected, the moment of its implementation differs as well as the consequences linked to a positive result.

The implementation of the test-and-cull strategy begins either as soon as a positive animal is introduced (Scenario T1) or based on results of clinical surveillance (Scenario T2). If the clinical surveillance is chosen, the test-and-cull strategy implementation occurs one month after the second clinically affected animal is detected in the herd. For scenarios T1 and T2, the default mean time of 6 months spent by clinically affected animals on the premises (Table I) is reduced to 2 months from the third clinical case as culling gets quicker usually when farmers become aware of the disease in their farm. A baseline scenario does not consider any test-and-cull strategy (Scenario T0). In fact, we chose to select a specific optimal situation for this study: systematic test-and-cull is indeed implemented from the day an infected animal is introduced. In reality, the tests have usually been implemented since a certain period of time (possibly years) before introducing an infected animal.

The test scheme selected is the following: annual testing in September (van Schaik, *et al.*, 2009) of all cows (parity 1 and more) with an ELISA test which sensitivity depends on the infection status (Table III).

After the annual test of the herd, a positive test result can end either in the 'immediate' or delayed (4 months) culling of 1/ the test-positive cow only (C1) or 2/ the test-positive cow and its two last calves if kept in herd (C2). Depending on the age of such female offspring, its culling occurs more or less rapidly to account for adaptation of the farmers' decision to the market (if the tests occur when the calf is below 2 months, the culling of the calf occurs at 150 days of age, otherwise, it occurs between 20 and 22 months of age). The 'immediate' culling occurs two weeks after the test (D1). One week is indeed needed to get the result and the farmer has one week to decide and organise the departure of the animal from the farm (expert opinion). A delay of 4 months (D2) has been chosen for a comparison as it is reported to be the mean time recommended for the elimination of an ELISA positive asymptomatic infected animal in France (Coursaget, 2009). Culling of heavy faecal shedding cows is also reported to occur at a median time of 4 months after sampling in a study based on American herds following a control programme (Ferrouillet, *et al.*, 2009). The different types of culling following a positive test have been chosen in order to take into account the fact that animals can shed *Map* before being detected and that calves are mainly susceptible at birth when they are in the same area as their dam.

Finally, the scenarios are implemented in herds with different levels of hygiene resulting in different levels of *Map* transmission: standard, low and high.

All the scenarios are gathered in Table V.

2.4.2 Outputs of the model

Both epidemiological criteria and economic criteria are considered in this study. Indeed, focusing on these criteria separately may not always lead to the optimal interpretation notably if there is a trade-off between economic attractiveness and epidemiological effectiveness.

Regarding the epidemiological criteria, the mean prevalence of infectious adults (Is+Ic) and the persistence of the infection are here studied. Mean values are based on the 100 iterations independently of the persistence of the infection (unless specified it is a mean calculated in infected herds only).

To assess the direct economic impact of paratuberculosis, costs (i.e. extra-resources used) and losses (i.e. reduced revenues) have to be quantified and aggregated. Control costs linked to *Map* infection correspond mainly to test costs, preventive measures (especially hygiene). Losses correspond to the economic impact of the reduced milk production of cows (which differs depending on whether the quota is reached or not), the impact of mortality or

Table V: Description of the scenarios of surveillance and control of Mycobacterium avium *subsp.* paratuberculosis *implemented in a dairy herd economic model*

Surveillance	Action in case of a positive test		Level of hygiene and scenario number		
	Animals culled	Delay before culling	Standard	Impaired	Improved
None (T0)	NA	NA	1	2	3
Active (systematic animal testing) (T1)	C1	D1	4	5	6
	C1	D2	7	8	9
	C2	D1	10	11	12
	C2	D2	13	14	15
Clinical (systematic annual testing triggered after 2 clinical cases) (T2)	C1	D1	16	17	18
	C1	D2	19	20	21
	C2	D1	22	23	24
	C2	D2	25	26	27

C1: culling of test-positive cows, C2: culling of test-positive cows and their 2 last calves, D1: culling 2 weeks after the test, D2: culling 4 months after the test, NA: not applicable.

additional culling, and the resulting decrease of the genetic progress. To allow a comparison of decisions for health management, we use a marginal approach, based on the comparison of the output/input ratio; the better control programme being the one that has larger losses avoided compared to the additional costs. Control measures studied here neither call the general structure of the farm into question nor the feeding system, the milk quota production, or the management options of the herd. It is thus assumed that fixed costs are not significantly modified. In ECOMAST/ECOMAM, economic results are expressed by calculation of a mean discounted gross margin produced for each simulated year, as well as a cumulated discounted gross margin. These two outputs are presented either in euros (€) or in € for 1000 litres of milk produced. In situations where milk quota is the main production constraint, these outputs are relevant measures of financial performance because of the extra costs of producing more or less than the milk quota (Kristensen, *et al.*, 2008). The gross margin is calculated as follows: Gross Margin = Total revenues – Total variable costs (excepted roughage production costs).

Revenues are composed of the profits due to sales of milk, calves, heifers, and culled cows. Variable costs are related to: i) feeding (concentrates, milk powder), ii) mastitis control, iii) reproduction (artificial inseminations, treatments associated with reproductive disorders), iv) other health disorders control, and v) diverse variable costs (bedding materials, dairy herd improvement association fees, …).

An annual discount rate (r) of 3% is applied on gross margins. A set of typical 2009-2010 French prices and costs are used in the calculations (Table VI). Total costs of the tests and losses of milk are also calculated.

In the figures, relative discounted (annual or cumulated) gross margins are presented. The discounted (annual or cumulated) gross margins of a herd with no paratuberculosis are calculated each year and are set as a baseline value of 100. Gross margins of all scenarios with paratuberculosis (whatever the control programme) are compared to these baseline values and transformed in the scale 0-100.

3 Results

3.1 Herd dynamics, production and *Map* prevalence when no tests for *Map* are implemented

After 15 years of simulation, a decrease of the mean number of cows kept to consistently achieve the milk quota was observed in herds with no paratuberculosis (Table VII). This decrease in the number of adults was related to the simulated genetic improvement in the herd. Replacement cows

Table VI: Prices and costs used for production and for Mycobacterium avium *subsp.* paratuberculosis *control* in a *dairy herd economic model*

Element	Value	Unit
Milk[*]	Min: 252.97 Max: 296.55 Mean: 279.52	€/1000L
Cow sale	Random culling: 600 Sale for breeding:1200 Other culling: 860	€
Slaughter culled heifers	Not pregnant: 800	€
Pregnant heifers	< 6 months: 1050 > 6 months: 1350	€
Males calves	150	€
Female calves	8 days: 120 < 15 days: 120 15-184 days: 350 185-365 days: 650	€
Dead cow	0	€
Paratuberculosis annual test (veterinary cost, laboratory cost)	11.5	€/test
Test used to confirm a clinical suspicion	15	€/animal
Increase in genetic merit for milk yield	Mean: 250; 0; 0.2 Standard deviation: 100; 0.2; 0.1	kg milk; g/l fat; g/l protein
Rearing costs		€
Male calf (8 days)	20 (alive), 10 (dead)	
Female calf (8 days)	25	
Heifer below 1 year	23	
Heifer between 1 and 2 years	30	
Heifer above 2 years	40	
Cow	65	
Artificial insemination forfeiting	40	
Feed costs	221.6	€/tonne
Milk powder	0.26	€/kg
Veterinarian costs intra-mammary infection costs excluded		€
Cow	12	
Random culling	50	
Random death	55	
Infertility treatment cost	25	
Intra-mammary infection costs		
Annual mastitis treatment cost[#]	80	€/cow/year
Annual mastitis prevention cost	23	€/cow/year
Systematic treatment at drying	12.5	€/treatment
Annual increase in prices	1	%

[*]: milk price varies according to season, [#]: other mastitis treatment costs depend on the level of production of the cow.

Table VII: Prevalence of Mycobacterium avium *subsp.* paratuberculosis *(*Map*), quota achievement, and herd characteristics: outputs of an economic model in year 15 after the introduction of one subclinically infected heifer when no tests for* Map *are implemented (mean values of the 100 iterations)*

Health characteristics of the herds	Output in year 15	Not *Map* infected herds	*Map* infected herds		
			Standard hygiene	Impaired hygiene	Improved hygiene
Herd with low prevalence of concomitant health disorders	Prevalence of infectious adults	0	0.59	0.86	0.10
	Quota achievement (%)	101.2	96.2	86.2	100.7
	Herd size (mean number of adults)	94.1	95.9	90.7	94.8
	Replacement rate	37.7	39.5	42.3	38.7
Herd with high prevalence of concomitant health disorders	Prevalence of infectious adults	0	0.54	0.86	0.12
	Quota achievement (%)	97.3	88.0	64.6	99.7
	Herd size (mean number of adults)	99.4	94.1	72.2	96.9
	Replacement rate	32.0	34.6	40.4	37.3

were indeed producing more milk over time and thus fewer cows were needed to reach the same milk production. An average replacement rate of 37.7% was observed in such herds.

In herds with low prevalence of other health disorders, at a similar level of mean prevalence of infectious adults after 15 years following the introduction of one subclinically infected heifer in standard herds, the mean number of adults stayed above the one of herds without paratuberculosis. However the milk quota was still close to be achieved (Table VII). In herds with impaired hygiene, culling due to paratuberculosis led to a decrease of herd size and the milk quota was frequently not reached. The situation in a herd with improved hygiene was close to the one of a herd without paratuberculosis. In these herds (improved hygiene), after 15 years of simulation, the mean prevalence of infectious adults reached 10%. The higher *Map* prevalence was in a herd, the higher the replacement rate was.

In herds with a high prevalence of reproduction and mastitis disorders and no paratuberculosis, a higher number of adults was needed to fulfil the milk quota. After 15 years of simulation, average quota fulfilment only reached 97% and the replacement rate was lower than in a herd with limited health disorders. In herds with paratuberculosis on top of reproduction and mastitis disorders, while a similar mean prevalence of infectious adults after 15 years was reached, the milk quota fulfilment, the mean number of adults, and the replacement rates were lower, whatever the level of hygiene compared to similar herds with no health disorders but paratuberculosis. In herds with improved hygiene, the differences were smaller.

The evolution of the prevalence of infected animals (L+Is+Ic), infectious (Is+Ic) and affected adults (Ic) of a standard herd with no other health disorder than paratuberculosis and in which no control measure is implemented is given in Figure 2.

3.2 Impact of systematic detection

Systematic test-and-cull reduced dramatically both the persistence of *Map* (% of infected herds) and the within-herd prevalence (Figure 3). By contrast, clinical surveillance resulted in a drop in prevalence but in no or little reduction in persistence. In herds with impaired hygiene, persistence and prevalence were higher, with little change in the differences between the scenarios. In herds with improved hygiene, persistence was even more reduced with systematic surveillance, and prevalence was low, whatever the scenario.

The difference between scenarios was all the more important that the control strategy (no test, systematic test implementation, or test implementation based on clinical surveillance) was implemented for a long period of time.

Cumulated discounted gross margin decreased compared to herds with no paratuberculosis, whatever the scenario implemented. In herds with standard

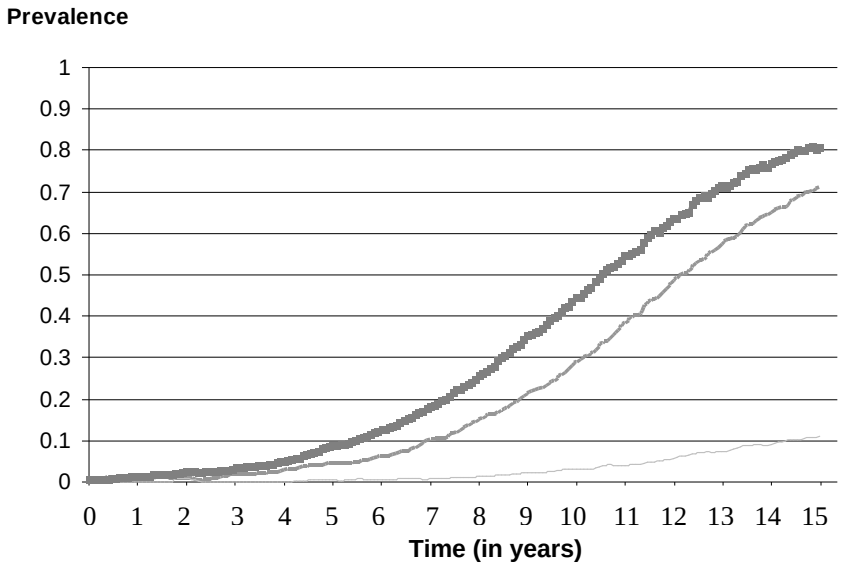

Figure 2: Evolution of the prevalence of infected animals (thick line), infectious adults (medium line) and clinically affected adults (thin line) over time in herds infected by Mycobacterium avium *subsp.* paratuberculosis *(*Map*): output of the dairy herd economic model when no tests for* Map *are implemented (standard hygiene, low prevalence of concomitant health disorders)*

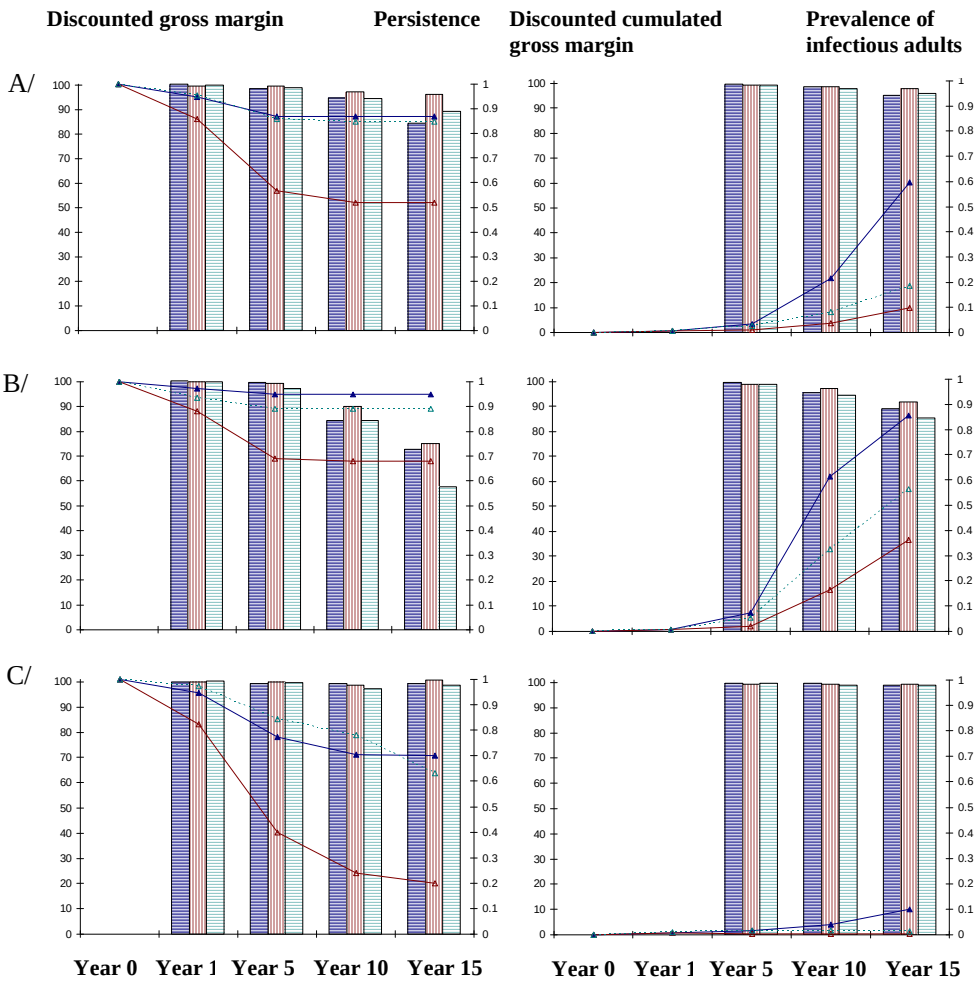

Figure 3: Persistence of Mycobacterium avium *subsp.* paratuberculosis (Map) *infection in the herds (left column) (lines), prevalence of infectious adults (right column) (lines), and evolution of the relative discounted gross margin per year (left) and cumulated (right) (compared to a herd with no paratuberculosis = baseline of 100) (histograms) in all herds: outputs over time of the dairy herds economic model for scenarios of* Map *control (T0: no control, T1: test-and-cull based on systematic surveillance, T2: test-and-cull triggered by clinical surveillance) with A/ standard level of hygiene, B/ impaired level of hygiene, C/ improved level of hygiene*

Surveillance T0 (scenarios 1 to 3) *Surveillance T1 (scenarios 4 to 6)* *Surveillance T2 (scenarios 16 to 18)*

hygiene, when systematic surveillance was implemented, the gross margin was sometimes slightly below at the beginning, but always ranked first of the different surveillance implemented after 15 years. Implementing a test-and-cull based on clinical surveillance appeared better than doing nothing in year 15 only. After 15 years, the relative mean gross margin produced decreased in infected herds from 1 to 15% depending on the level of hygiene. Differences between scenarios were small when hygiene was improved.

Implementing systematic test-and-cull was more efficient than clinical surveillance or doing nothing after 7 to 13 years (Figure 4). Return on investment (ROI) of systematic surveillance was higher and earlier when hygiene was impaired (higher risk of *Map* transmission). The differences were small when hygiene was improved.

Direct losses due to *Map* and components of the gross margin in year 15 are displayed in Table VIII. The number of cows culled for paratuberculosis was lower in herds with systematic testing. Milk and slaughter value losses were dramatically reduced with both surveillance scenarios compared to no control. Decrease in losses was higher in herds with systematic test and cull implementation, whatever the level of hygiene of the herd. As a consequence of systematic testing, most of the cows culled for paratuberculosis were test-positive cows and not clinically affected animals. In year 15, test costs were similar in scenarios T1 and T2 (slightly lower for T2) and low compared to differences in losses. The costs were lower in herds with impaired hygiene. When no control was implemented, the number of clinical culling was high. Such cullings were lower when systematic surveillance or clinical surveillance was implemented. The differences in production losses and test costs did not add directly into a difference of gross margin especially because milk losses are partly compensated by herd management rules to reach the quota.

Impact of the action following a positive test or the detection of clinically affected cattle

When the last two calves of a test-positive animal or a clinically affected animal were culled instead of culling only the positive animal, the impact on both the prevalence and the persistence was almost null (Figure 5). Both criteria were slightly higher whatever the level of hygiene.

Mean discounted gross margins (annual or cumulated) became lower over time than for similar scenarios in which only test-positive animals and clinically affected animals were culled. Differences between scenarios were smaller if hygiene is improved (results not shown). From year 10, the difference of gross margin reported is against the option ‘culling test-positive animal and the last two calves’, whatever the surveillance implemented.

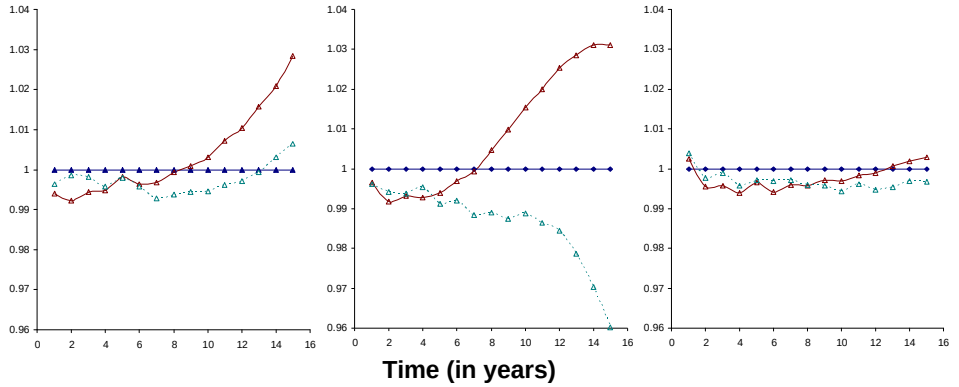

Figure 4: Ratio of the cumulated discounted gross margins in all herds of two surveillance scenarios compared to no control of Mycobacterium avium *subsp.* paratuberculosis *(*Map*): outputs of a dairy herd economic model simulated with standard (left), impaired (middle), or improved level of hygiene (right)*

T0: no control, T1: test-and-cull based on systematic surveillance, T2: test-and-cull triggered by clinical surveillance

Scenario T0 (no control); Scenario T1 (test-and-based on systematic surveillance); Scenario T2 (test-and-cull triggered by clinical surveillance)

Table VIII: Direct losses, control costs and economic results with 3 different control strategies against Mycobacterium avium *subsp.* paratuberculosis (Map) *infection (T0: no control, T1: test-and-cull based on systematic surveillance, T2: test-and-cull triggered by clinical surveillance): outputs of a dairy herd economic model in year 15 after the introduction of an infected cow in a susceptible herd with standard level of hygiene, impaired level of hygiene, or improved level of hygiene (mean values of 100 iterations)*

Output variable	Standard hygiene			Impaired hygiene			Improved hygiene		
	T0	T1	T2	T0	T1	T2	T0	T1	T2
Milk losses (€)	56,900	7,950	14,130	78,300	20,500	27,100	9,164	363	1,146
Slaughter value losses (€)	7,910	2,120	4,080	11,800	5,820	7,800	1,270	100	370
Test costs (€)	0	1,040	930	0	920	650	0	1,030	900
Total number of animals culled for paratuberculosis	16.2	6.8	13.2	26.2	19	25.1	1.9	0.2	1
Total number of Ic animals culled for paratuberculosis	16.2	1.2	3.2	26.2	5.2	7.1	1.9	0.1	0.3
Total variable costs (€)	63,000	63,500	62,090	58,300	51,800	41,300	63,700	64,500	65,000
Total revenues (€)	277,000	307,000	288,000	242,000	241,000	187,000	315,000	320,000	315,000
Gross margin (€)	214,000	244,000	226,000	184,000	190,000	146,000	251,000	255,000	250,000
Discounted gross margin (€)	141,000	161,000	149,000	121,000	125,000	96,400	166,000	169,000	165,000
Discounted gross margin (€/1000L)	179	204	195	165	189	179	208	212	209
Standard deviation of the gross margin (€/1000L)	26.6	18.5	17.4	19.5	28.2	23.3	15.7	12.6	13.4
Standard deviation of the prevalence of infectious adults	0.28	0.12	0.13	0.21	0.27	0.22	0.11	0.01	0.02
Quota achievement (%)	96.2	99.4	96.0	86.2	78.7	60.3	101.3	101.6	101.0

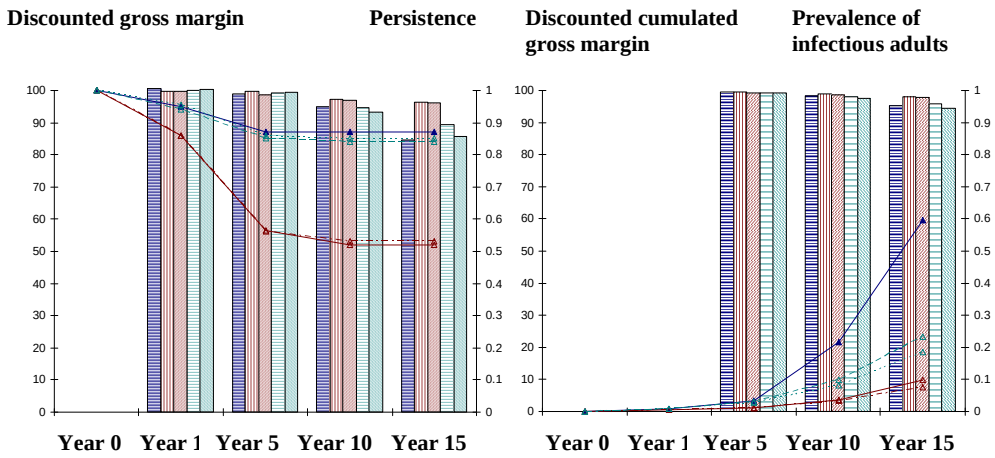

Figure 5: Effect of the selection of culled animals. Persistence of Mycobacterium avium *subsp.* paratuberculosis (Map) *infection in the herds (left column) (lines), prevalence of infectious adults (right column) (lines), and evolution of the relative discounted gross margin per year (left) and cumulated (right) (compared to a herd with no paratuberculosis which has a baseline gross margin of 100) (histograms) in all herds: outputs over time of the dairy herds economic model for scenarios in which the action performed following a positive test varies (C1: culling of test-positive cows, C2: culling of test-positive cows and their 2 last calves) within a structured dairy herd with standard level of hygiene*

T0: no control, T1: test-and-cull based on systematic surveillance, T2: test-and-cull triggered by clinical surveillance

Scenario 1 (T0); Scenario 4 (T1 C1); Scenario 10 (T1 C2); Scenario 16 (T2 C1); Scenario 22 (T2C2)

3.3 Impact of time spent before culling a test-positive animal

Increasing the delay before culling (from 2 weeks to 4 months) limited the decrease of both the prevalence and the persistence when test-and-cull programmes are implemented compared to doing nothing (Figure 6). This was observed from year 5, whatever the level of hygiene (results not shown). The higher the level of hygiene was, the smaller the differences. Even when delaying the decision of culling test-positive cattle, implementing a test-and cull programme (whatever the surveillance implemented) was always better than doing nothing in terms of prevalence. In terms of persistence, the same conclusion applies, except for herds with improved hygiene. In these last herds, the persistence was similar or even higher than the one for the scenario with no control programme.

Mean discounted gross margins (annual or cumulated) became lower over time than for similar scenarios in which culling occurred after 2 weeks. The differences between scenarios were smaller if hygiene is improved (results not shown). From year 10, the difference of gross margin reported was against the option 'delaying the culling', whatever the surveillance implemented.

3.4 Impact of concomitant health disorders

When other health disorders were present in herds with impaired hygiene, milk quota was not reached over time and the size of the herd decreased dramatically (results not shown).

Similar results on ranking scenarios than before were found in herds with a high prevalence of concomitant health disorders whatever the level of hygiene implemented but for herd with impaired hygiene. Based on epidemiological criteria, implementing a systematic test-and-cull programme led to lower persistence and prevalence than implementing the same test-and-cull programme based on clinical surveillance. Both test-and-cull programmes led to lower prevalence and persistence over time than when nothing is implemented. However, the decrease in prevalence and persistence were lower here (high prevalence of concomitant health disorders) than in herd with low prevalence of concomitant herd disorders.

The decrease of relative discounted gross margins over time of scenarios implementing control programmes *versus* doing nothing was higher when the prevalence of concomitant health disorders increased. As before, this decrease of gross margin was lower when the level of hygiene in the herd increased. Differences between scenarios were higher when the prevalence of concomitant health disorders increased and were almost null for herds with improved hygiene. Implementing a control programme (whatever the surveillance used) was never profitable in economic terms for herds with impaired hygiene. It was profitable for herds with standard hygiene by year 15.

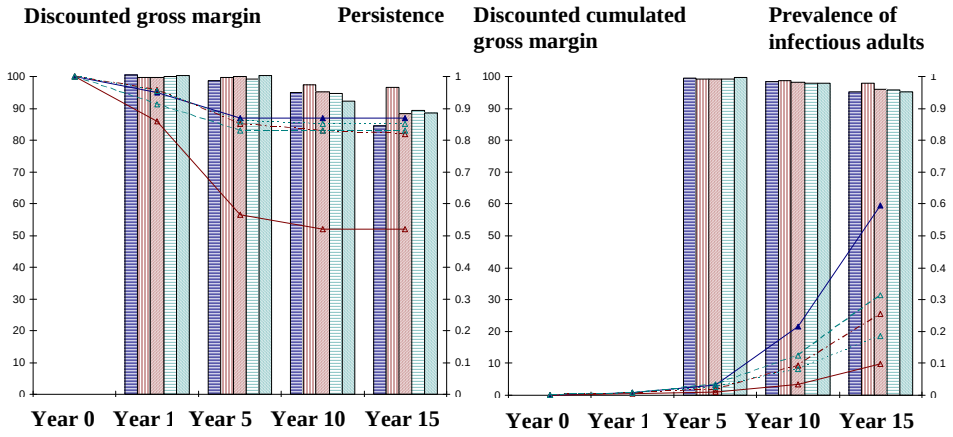

Figure 6: Effect of the time to culling positive animals. Persistence of Mycobacterium avium *subsp.* paratuberculosis (Map) *infection in the herds (left column) (lines), prevalence of infectious adults (right column) (lines), and evolution of the relative discounted gross margin per year (left) and cumulated (right) (compared to a herd with no paratuberculosis which has a baseline gross margin of 100) (histograms) in all herds: outputs over time of the dairy herds economic model for scenarios in which the delay before culling following a positive test varies (D1: culling 2 weeks after a test is performed, D2: culling 4 months after a test is performed) within a structured dairy herd with standard level of hygiene*

T0: no control, T1: test-and-cull based on systematic surveillance, T2: test-and-cull triggered by clinical surveillance

Scenario 1 (T0); Scenario 4 (T1 D1); Scenario 7 (T1 D2); Scenario 16 (T2 D1); Scenario 19 (T2 D2)

In terms of relative discounted gross margins, the differences between implementing a test-and-cull programme (whatever the surveillance) or doing nothing were even less important. It was not better or worst to implement test-and-cull programmes in economic term. Differences between scenarios were smaller if hygiene is improved.

In standard herds, the impact in terms of milk quotas and herd size was smaller when a high prevalence of concomitant health disorders was simulated. The fulfilment of the milk quota was better for T1, than for T0 and even more than for T2. Herd size varied between 90 and 105 adults. In herds with improved hygiene, the quota was fulfilled, while the number of adults varied between 94 and 104.

Return of investment occurred later in herds with concomitant health disorders than in herds with paratuberculosis only. Implementing systematic test-and-cull became economically viable from 10 years after the introduction of an infected heifer in an initially susceptible herd with impaired level of hygiene. Within the time frame of 15 years, it was never economically viable to implement the test-and-cull control programme based on clinical surveillance in these herds.

4 Discussion

With our model, it was possible to compare simultaneously the impact of selected control measures on the persistence of *Map*, the within-herd prevalence of infectious adults and the discounted gross margins (annual or cumulated). In an infected herd, implementing a test-and-cull programme was better than doing nothing in epidemiological terms. In an infected herd, implementing a systematic test-and-cull programme from the introduction of one infected cattle in an initially susceptible herd decreased the prevalence (from year 5) and persistence (from year 1) over time compared to doing nothing. The differences in persistence and prevalence between scenarios increased when the level of hygiene of the herd increased and decreased, respectively. A test-and-cull programme triggered by clinical surveillance also resulted in a drop in prevalence but in no or little reduction in persistence. Implementing a control programme did not improve or impair the situation in economic terms. Implementing a systematic surveillance became economically viable when the level of hygiene of the herd decreased. If the culling was also implemented on the last 2 calves of a dam tested as infected, systematic test-and-cull implementation was still beneficial in epidemiological terms, but even less than before in economic terms. Similarly, if the delay before culling was increased, systematic test-and-cull implementation was still beneficial (but less than before) in epidemiological terms, but, again, less than before in economic terms. In herds with a high prevalence of concomitant health disorders, similar conclusions could be drawn in epidemiological terms. The economic impact was even smaller (longer return on investment when existing). Finally, the differences between

scenarios remained small (in %). This could be different if an impact of paratuberculosis (health status of the herd) on the price of the milk sold or of the animals sold for breeding was simulated.

According to the criteria (epidemiological or economic) chosen to rank the scenarios studied, results were obtained at different time horizons. The return of implementing a test-and-cull programme was faster for epidemiological outputs than for economic ones. However, whatever the aim targeted by the farmer, paratuberculosis control needs a long investment, measurable in years. It appeared that 5 years were necessary for the farmer to notice a result in epidemiological terms versus 7 to 15 years for the return on investment, in the conditions simulated here. A farmer should thus know that no returns can be expected before several years if he decided to implement a control programme such as the ones studied here. The higher the risk of *Map* transmission (poor hygiene), the higher and earlier return on investment is.

The hygiene level of studied herds had an impact on the gross margin: the higher the level of hygiene, the higher the relative discounted gross margin was. Knowing the cost of one hour of work, and assuming that the model represents well the link between hygiene level and risk of transmission, a next step in this research could be to assess the length of time a farmer can spend cleaning in order to improve hygiene and thus decrease the risk of transmission, while remaining profitable. The simulation model did not take into account for the reduction of economic losses due to other diseases resulting from improved herd hygiene. Whatever the level of hygiene studied, other health disorders were considered at a similar level. However, the global costs associated with an improved hygiene were not considered either. Additional work of the farmer linked to *Map* infection that could benefit the prevention or treatment of other conditions was also not considered.

An analysis of the complex relation between reproduction, replacement, health disorders such as paratuberculosis, and milk quotas especially at herd level requires a model representing a realistic culling strategy, a production function and its interaction with paratuberculosis infection. Such qualities are available in ECOMAM/ECOMAST after its adaptation to incorporate *Map* infection. This is here all the more important that the management of milk quota has an influence on the culling strategy especially. If the gross margin for 1000 litres of milk produced is studied, there is a risk of under-estimating the impact of a control strategy if the quota is not reached. In herds with high prevalence of concomitant health disorders such as mastitis and reproduction disorders, it becomes less relevant to control paratuberculosis in economic terms compared to a similar herd with no paratuberculosis. Because of the culling rules, more culling for other reasons than paratuberculosis occur and it is therefore more difficult to produce enough milk to reach the milk quota. Infected but not detected animals are not preferentially culled because a decrease of milk production as other health disorders count more in terms of culling priority. Herd size is affected, while the replacement rate does not increase (and is even lower) as we work here in closed dairy herds. Decreasing replacement rate increases the average age of the herd.

Furthermore, we work here in a really specific situation: the one of the French milk quotas (no purchase of quota possible during the year and penalties if the quota is exceeded). In order to maintain herd size and to fulfil the milk quota, it could be relevant in a future study to change the culling or purchase rules. This could have an impact also on the gross margin and other studied outputs.

Such a complex model working over very long time periods cannot realistically be validated by comparing simulated outputs with field data. Here, we could check that the range of value of the prevalence of subclinically infected adults and clinically affected adults in herds with the 3 levels of hygiene studied is within the range of value of the data found in the literature. The true within-herd prevalence of infected animals, infectious and affected adults reached 80%, 70%, and 10%, respectively, 15 years after the introduction of one infected heifer in a herd with standard hygiene in which no control measures are implemented. Even if such values can be considered to be high, field studies usually report apparent prevalence not accounting for the low sensitivity of the tests available. Even though, some field studies report high individual apparent within-herd prevalence such as 60% before the start of a control programme (VanLeeuwen, *et al.*, 2001; Hendrick, *et al.*, 2005a; VanLeeuwen, *et al.*, 2006; Benedictus, *et al.*, 2008; Guicharnaud, 2009; Woodbine, *et al.*, 2009; Guatteo, *et al.*, 2010).

The study we present here is the first one presenting in the mean time epidemiological and economic outputs of the impact of *Map* control measures from the introduction of one infected cattle in an initially susceptible dairy herd. The fact that doing nothing is worst than implementing any control measures in epidemiological terms has been observed on the field (Pillars, *et al.*, 2009), as well as in other *Map* transmission models (Cho, *et al.*, 2010). It is reported that culling of infectious animals with a longer culling interval is less effective (epidemiologically) to control *Map* (Lu, *et al.*, 2008). By modelling, Collins *et al.* showed that farms with poor hygiene (higher effective contact rate) would economically benefit more from a test-and-cull programme than herds with management that diminish the risk of disease transmission from cows to calves (Collins and Morgan, 1991). However, most of the studies dealt with infected herds at different levels of prevalence and not at the introduction of an infected animal in a susceptible herd. They did not consider the persistence of *Map* in the herds or did not consider in the mean time economic and epidemiological outputs. In *Map* infected herds, test-and-cull programmes are usually reported to do little (or insufficient results) to reduce prevalence and not to reduce total costs over a 10-year period, while improved calf hygiene strategies are found to be critically important (Groenendaal and Galligan, 2003; Groenendaal, *et al.*, 2003; Kudahl, *et al.*, 2007a; Kudahl, *et al.*, 2007b; Kudahl, *et al.*, 2008; Bennett, *et al.*, 2009). In our study, while the total costs are not reduced, implementing a systematic test-and-cull programme decreased the prevalence, the persistence, and the economic impact of *Map* on gross margins. However, the test-and-cull programme is implemented from the introduction of an infected heifer or from the moment 2 clinically affected animals are present. We did not wait the

prevalence to reach high levels before implementing the programme. In our model also, hygiene levels have a higher impact on the prevalence than the test programme implemented.

There is little financial incentive for dairy producers to invest in controlling *Map* infection except in particular circumstances (Stott, *et al.*, 2005). Often, those initiatives are focused on herds already infected with *Map* (prevalence higher than 20% in above study). The above conclusion would then need to be tested in non-infected herds which could purchase the infection. It is necessary to remind that paratuberculosis is a disease that causes losses even when not clinically affected animals are observed, and it will not just go away without some efforts. Our aim was here to assess whether an early systematic test-and-cull control programme could notably decrease setting of the disease in a herd, as it is difficult and expensive to get rid of the disease once it is present. However, even with an early action, the financial incentive in the short term remains small and at least several years are necessary in herds with standard hygiene to reach a positive value added by the programme (at least 7 years in the conditions simulated here). Control of *Map* infection requires persistence, patience and motivation from the farmers.

The scope of this study is limited by the moment defined to implement the systematic test programme compared to the moment of the introduction of an infected animal in an initially susceptible herd. The comparison between systematic implementation and implementation following a clinical surveillance are highly dependent on the beginning of the simulation. We chose to select a specific optimal situation for this study: systematic test-and-cull was indeed implemented from the day an infected animal is introduced. In reality, it is not known whether a purchased animal is infected or not. Further step is to take into account the probability p of introducing an infected animal. Depending on the value of p, the conclusions of this study could change. Furthermore, a specific test scheme is implemented (annual ELISA test performed on all cows). This scheme has been selected based on its tractability, cost and rapidity of results collection. It corresponds to the current procedures recommended in Western France. Moreover, a situation with milk quotas is studied here, milk quota achievement having a large impact on culling rules especially. Finally, most of the results are obtained in herds with low prevalence of concomitant health disorders, which is not common in the field. It has been demonstrated that estimating a cost of a disease might be underestimated if the indirect costs such as increased or decreased risk of associated health disorders and increased risk of culling are ignored (Kossaibati and Esslemont, 1997; Ostergaard, *et al.*, 2003). The results obtained here cannot be generalised to all test schemes, and all herd management types.

The model described and used here is a tool that could be used to study other control programmes. Tests at herd level (pooled tests or test of targeted population within a herd) could also be implemented, potentially reducing test costs (van Schaik, *et al.*, 2003). Vazquez et al. indeed report that sampling within the 3-4 years of age group could improve the chance of herd infection

detection with a minimum number of samples (Vazquez, *et al.*, 2009). Finally, birth clusters of *Map* infection have been shown to be an important component of maintaining endemic infection levels of dairy farms (Van Genugten, *et al.*, 2009; Woodbine, *et al.*, 2009). Assessing the effectiveness of culling all the calves born at the same moment than an infectious cow gave birth could also be assessed with the model in order to improve the impact of *Map* infection control programmes.

5 Conclusions

Implementing a test-and-cull programme (either systematically from the introduction of an infected cattle in a susceptible herd or based on clinical surveillance) always improves the situation from an epidemiological perspective. The persistence and prevalence improvements do not correlate to a marked improvement of profitability in all the scenarios tested. Economic improvement when implementing a control programme can be noticed when the level of hygiene of the herd decreases. A systematic test-and-cull limits the persistence of *Map* infection and maintains low within-herd prevalence at a lower cost than doing nothing. There is no value added of culling the last two calves of test-positive cows. The impact of postponing the culling once an animal is detected positive is null on *Map* prevalence and persistence and negative on gross margins. Whatever the programme implemented, obtaining both better epidemiological and better economic results takes time.

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References

Abbas, B., Riemann, H. P. and Hird, D. W., Diagnosis of Johne's disease (paratuberculosis) in Northern California cattle and a note on its economic significance, California Vet. (1983) 8:20-24.

Aly, S. S., Anderson, R. J., Adaska, J. M., Jiang, J. and Gardner, I. A., Association between *Mycobacterium avium* subspecies *paratuberculosis* infection and milk production in two California dairies, J. Dairy Sci. (2010) 93:1030-1040.

Beaudeau, F., Belliard, M., Joly, A. and Seegers, H., Reduction in milk yield associated with *Mycobacterium avium* subspecies *paratuberculosis* (*Map*) infection in dairy cows, Vet. Res. (2007) 38:625-634.

Benedictus, A., Mitchell, R. M., Linde-Widmann, M., Sweeney, R., Fyock, T., Schukken, Y. H. and Whitlock, R. H., Transmission parameters of *Mycobacterium avium* subspecies *paratuberculosis* infections in a dairy herd going through a control program, Prev. Vet. Med. (2008) 83:215-227.

Benedictus, G., Dijkhuizen, A. A. and Stelwagen, J., Economic losses due to paratuberculosis in dairy cattle, Vet. Rec. (1987) 121:142-146.

Bennett, R., McClement, I. and McFarlane, I., An economic decision support tool for simulating paratuberculosis control strategies in a UK suckler beef herd, Prev. Vet. Med. (2009) 93:286-293.

Cho, J., Tauer, L. W., Schukken, Y. H., Smith, R. L., Lu, Z. and Grohn, Y. T., Economic Analysis of Johne's Disease Control Strategies in Dairy Herds, Poster prepared for presentation at the Agricultural & Applied Economics Association 2010 AAEA, CAES, & WAEA Joint Annual Meeting, Denver, Colorado, (2010), pp.

Collins, M. T. and Morgan, I. R., Economic decision analysis model of a paratuberculosis test and cull program, J. Am. Vet. Med. Assoc. (1991) 199:1724-1729.

Coursaget, S., Review of paratuberculosis action programmes in Western France [in French], Doctorate in Veterinary Medicine Thesis, Nantes, France, (2009).

Ezanno, P., Fourichon, C., Beaudeau, F. and Seegers, H., Between-herd movements of cattle as a tool for evaluating the risk of introducing infected animals, Anim. Res. (2006) 55:189-208.

Ferrouillet, C., Wells, S. J., Hartmann, W. L., Godden, S. M. and Carrier, J., Decrease of Johne's disease prevalence and incidence in six Minnesota, USA, dairy cattle herds on a long-term management program, Prev. Vet. Med. (2009) 88:128-137.

Gonda, M. G., Chang, Y. M., Shook, G. E., Collins, M. T. and Kirkpatrick, B. W., Effect of *Mycobacterium paratuberculosis* infection on production,

reproduction, and health traits in US Holsteins, Prev. Vet. Med. (2007) 80:103-119.

Groenendaal, H. and Galligan, D. T., Economic consequences of Johne's disease control programs, School of Veterinary Medicine, University of Pennsylvania. (1999) Center of Animal Health and Productivity Technical Report, pp. 52.

Groenendaal, H. and Galligan, D. T., Economic consequences of control programs for paratuberculosis in midsize dairy farms in the United States, J. Am. Vet. Med. Assoc. (2003) 223:1757-1768.

Groenendaal, H., Nielen, M. and Hesselink, J. W., Development of the Dutch Johne's disease control program supported by a simulation model, Prev. Vet. Med. (2003) 60:69-90.

Groenendaal, H. and Wolf, C. A., Farm-level economic analysis of the US National Johne's Disease Demonstration Herd Project, J. Am. Vet. Med. Assoc. (2008) 233:1852-1858.

Guatteo, R., Marcé, C., Lehébel, A., Vermesse, R., LeDréan, E. and Fourichon, C., Paratuberculosis in cattle: proportion of infected animals which are not detected in infected dairy herds. (2010) In Preparation.

Guicharnaud, M., Prevalence of paratuberculosis in dairy cattle herd worldwide: review and analysis [in French], Doctorate in Veterinary Medicine Thesis, Nantes, France, (2009).

Haddad, J. P. A., Dohoo, I. R., VanLeeuwen, J. A., Tiwari, A., Keefe, G. P. and Tremblay, R., Effects of seropositivity for agents causing enzootic bovine leucosis, neosporosis, and Johne's disease on calving to conception interval in Canadian dairy cows, Proceedings of the 10th International Society for Veterinary Epidemiology and Econonomics Conference, Vina Del Mar, Chili, (2003), pp. 252.

Hendrick, S., Duffield, T., Leslie, K., Lissemore, K., Archambault, M. and Kelton, D., The prevalence of milk and serum antibodies to *Mycobacterium avium* subspecies *paratuberculosis* in dairy herds in Ontario, Can. Vet. J. (2005a) 46:1126-9.

Hendrick, S. H., Kelton, D. F., Leslie, K. E., Lissemore, K. D., Archambault, M. and Duffield, T. F., Effect of paratuberculosis on culling, milk production, and milk quality in dairy herds, J. Am. Vet. Med. Assoc. (2005b) 227:1302-1308.

Hortet, P., Fourichon, C., Sorensen, J. T. and Seegers, H., A simulation model for assessment of economic consequences of mastitis in dairy herds, Proceedings of the 8th International Symposia on Veterinary Epidemiology and Economics, Paris, France, (1997), pp.

Hortet, P., Ex-ante assessment of the economic efficiency of mastitis control plans in dairy herd [in French], PhD Thesis, Rennes, France, (1999).

Hutchinson, L. J., Economic impact of paratuberculosis, Vet. Clin. North Am. Food Anim. Pract. (1996) 12:373-381.

Johnson-Ifearulundu, Y. J. and Kaneene, J. B., Epidemiology and economic impact of subclinical Johne's disease: a review, Vet. Bull. (1997) 67:437-447.

Johnson-Ifearulundu, Y. J., Kaneene, J. B., Sprecher, D. J., Gardiner, J. C. and Lloyd, J. W., The effect of subclinical *Mycobacterium paratuberculosis* infection on days open in Michigan, USA, dairy cows, Prev. Vet. Med. (2000) 46:171-181.

Johnson, Y. J., Kaneene, J. B., Gardiner, J. C., Lloyd, J. W., Sprecher, D. J. and Coe, P. H., The effect of subclinical *Mycobacterium paratuberculosis* infection on milk production in Michigan dairy cows, J. Dairy Sci. (2001) 84:2188-2194.

Jubb, T. F. and Galvin, J. W., Effect of a test and control program for bovine Johne's disease in Victorian dairy herds 1992 - 2002, Aust. Vet. J. (2004) 82:228-232.

Kossaibati, M. A. and Esslemont, R. J., The costs of production diseases in dairy herds in England, Vet. J. (1997) 154:41-51.

Kostoulas, P., Leontides, L., Billinis, C., Amiridis, G. S. and Florou, M., The association of sub-clinical paratuberculosis with the fertility of Greek dairy ewes and goats varies with parity, Prev. Vet. Med. (2006) 74:226-238.

Kristensen, E., Ostergaard, S., Krogh, M. A. and Enevoldsen, C., Technical Indicators of Financial Performance in the Dairy Herd, J. Dairy Sci. (2008) 91:620-631.

Kudahl, A., Nielsen, S. S. and Sorensen, J. T., Relationship between antibodies against *Mycobacterium avium* subsp. *paratuberculosis* in milk and shape of lactation curves, Prev. Vet. Med. (2004) 62:119-134.

Kudahl, A. B., Ostergaard, S., Sorensen, J. T. and Nielsen, S. S., A stochastic model simulating paratuberculosis in a dairy herd, Prev. Vet. Med. (2007a) 78:97-117.

Kudahl, A. B., Sorensen, J. T., Nielsen, S. S. and Ostergaard, S., Simulated economic effects of improving the sensitivity of a diagnostic test in paratuberculosis control, Prev. Vet. Med. (2007b) 78:118-129.

Kudahl, A. B., Nielsen, S. S. and Ostergaard, S., Economy, efficacy, and feasibility of a risk-based control program against paratuberculosis, J. Dairy Sci. (2008) 91:4599-4609.

Kudahl, A. B. and Nielsen, S. S., Effect of paratuberculosis on slaughter weight and slaughter value of dairy cows, J. Dairy Sci. (2009) 92:4340-4346.

Lombard, J., Fossler, C. and Carter, M., Results from the US JD demonstration herd project: Key findings for disease control (Control programs Keynote lecture), Proceedings of the 10th International Colloquium on Paratuberculosis, Minneapolis, Minnesota, USA, (2009), pp. 195.

Lombard, J. E., Garry, F. B., McCluskey, B. J. and Wagner, B. A., Risk of removal and effects on milk production associated with paratuberculosis status in dairy cows, J. Am. Vet. Med. Assoc. (2005) 227:1975-1981.

Lu, Z., Mitchell, R. M., Smith, R. L., Van Kessel, J. S., Chapagain, P. P., Schukken, Y. H. and Grohn, Y. T., The importance of culling in Johne's disease control, J. Theor. Biol. (2008) DOI:10.1016/j.jtbi.2008.05.008.

Lu, Z., Schukken, Y. H., Smith, R. L. and Grohn, Y. T., Stochastic simulations of a multi-group compartmental model for Johne's disease on US dairy herds with test-based culling intervention, J. Theor. Biol. (2010) DOI:10.1016/j.jtbi.2010.03.034.

Marcé, C., Beaudeau, F., Bareille, N., Seegers, H. and Fourichon, C., Higher non-return rate associated with *Mycobacterium avium* subspecies *paratuberculosis* infection at early stage in Holstein dairy cows, Theriogenology. (2009) 71:807-816.

Marcé, C., Ezanno, P., Seegers, H., Pfeiffer, D. U. and Fourichon, C., Modelling the spread of *Mycobacterium avium* subsp. *paratuberculosis* towards fadeout or endemic infection in a dairy herd, Vet. Res. (2010) Submitted.

Matthews, H. T., On Johne's disease, Vet. Rec. (1947) 59:397-401.

McNab, W. B., Meek, A. H., Martin, S. W. and Duncan, J. R., Associations between dairy production indices and lipoarabinomannan enzyme-immunoassay results for paratuberculosis, Can. J. Vet. Res. (1991) 55:356-361.

Nielsen, S. S., Enevoldsen, C. and Toft, N., Milk production losses associated with bovine paratuberculosis diagnosed from repeated testing, Proceedings of the 11th International Symposia on Veterinary Epidemiology and Economics, Cairns, Australia, (2006), pp. 619.

Nielsen, S. S. and Ersboll, A. K., Age at occurrence of *Mycobacterium avium* subspecies *paratuberculosis* in naturally infected dairy cows, J. Dairy Sci. (2006) 89:4557-4566.

Nielsen, S. S. and Toft, N., Ante mortem diagnosis of paratuberculosis: A review of accuracies of ELISA, interferon-γ assay and faecal culture techniques, Vet. Microbiol. (2007) 129:217-235.

Nielsen, S. S., Transitions in diagnostic tests used for detection of *Mycobacterium avium* subsp. *paratuberculosis* infections in cattle, Vet. Microbiol. (2008) 132:274-282.

Nielsen, S. S., Programmes on Paratuberculosis in Europe, Proceedings of the 10th International Colloquium on Paratuberculosis, Minneapolis, Minnesota, USA, (2009), pp. 101-108.

Nielsen, S. S., Krogh, M. A. and Enevoldsen, C., Time to the occurrence of a decline in milk production in cows with various paratuberculosis antibody profiles, J. Dairy Sci. (2009) 92:149-155.

Nielsen, S. S. and Toft, N., Management practices reducing the test-prevalence of paratuberculosis in Danish dairy herds, Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine, Nantes, France, (2010), pp. 189-200.

Ostergaard, S., Sorensen, J. T. and Houe, H., A stochastic model simulating milk fever in a dairy herd, Prev. Vet. Med. (2003) 58:125-143.

Pillars, R. B., Grooms, D. L., Wolf, C. A. and Kaneene, J. B., Economic evaluation of Johne's disease control programs implemented on six Michigan dairy farms, Prev. Vet. Med. (2009) 90:223-232.

Raizman, E. A., Fetrow, J., Wells, S. J., Godden, S. M., Oakes, M. J. and Vazquez, G., The association between *Mycobacterium avium* subsp. *paratuberculosis* fecal shedding or clinical Johne's disease and lactation performance on two Minnesota, USA dairy farms, Prev. Vet. Med. (2007) 78:179-195.

Raizman, E. A., Fetrow, J. P. and Wells, S. J., Loss of income from cows shedding *Mycobacterium avium* subspecies *paratuberculosis* prior to calving compared with cows not shedding the organism on two Minnesota dairy farms, J. Dairy Sci. (2009) 92:4929-4936.

Seegers, H., Fourichon, C., Sorensen, J. T., Hortet, P. and Billon, D., Modelling the cost-benefits of prevention and control of mastitis in dairy herds, Econ. Modelling Anim. Health Farm Manag., Wageningen, Netherlands, (2000), pp. 55-61.

Seegers, H., Fourichon, C., Billon, D. and Hortet, P., Cost-benefit analysis of mastitis control by simulation: model description and profitability of strategies targeting subclinically infected cows, Proceedings of the Society of Veterinary Epidemiology and Preventive Medicine, Martigny, Suisse, (2004), pp. 179-186.

Smith, R. L., Grohn, Y. T., Pradhan, A. K., Whitlock, R. H., Van Kessel, J. S., Smith, J. M., Wolfgang, D. R. and Schukken, Y. H., A longitudinal study on the impact of Johne's disease status on milk production in individual cows, J. Dairy Sci. (2009) 92:2653-2661.

Sockett, D. C., Johne's disease eradication and control: regulatory implications, Vet. Clin. North Am. Food Anim. Pract. (1996) 12:431-440.

Sorge, U., Kelton, D., Lissemore, K., Godkin, A., Hendrick, S. and Wells, S., Attitudes of Canadian dairy farmers toward a voluntary Johne's disease control program, J. Dairy Sci. (2010) 93:1491-1499.

Stott, A. W., Jones, G. M., Humphry, R. W. and Gunn, G. J., Financial incentive to control paratuberculosis (Johne's disease) on dairy farms in the United Kingdom, Vet. Rec. (2005) 156:825-831.

Taisne, D., A control programme for paratuberculosis in infected dairy herds in the Ille-et-Vilaine county [in French], Doctorate in Veterinary Medicine Thesis, Nantes, France, (2009).

Van Genugten, F., Mitchell, R. M., Stehman, S. M. and Schukken, Y. H., Birth clusters of animals infected with *Mycobacterium avium* subspecies *paratuberculosis* in a New York State dairy herd, Proceedings of the 10th International Colloquium on Paratuberculosis, Minneapolis, Minnesota, USA, (2009), pp. 155-158.

Van Roermund, H. J. W., Bakker, D., Willemsen, P. T. J. and De Jong, M. C. M., Horizontal transmission of *Mycobacterium avium* subsp. *paratuberculosis* in cattle in an experimental setting: Calves can transmit the infection to other calves, Vet. Microbiol. (2007) 122:270-279.

van Schaik, G., Stehman, S. M., Schukken, Y. H., Rossiter, C. R. and Shin, S. J., Pooled fecal culture sampling for *Mycobacterium avium* subsp. *paratuberculosis* at different herd sizes and prevalence, J. Vet. Diagn. Invest. (2003) 15:233-241.

van Schaik, G., Swart, W., Van Maanen, K. and Weber, M. F., The validity of repeated serological and culture results to determine the true infection status for *Mycobacterium avium* subsp. *paratuberculosis*, Proceedings of the 10th International Colloquium on Paratuberculosis, Minneapolis, Minnesota, USA, (2009), pp. 193.

VanLeeuwen, J. A., Keefe, G. P., Tremblay, R., Power, C. and Wichtel, J. J., Seroprevalence of infection with Mycobacterium avium subspecies paratuberculosis, bovine leukemia virus and bovine viral diarrhea virus in Maritime Canada dairy cattle, Can. Vet. J. (2001) 42:193-198.

VanLeeuwen, J. A., Tiwari, A., Plaizier, J. C. and Whiting, T. L., Seroprevalences of antibodies against bovine leukemia virus, bovine viral diarrhea virus, *Mycobacterium avium* subspecies *paratuberculosis*, and *Neospora caninum* in beef and dairy cattle in Manitoba, Can. Vet. J. (2006) 47:783-6.

Vazquez, P., Molina, E., Alonso-Hearn, M., Geijo, M. V., Sevilla, I. A., Estonba, A., Ruiz, O., Garrido, J. M. and Juste, R. A., Age structure of *Mycobacterium avium* subsp. *paratuberculosis* infection in culled Friesian cattle, Proceedings of the 10th International Colloquium on Paratuberculosis, Minneapolis, Minnesota, USA, (2009), pp. 185-188.

Wells, S. J., Hartmann, W. L. and Anderson, P. L., Evaluation of progress made by dairy and beef herds enrolled in the Minnesota Johne's Disease Control Program, J. Am. Vet. Med. Assoc. (2008) 233:1920-1926.

Whittington, R. J. and Windsor, P. A., In utero infection of cattle with *Mycobacterium avium* subsp. *paratuberculosis*: A critical review and meta-analysis, Vet. J. (2009) 179:60-69.

Woodbine, K. A., Schukken, Y. H., Green, L. E., Ramirez-Villaescusa, A., Mason, S., Moore, S. J., Bilbao, C., Swann, N. and Medley, G. F., Seroprevalence and epidemiological characteristics of *Mycobacterium avium* subsp. *paratuberculosis* on 114 cattle farms in south west England, Prev. Vet. Med. (2009) 89:102-109.

Chapter 6

General Discussion

The aim of this thesis was to investigate the epidemiological and economic effectiveness of control measures of *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) infection for limiting the prevalence and persistence of the infection in dairy herds. To achieve this aim, a modelling approach has been used linking an epidemiological with an economic model. Three objectives were defined to reach the overall objective:

1/ to develop a computer model that takes into account the current scientific knowledge on epidemiological attributes of *Map* infection and *Map* infection control within dairy herds;

2/ to better understand the transmission of *Map* within a dairy herd specifically in terms of prevalence and persistence of the infection, relative contribution of the different routes of transmission, and impact of the contact between susceptible and infectious animals;

3/ to obtain insight into the economic and epidemiological effects of selected control programmes for *Map* infected herds by linking the epidemiological with an existing economic model adapted to study paratuberculosis.

The general discussion summarizes in section 1 the major findings of the thesis. Section 2 discusses the general research approach followed**.** Section 3 deals with uncertainty and variability and how both were considered in this thesis, while section 4 focuses on the linking of epidemiological and economic models. In section 5, the validity of the final model and its results are discussed. Finally, in section 6, implications, perspectives and recommendations for future research are provided.

1 Major findings

Two new models are now available to study *Map* transmission in a dairy cattle herd and the economic effectiveness of different control programmes.

A review of previously existing epidemiological models of within-herd *Map* transmission in dairy cattle showed that assumptions about the routes of transmission and their contribution within a herd vary greatly among authors. Gaps of knowledge such as variation of susceptibility with age and variability of pattern of shedding were identified and should be targeted to improve the validity of models. There was a need for new models which consider indirect transmission via the environment, *Map* survival, and possible direct or indirect contacts between animals in a herd. There also was a need for outputs on persistence and relative importance of the routes of transmission in order to be able to provide critical features for the construction of future *Map* transmission models in terms of herd management and herd structure characteristics.

Therefore, we developed a new epidemiological model which takes into account these recommendations (Chapter 2). *Map* infection in a herd in the

absence of control measure is described. In a baseline scenario for transmission parameters, herd management and housing facilities, fadeout occurred in 70% of the runs whereas persistent infection was established within 11 years, with no later fadeout, in 30% of the remaining runs. Runs with future fadeout or future persistent infection were differentiated by the number of clinically affected animals which was rarely above one when fadeout spontaneously occurred. In persistently infected herds, the two main routes of transmission were transmission via the environment of the farm mainly contaminated by adults, and *in utero* transmission. Calf-to-calf transmission was minor, as well as milk and colostrum contributions to the transmission of the infection. Aerosol transmission was not specifically considered as this route of transmission only has been hypothesized (Eisenberg, *et al.*, 2010).

The contacts between calves did not influence *Map* transmission in the conditions modelled here. *Map* transmission in the herd was mainly influenced by the exposure of calves to the environment contaminated by adults' faeces. Delaying this exposure is thus recommended as a measure for decreasing *Map* prevalence. This can be achieved through systematic separation of adults from calves in addition to effective hygiene measures. Early culling of clinically affected adults led to a lower prevalence of infectious adults over time. Early culling of such animals should thus also be targeted as this will decrease *Map* environmental contamination.

In a herd with limited incidence of reproduction and mastitis disorders, a systematic implementation of a test-and-cull programme towards *Map* infection was more effective than performing a clinical surveillance where screening is triggered by the occurrence of cases. Whatever the moment of implementation, the same herd screening and culls was implemented. It was profitable 7 to more than 15 years after the introduction of *Map*, depending on the exposure of calves to adult faeces via the environment. Several factors that could influence the conclusions have been explored such as the delay before culling execution, the action performed after a positive test, or the level of hygiene. Similar conclusions could be done in a herd where health disorders other than paratuberculosis occur at the exception that milk quotas were not fulfilled anymore and that herd size decreased dramatically. A specific situation was studied here: the systematic implementation of the programme begins the day an infected animal is introduced in a fully susceptible herd. The probability of introducing an infected purchased animal or the length of the period during which the test-and-cull programme is implemented before the introduction of an infected animal should now be considered to further evaluate economically such a programme.

2 General approach

A modelling approach which allows studying a complex system such as the transmission of an infectious disease in a herd was selected as the basis for this thesis. Both, epidemiological and economic features were considered

during this process. Modelling can indeed be a powerful tool for the analysis of infectious disease spread and the evaluation of control programmes, by optimizing the use of limited resources or by targeting control measures more efficiently (Keeling and Rohani, 2008). Assumptions can be tested, outcomes of the host-pathogen interactions in a population can be predicted, and available knowledge on transmission can be represented and summarized thanks to dynamic models (Anderson and May, 1999). Finally, models can be used not only as a predictive tool but also to understand, to provide explanation, and to identify new research questions and important gaps of knowledge (Epstein, 2008; Lander, 2010).

Another alternative approach to investigating the aim of this research project would have been to perform epidemiological studies in infected herds. However, such an approach is time consuming and costly, particularly for the study of paratuberculosis which is characterized by a very long incubation period and poorly discriminative diagnostic tests. The use of modelling allows for testing a large number of scenarios as well as hypotheses on the transmission of *Map*. Actually, both approaches (epidemiological studies and modelling) are complementary and ideally should be combined. Models are very suitable for identifying gaps of knowledge or testing hypotheses of epidemiological relationships that can then be investigated through epidemiological studies. The knowledge generated by these studies is essential for constructing and parameterising the models.

Two different modelling approaches can be used: analytical modelling or simulation modelling. Simulation is defined as the process of designing a model of a real system based on a set of assumptions and conducting experiments with this model with the purpose of either understanding the behaviour of the system or evaluating various strategies for the operation of the system (Shannon, 1975; Winston, 1987). While analytical models provide exact mathematical solutions, simulation models need to run the model several times to generate representative samples of measures of performance (Winston, 1987; Frigg and Hartmann, 2006). When models are complex, simulation is the only possibility. The integrated approach we chose required representing the whole system (population dynamics and infection process), resulting in a complex simulation approach. This simulation approach necessitates defining simulated scenarios.

The construction of both models presented in this thesis (epidemiological and bioeconomic) was guided by our specific research questions. First, discussions with stakeholders revealed that when a herd is infected by *Map*, it is difficult to eradicate the bacteria. We therefore chose to focus our work on infected herds before the prevalence reached a high level and more precisely on the early stage of the disease, when a herd becomes infected. Then, in order to prioritise the various control measures that would be studied (should we focus on hygiene measures, or feeding measures, or *in utero* transmission?), we decided to assess the contribution of the different routes of transmission. This required representing all known routes of transmission in our model. Finally, calf-to-calf transmission was recently demonstrated. We

therefore attempted to assess the contribution of this route of transmission and whether the contact pattern had an impact on *Map* transmission during the first weeks of age. Studying the impact of calf housing (direct contact / shared environments) the first weeks of age required us to use an individual-based model for calves in individual pens and a detailed representation of housing facilities (compartmental model) until animals are resistant. These research questions were studied with the epidemiological model in order to improve the understanding of the disease dynamics and its determinants at the herd level and to identify current lack of knowledge.

Here, we chose a stochastic approach. While the deterministic representation is appropriate for large populations where variability between individual animals have little impact on the population-scale dynamics, the stochastic representation is more appropriate for small populations and rare events, and allows approaching the variability in the system more meaningfully. Here, the stochastic approach allowed distinguishing fadeout and persistent infection. Control measures that would decrease the persistence of the infection were thus targeted. However, as paratuberculosis was the issue, population dynamics did not include stochastic components and were based on the most common European dairy herd dynamics and on the situation in French herds when specific parameter values were necessary.

The models presented in this thesis are flexible tools that can be easily adapted to study other research questions. Other choices and decisions taken during the modelling process could be necessary. It was indeed necessary to decide on the timing of implementation of selected control measures. In our simulations, we chose to study more specifically the initial infection of a susceptible herd and not already persistent *Map* infected herds in order to be able to provide also recommendations to farmers which are currently not infected but exposed to *Map* introduction: will it be possible to remove the infection if one infected animal is introduced, or will the infection persist over time? With the model, we could implement control measures when an endemic situation (equilibrium of prevalence) is reached. However, this would assume that, in the field, equilibrium is usually reached. This is only a hypothesis, and available knowledge does not allow deciding whether it generally is the case. Observed prevalence in infected herds is generally (but not always) low, suggesting detection before equilibrium is reached. We could also implement control measures modelling a herd with a specific prevalence level (for example 5, 10 or 20% of infected animals) and then study how *Map* spreads according to the chosen value. We decided not to select these two options as it has already been studied (Groenendaal, *et al.*, 2002; Kudahl, *et al.*, 2007a). Our modelling study was conducted for closed dairy herds where a single infected animal had been introduced. Open herds could have been studied, but it was preferred to limit the introduction of infected animals to a single event to better understand the mechanisms of *Map* spread. The model presented here can also be used to examine hypotheses in relation to infection dynamics other than the ones tested in this thesis. A more accurate explanation of within-herd infection dynamics could indeed be possible if infection states were more discriminated. This is particularly true for super-

shedders for which several biological hypotheses exist: super-shedders could be specific animals (a specific compartment is considered for super-shedders) or it could only be an intermittent state of subclinically infected animals (a few I_S cattle can sometimes shed a large amount of *Map*). The model could thus be adapted for the study of the 2 corresponding scenarios in order to assess the influence of super-shedders on *Map* transmission for example. In the first case, targeting super-shedders with control measures could highly influence *Map* transmission. This modelling study would need to be completed by a comparison of both scenarios' outputs with field data.

In addition to the epidemiological outputs, an evaluation of the economic effectiveness was performed. Epidemiological and economic effectiveness of a control strategy can indeed differ. An intensive test-and-cull programme can potentially rapidly reduce the prevalence but be at too high an economic cost for the farmer. To help decision makers, both epidemiological and economic results are necessary. Two options were possible for integrating epidemiological and economic outputs: adding an economic layer to the epidemiological model, or adapting a more complex economic model to the study of *Map* infection. While the first option would have been easier to implement (for example by adding losses and control costs) provided major simplifications are performed, the second option was selected in this thesis specifically, since it allowed to study the impact of the farmers' decisions (culling and replacement), of detailed simulation of the production process in the herd, of the herd management, and of milk quotas. For example, the study performed in chapter 5 showed that the differences of losses were not directly differences of revenues and they did not result in equivalent differences of gross margins. An existing bioeconomic simulation model (ECOMAM/ECOMAST) was adapted to studying *Map* transmission. In contrast to the epidemiological model, the population dynamics and herd management are represented in this economic model (stochastic components) in some detail, while the infection process is kept simple. This individual-based economic model allows to consider suboptimal culling of cows and milk quotas. In order to consider possible interactions of culling for other reasons than paratuberculosis control, the culling rules represented in that model are more complex than in the epidemiological model, where cows are grouped in compartments based on their parity and their paratuberculosis infection status. The epidemiological model does not include any detailed information about individual milk production or health disorders other than paratuberculosis. Moreover, in ECOMAM/ECOMAST, the structure of the model enables the study of measures targeting cohorts or dam and calf couples (for example culling a test-positive dam and its calf, or all the calves born at the same period of time). However, it is not possible to specifically study the effect of measures based on milk or colostrum management as only two routes of transmission are represented for paratuberculosis: *in utero* transmission, or transmission during the first year of age (whatever the route). The costs of studied control measures were quantified over time in order to integrate the possible changes of effectiveness of intervention and to determine the time to return on investment. Only looking at the initial and

final situations would not have allowed understanding the mechanisms implemented and only provided an incomplete impact: the costs of intermediate steps can be very high.

Different choices were performed in terms of selection of scenarios. It was indeed necessary to select the type of measures implemented, the delay before their implementation, their consequences, and other factors that could influence the results. A wide variety of scenarios were selected, even if not realistic, to assess the potential impact of the different measures. Studied control programmes are based on control measures implemented in the field such as the use of diagnostic tests followed by culling of any test-positive animal. However, to assess the relevance of studying early detection, we chose to study the systematic implementation of such a measure from the time of introduction of an infected animal into a herd. In the field, the true infection status of a purchased animal is not known by the farmer. Mainly for the economic part, the modelling study performed here needs to be followed by another study in which we consider the probability of introducing an infected animal and compare scenarios in which tests are implemented for a certain period of time (possibly years) before the time of introduction of an infected animal into the herd. Furthermore, the impact of different risk of transmission within a herd has been assessed by studying different levels of hygiene implemented in a herd, resulting in different conclusions. This pinpoints the importance of controlling the exposure of calves to adult faeces. Studied levels have been arbitrarily defined. It is again a hypothetical scenario as we do not know which cleaning-disinfection measures would allow reaching such levels. Finally, we assumed that the time necessary for a farmer to detect and cull a clinically affected animal does not change over the simulated period. This was done to study a situation with absolutely no control measures implemented, when the epidemiological model showed that early culling clinically affected animals had a dramatic impact on *Map* prevalence. Such a scenario in which no control measure is implemented is not realistic. Indeed, from the time a farmer notices a disorder of diarrhoea on his farm, he will preferably cull these animals and will not, for example, delay similarly the culling of the 1st and the 10th clinically affected animal. However, range of model outputs for prevalence reached 8 to 10 years after the introduction of one infected cattle into an initially susceptible herd fitted with range of corrected observed prevalence in the field when farmers decide to begin a control programme. Interestingly, at this time, spontaneous fadeout is almost null in our outputs when no control measure is implemented. In the bioeconomic model, spontaneous fadeout occurred less frequently compared to the epidemiological model, either when no control measures or when test-and-cull programmes were implemented. This may be explained by the fact that cullings were considered in a different way in both models (see below part 4.).

3 Uncertainty and variability

Biological systems are characterized by their variability and uncertainty. Such characteristics need to be considered to obtain results that can be used for decision making.

Uncertainty is related to the lack of knowledge we have on fixed parameters that characterise a disease (Harwood and Stokes, 2003). It is not an effect of chance. For example, uncertainty exists in the knowledge we have on the upper limit in age for susceptibility to *Map* infection, or on the transmission rates of *Map* within a herd via the different routes of transmission.

Variability can be measured, analysed and possibly explained. It is composed of both the variability between individuals in a population and within an individual (Banks and Potter, 2004). An example of variability for paratuberculosis is the amount of *Map* shed by individuals, which differs both between individuals and within an individual over time. Variability can also be a consequence of randomness. For an animal, depending on the sample of faeces performed, the count of *Map* differs (as well as the diagnostic). This is also known as the stochastic variability. Such heterogeneity should be considered, especially when specific categories of animals are targeted by a control programme. When it comes to susceptibility or shedding, heterogeneity can be modelled by considering as many categories of individuals (compartments) as necessary to describe variability.

Both uncertainty and variability represent the unpredictability of the system and reflect the variations of the outputs that could be expected. Uncertainty can be limited using expert opinion or available data (selection of plausible intervals). Variability can be quantified and modelled thanks to repeated observations. Sensitivity analysis allows assessing how a model behaves towards uncertainty or variability of parameters in order to define whether it is possible to trust the prediction of this model (stability of the conclusions) (Hyman, *et al.*, 2001). Indeed, sensitivity analysis investigates the effect of a change in any of the input parameters on the outcome. It is thus possible to determine which input parameters have the biggest influence on the outcome. If the outcome differs depending on the value of an unknown input parameter, the model cannot be used as a predictor (at least not quantitatively). If interactions exist between unknown parameters, even qualitative predictions (e.g. ranking of control measures) are risky.

If there is complete knowledge on the system and no variability, a deterministic model can be used. Otherwise, a stochastic model should be used. A stochastic approach has been chosen here to represent variability (probability of occurrence) in both the epidemiological and the economic models. In the epidemiological model, the stochastic part was limited to the infection process (Table I). For the population dynamics, a deterministic approach was used as uncertainty and variability were limited for the purpose of our study (a specific situation was indeed chosen for the population dynamics). We chose to express the stochastic valence of our model by using

Table I: Differences in terms of how variability and uncertainty are taken into account in both models and the simulations

Model component	Epidemiological model (compartmental model)		Economic model (individual based model)	
	Variability	Uncertainty	Variability	Uncertainty
Population dynamics and dairy herd management*	Deterministic model Variability of housing and contact structure studied by simulation	Not considered	Stochastic process for the herd dynamics and production (parameters chosen in distributional laws) and 2 levels of other health disorders influencing replacement of the herd	Not considered No variation (variability and uncertainty) on prices
Map infection process	Stochastic process (probability distributions applied to fixed parameters)	Sensitivity analysis performed on the following uncertain parameters: upper limit in age susceptibility, age-dependent relationship, survival of *Map* in the environment, vertical and horizontal transmission rates Lack of or limited knowledge in terms of: ▪ Upper limit in age susceptibility ▪ Age-dependent dose-response relationship ▪ *Map* transmission parameters ▪ Survival of *Map* in the environment ▪ Shedding levels ▪ Existence of super-shedders and passive shedding	Cf epidemiological model The time spent in the different infection states are drawn in distributional laws (mean and standard deviation provided in Chapter 5) Three different levels of hygiene modifying the transmission parameters are studied	
Initial conditions	▪ 25 initial susceptible herds ▪ The infected cattle is introduced in the same compartment (same parity and infection status but different length in I_S)	Not considered	▪ 1 initial susceptible herd ▪ The infected cattle introduced is the same for all simulations (same length in the I_S status especially)	Not considered

*French dairy herd population dynamics are specifically studied

likelihood of occurrence of events (fixed parameters) linked to the infection process. We did not use distributions on inputs in order to better control the variability of the system, and because such distributions are poorly or not known. Moreover, 25 initial herds (without infection) representative of a usual herd from Western France were selected to represent the variability of the system. This limited the chance of selecting a particular herd for the initial condition before introducing an infected animal. In the economic model, the population dynamics included more stochastic components but all scenarios were run from the same initial herd. Variability was limited in the economic model as the same infected animal was introduced for all scenarios. This was possible because the economic model is an individual-based model in which the characteristics of each cow can be defined precisely. This is not the case for the epidemiological model.

Uncertainty in the epidemiological model was studied through a sensitivity analysis. Several uncertain parameters were identified as influencing the results: the percentage of *Map* removed from the environment, the level of exposure of calves to adult faeces, and the infection probability if one infectious dose is shed by any animal in the environment (between-group transmission rate via the environment). More knowledge on these uncertain parameters would be of interest.

As the outputs of the epidemiological model were used to define the parameters of the economic model for *Map* infection process, the uncertainty existing in the first model was assumed to be the same in the second one.

Inclusion of variability gives a more realistic picture and allows appreciating the variation of the situations in terms of epidemiological and economic consequences of *Map* infection control. However, modelling is a compromise between simplicity, tractability, and realism of the system we want to represent and study. Interaction between biology and modelling is essential to identify and represent uncertainty and variability, to interpret the outputs, and to define the scenario that are relevant to be studied.

4 Linking epidemiological and economic models

Linking epidemiological and economic models adds value to the decisions based on the outputs notably when conclusions differ depending on the model outputs used.

While epidemiological models take little or no account of economic constraints or incentives (such as milk quotas and culling decision rules), economic models mostly ignore the temporal dynamics of the disease. Linking models can be done by adding economic values to identified losses and costs in existing epidemiological models. This has been up to now the most frequent approach used in animal health economics. Partial budget approach is a simplified approach looking at the differences between an initial

and a final situation. Following the economic outputs over time and regulating the comportment of the model depending on economic outputs as it is done in this thesis is more complex. Following economics outputs over time while adapting the associated epidemiological model to the economic outputs is an original approach that has not been often used until now (Kudahl, *et al.*, 2007b; Rat-Aspert and Fourichon, 2010). We here chose to allow regulations of the behaviour of the model depending on economic constraints such as milk quotas and culling decision rules.

A metamodel was built in order to describe the relation between prevalence of infectious adults and incidence of *Map* infection in susceptible animals, without looking specifically at the routes of transmission after birth, as they are not specifically represented in the economic model. Two other research teams working on paratuberculosis (Groenendaal, *et al.*, 2002; Kudahl, *et al.*, 2007a) did a similar choice when adapting an existing model to paratuberculosis, or when building an epidemiological model in which the economic part is particularly developed. However, the time step of 6 month used in the Dutch model does not allow as fine fittings as our model in terms of economic outputs and management decision influencing *Map* transmission (Groenendaal, *et al.*, 2002). Furthermore, this model does not consider explicitly the management of culling and replacement which is expected to be strongly influenced by the disease and vice-versa as well as by other diseases, and to strongly influence the economic results.

A difficulty in the process chosen comes from the validation of the economic model in terms of paratuberculosis progression (black box approach based on incidence and prevalence only). The persistence of the infection in the epidemiological and in the economic models differs notably. The main difference between the two models lies in how culling is performed. In the economic model, culling decision rules are complex. In the epidemiological model, there is no preferential culling of subclinically affected animals due to the decrease in milk production while it is the case in the economic model in which culling decisions are more refined. In the sensitivity analysis of the epidemiological model, the time spent in the herd by clinically affected animals before culling was found to largely affect the persistence, with a lower persistence when clinically affected animals stay during a shorter period of time. While one could expect that clinically affected animals stay less time in the economic model notably because of the losses in milk production, persistence occurred to be higher in this last model. This could be explained by the fact that in the epidemiological model, the initial case (infected animal introduced) is rapidly culled, preventing the transmission of the infection from the beginning. In the economic model, the introduced infected heifer is relatively protected from culling because of its age and of increased genetic merit due to average improvement observed between generations (as parameterized in the rules of the model). As the animal introduced is subclinically infected, the milk losses are low and do not induce the immediate culling of this animal.

5 Model validation and validity of the results

Simulation models of a complex system can only be simplified approximations of the system. Depending on available knowledge and on the question to be answered, models can be used in different manners. First, they can be used to summarise available knowledge, to identify lack of knowledge and to construct a formal representation of a system to better understand it. Second, they can be used to test biological hypotheses. Third, they can be used to compare different scenarios and if they are validated against data, they can be used to predict future states of a system. Such different uses necessitate different levels of validation of a model.

When it comes to paratuberculosis, the epidemiological model of the thesis has been used in different manners: to summarise available knowledge, to identify lack of knowledge, to test biological hypothesis and finally to evaluate scenarios of control. The internal and external validity of the results has indeed been assessed as well as the choice of the assumptions. Internal validity corresponds to the trust in the models and simulation outputs that is assessed thanks to the validation process. External validity corresponds to the possible extrapolation of all or part of the results in a broader frame than the one studied.

Model validity needs to be assessed for the model to be used (validation process). This can be performed through a sensitivity analysis, by verifying the hypothesis performed and by confronting the outputs with field data or expert opinions. Quantitative validation is seldom possible. It is indeed difficult to obtain field data for situation without control measures implemented, especially due to ethical and economic reasons. Furthermore, it can be difficult to know the real status of animals, especially when diagnostic tests are poorly discriminative as it is for *Map*. Moreover, such field observations can take a long time to collect. Furthermore, if variability is high, a huge number of observations need to be performed for a similar situation, everything else being constant. If models are not quantitatively validated they cannot reliably be used for prediction. However a partially validated model can be used for other purposes such as decision making. If so, all model users need to be aware of the model limits and be critical towards the outputs and the hypothesis on which the outputs are based. There is no perfect model and no definite answer (Epstein, 2008; Lander, 2010). Model outputs should always be reported with the assumptions on which they are based, the objective targeted, and the conditions in which the work has been performed. Here again, a trade-off between available knowledge, assumptions, objective and outputs needs to be found.

Both models were validated by comparing simulated outputs such as prevalence of infected adults with field data. The range of prevalence of infected adults found in both models was up to 90% after 25 years of simulation without any control measure. Even if such values can be

considered to be high, it is necessary to remind that true prevalence is reported in the models and again that no control measure is implemented. Furthermore, field studies report also high individual within-herd prevalence such as 60% before the start of a control programme (Benedictus, *et al.*, 2008). The conclusion that if calves were not exposed to any environment contaminated by adults, the other routes of transmission are not sufficient to maintain the infection was confirmed by the results of two studies: Benedictus *et al.* (2008) indeed reported that *in utero* transmission was not sufficient to maintain the infection, while calf-to-calf transmission was also reported not to be sufficient neither (Van Roermund, *et al.*, 2007).

Different assumptions have been set and should be reminded when looking at the outputs.

- Simplifications that could decrease *Map* transmission compared to reality have been done. It is indeed assumed that even if we consider the existence of *Map* survival in the environment, pastures are free of *Map* when animal go grazing every year in April. There is no *Map* residual from one year to the following one. The quantity of *Map* on pastures is perhaps under-estimated, notably if pastures are fertilized with bovine slurry from *Map* infected herds. However, only animals above 6 months graze in our dairy cattle model, while the most susceptible animals are younger. This is corroborated by recent studies on the persistence of *Map* in pastures fertilized with bovine slurry that reported that the contamination risk for field dry hay, although possible, is of limited importance for the infection spread (Arrigoni, *et al.*, 2009; Kruze, *et al.*, 2009). However, this could be adapted to study beef herds or dairy herds grazing all year long (Grewal, *et al.*, 2006; Salgado, *et al.*, 2009). It is furthermore assumed that during the grazing season, calves are not exposed to any environment contaminated by adults even if adults come inside twice a day for milking. It is considered that the milking parlour is not close to calf housing facilities or that milking cows does not result in an exposure of calves to adult faeces.

- Culling rates are fixed in the epidemiological model. In reality, if there is more culling because of paratuberculosis, other usual culling (random process, low milk production, reproduction disorders notably) decreases, and thus culling rates can vary depending on parities in order to keep a constant herd size or to fulfil the milk quota. Also, from the moment a farmer realizes he has paratuberculosis in his herd, he will preferably cull the affected cows. Thus, affected animals will stay a shorter time in the herd than at the beginning of the infection. This is not considered in the epidemiological model, but it is in the economic model. Different objectives are indeed targeted depending on the model and such precisions are not equally useful.

- We made simplifications: i) on infection states and shedding. A recent genetic study refutes the 'pass through' (passive faecal shedding of *Map*) assumption (Pradhan, *et al.*, 2009) while another study corroborates the assumption based on faecal and tissue culture (Whitlock, *et al.*, 2009). By comparing the results of *Map* strain-typing and shedding levels, Pradhan *et al.* concluded that very few cows had characteristics of a possible pass-through animal, and many more cows were actively infected. However, the sharing of the same strain by low shedders with the contemporary super-shedders suggested that low shedders may be infected as adults by the super-shedders. It would thus probably be relevant to consider such categories of animals; ii/ on horizontal transmission and more precisely on the influence on indirect faecal contamination of colostrum and milk of the level of hygiene implemented on farm (cleaning measures). Milk and colostrum indirect contamination only depended here on the level of shedding of cows present on the farms; iii/ on *Map* impacts on reproduction. Literature is contradictory on this issue, and recent studies did not report significant negative impact of *Map* infection on reproduction (Marcé, *et al.*, 2009; Smith, *et al.*, 2010).

- It should be reminded that the *in utero* transmission considered in both models in fact takes into account *in utero* transmission *sensu stricto* and transmission during the 24-48 hours after birth before the calf is separated from its dam. Information were indeed lacking to model separately *in utero* transmission *sensu stricto* and transmission around birth. It was also considered that the feasibility of a control measure targeting the separation of dam and calf within one hour after the birth was low.

- Several assumptions have been set on animal susceptibility to *Map* infection: the slope of the susceptibility function could be changed. However, the sensitivity analysis performed on susceptibility did not point out a strong influence on the results. No genetic variability has been considered for susceptibility.

Finally, to improve the internal validity of the models, the main knowledge gaps that need to be addressed are the following: the resistance of *Map* in the environment, the existence of pass-through, and the existence and characteristics of super-shedders.

A sensitivity analysis has been performed on the epidemiological model for parameters linked to contact patterns (Chapter 3). Then, outputs have been confronted to field data in order to perform a partial validation (Chapter 2). A better validity of the model could be reached by implementing an epidemiological study adapted for model validation. However, this would necessitate several years of follow-up and a lot of observations. That was not possible within the time available for the thesis and no data corresponding to the modelled situation are available as it is really rare that a farmer does not

implement anything in an infected herd. Another option would be to compare the outputs of a scenario in which control measures are implemented with field data where the same control measures are implemented. However, it is difficult to control whether the measures are well implemented in the field. And most importantly, observed individual and true *Map* prevalences differ because of the low sensitivity of currently available tests. Conversely, it is possible to compare observed and simulated distributions by stochastic modelling. More easily, it also is possible to verify that observed outputs are in the range of credible simulated outputs.

The transmission rates of the epidemiological model are here based on one experimental study (Van Roermund, *et al.*, 2007). The next step would be to validate these parameters from other experimental studies when they will become available. A sensitivity analysis was performed on these parameters (results not shown). It showed that the between-group transmission rate via the environment (infection probability if one infectious dose is shed by any animal) had an influence on the outputs while other transmission rates had no influence. Dividing by ten these different infection probabilities had no impact on the prevalence and persistence, and on the other conclusions of our work. For the economic model, the population dynamics has been validated through previous studies done with this model. The paratuberculosis infection process is based on the outputs of the epidemiological model. While it is possible to compare prevalence outputs with field data (with the limitations of the difference between true and observed prevalence linked to the difficulty to detect animals owing to the low sensitivity of available tests), it is not possible to get field data on persistence. Fadeout probably exists without any control measure is implemented, but it is usually not reported or observable.

Based on these validation steps, the two models presented in this thesis can be used to test assumptions and to qualitatively compare scenarios. They have been used to quantify the submerged part of the iceberg by providing the repartition of the animals in the different infection states such as latent or subclinical infection (Chapter 2). However, all the conclusions of this thesis do not have the same external validity. While different herd sizes have been studied, only one contact structure was assessed for example. It would be necessary to perform similar scenarios adapted to beef herds, to other dairy farming systems, to other breeds to conclude for other types of cattle farming systems in terms of contribution of the different routes of transmission, of influence of contact structure, of stochastic dynamics of *Map* infection, or on test-and-cull strategies impact. For the economic part, quantitative results are linked to the prices chosen. The ranking can be extrapolated, but not the value itself of the economic effectiveness. By multiplying the scenarios studied, a range of quantitative conclusions could be given.

6 Implications

Several studies have reported a low compliance of control programmes when several measures are advised to farmers without pointing out priority

measures (Coursaget, 2009; Taisne, 2009; Nielsen and Toft, 2010; Sorge, *et al.*, 2010). The outputs of the research performed during this thesis can help defining priority measures. It has indeed been shown that the two main routes of transmission are transmission via the global environment of the farm mainly contaminated by adults, and *in utero* transmission (Chapter 2). Furthermore, *Map* transmission in a dairy herd is mainly influenced by the exposure of calves to any environment contaminated by adults' faeces. Delaying this exposure is thus recommended to decrease *Map* prevalence. This can be obtained through systematic strict and early separation of calves from adults in addition to effective hygiene measures.

Return on investment (ROI) was never below 7 years on average in the scenarios simulated in this thesis. This minimum ROI was reached in herds with impaired hygiene when systematic test-and-cull is implemented. Thereby, farmers need to be warned from the start of a control programme that controlling *Map* takes time and that they will need to implement the measures for 5 to 10 years to obtain profitable results. A communication effort is necessary to keep the farmers motivated, to avoid *Map* reintroduction when purchasing cattle, and even to create a desire of getting involved in a control programme. Furthermore, it appeared that the results in terms of prevalence and incidence were better in herds with improved hygiene, while the ROI was longer for such herds. However, it is probable that the hygiene measures implemented towards *Map* infection also are profitable for other diseases and induce a decrease in their incidence. This was not considered in the bioeconomic model used here. It appeared that it was better to implement a test-and-cull strategy than doing nothing in the conditions studied here (infected herds). This was all the more accurate that studied herd presents other concomitant health disorders. Further work would however be relevant to study the impact of the concomitant health disorders both on epidemiological and economic outputs in situations with and without milk production quota. In herds with improved hygiene, test-and-cull strategies were not economically efficient (really long time-lag before ROI). Actually, a decrease of transmission had more impact than test-and-cull strategies. However, the variation of parameters with hygiene is not known. Thus, quantitative conclusions should be taken carefully while qualitative results are to be considered. Observed differences between scenarios in terms of ROI were small.

Economic values on gross margins and return on investment would need to be adjusted if the prices of sale products (milk, cattle especially) depended on the herd status for *Map*. This is currently not the case in the bioeconomic model but could become accurate on the field in the future. Finally, in order to assess the relevance of test-and-cull strategies for any farmer (with a non-infected herd especially), it would be necessary to consider also the probability of purchasing an infected cattle (both for the probability of a unique *Map* introduction and for the probability of *Map* reintroduction).

We here performed a cost-effectiveness analysis. Mean values of discounted gross margins were provided especially. Other outputs could be of interest for

decision making. The variability of the gross margin would be relevant. Knowing the shape of the distribution of this output would indeed enable considering risk aversion. For 2 scenarios resulting in the same mean gross margin, a farmer can indeed chose one or the second strategy based on the minimum / maximum expected gross margins and their probability of occurrence, depending on his risk aversion. Such a variability of the output can be considered in our stochastic bioeconomic model. Furthermore, a farmer can base his decision not only on the result of a cost-benefit analysis, but also on the costs alone which can prevent him implementing one expensive strategy. Such outputs are available in the bioeconomic model. Lastly, non-monetary outputs such as the work load (not considered in our model) or the frequency of the disease can influence a decision. Here, for example, strategies implemented in herds with improved hygiene were not economically profitable, but decreased the frequency of *Map* infection.

Both models can now be used for immediate or longer term purposes. An immediate utilization can be for research. An analysis of the influence of super-shedders is scheduled. The epidemiological model will serve to test the two following biological assumptions: are super-shedders specific animals which always shed a larger load of *Map*, or are they animals that sometimes shed a large amount of *Map* in their faeces, whatever the load they shed the time step before? Depending on the conclusion of the study and after confronting outputs to field data, conclusions and advised control measures could differ. If it is acknowledged that super-shedders are specific animals, it would then be necessary to try to characterize these animals in terms of shedding (especially how much and from when) and if possible to target such animals in order to cull them as soon as possible. Otherwise, it will be more difficult to define targeted control measures. On the other hand, the epidemiological model can also be adapted to study the assumption of passive shedding if new biological knowledge confirms this assumption. Impact of selective culling of most susceptible calves or future super-shedders can also be studied when information on genetic markers will become available as well as the impact of herd size. To meet farmers' demand, the economic model can be adapted to test other detection strategies that are less expensive, such as pooled tests or tests targeting only specific categories of animal. For example, parity 2 and older cows could be targeted in order to limit the cost of the tests as young animals are difficult to detect. Test sensitivity for parity 1 cows is currently really low, and the costs of using such tests on young cattle are probably higher than the advantages. Environmental tests could also be implemented. However, the model is currently designed to study individual testing and would need to be adapted to study herd level outputs.

Another perspective could be to study cohorts and cull all the calves that are born close to the calving of an infectious cow (Benedictus, *et al.*, 2008; Van Genugten, *et al.*, 2009). It has indeed been reported recently that birth clusters of *Map* infection are an important component of maintaining persistent infection levels of dairy farms (Van Genugten, *et al.*, 2009). Vaccination strategies could also be studied.

Finally, working at herd level and considering the probability of introducing an infected animal can influence the individual decision of a farmer. A larger scale could also be targeted with measures at the country or regional levels having impacts on prevalence and on transactions between farms (joint programmes).

References

Anderson, R. and May, R., Infectious diseases of humans: Dynamics and control, Cambridge, U.K., (1999), pp. 768.

Arrigoni, N., Cammi, G. and Belletti, G. L., Persistence of *Mycobacterium avium* subsp. *paratuberculosis* (Map) in field dried hay fertilized with bovine slurry from Map infected herds, Proceedings of the 10th International Colloquium on Paratuberculosis, Minneapolis, Minnesota, USA, (2009), pp. 172-175.

Banks, H. T. and Potter, L. K., Probabilistic methods for addressing uncertainty and variability in biological models: application to a toxicokinetic model, Math. Biosci. (2004) 192:193-225.

Benedictus, A., Mitchell, R. M., Linde-Widmann, M., Sweeney, R., Fyock, T., Schukken, Y. H. and Whitlock, R. H., Transmission parameters of *Mycobacterium avium* subspecies *paratuberculosis* infections in a dairy herd going through a control program, Prev. Vet. Med. (2008) 83:215-227.

Coursaget, S., Review of paratuberculosis action programmes in Western France, Doctorate in Veterinary Medicine Thesis, Nantes, (2009).

Eisenberg, S. W. F., Nielen, M., Santema, W., Houwers, D. J., Heederik, D. and Koets, A. P., Detection of spatial and temporal spread of *Mycobacterium avium* subsp. *paratuberculosis* in the environment of a cattle farm through bio-aerosols, Vet. Microbiol. (2010) 143:284-292.

Epstein, J. M., Why model?, J. A. S. S. S. (2008) 11:12.

Frigg, R. and Hartmann, S., Models in Science, in: E. N. Zalta (Ed.), The Stanford Encyclopedia of Philosophy, The Metaphysics Research Lab, Center for the Study of Language and Information, Stanford University, (2006).

Grewal, S. K., Rajeev, S., Sreevatsan, S. and Michel, F. C., Persistence of *Mycobacterium avium* subsp. *paratuberculosis* and other zoonotic pathogens during simulated composting, manure packing, and liquid storage of dairy manure, Appl. Environ. Microbiol. (2006) 72:565-574.

Groenendaal, H., Nielen, M., Jalvingh, A. W., Horst, S. H., Galligan, D. T. and Hesselink, J. W., A simulation of Johne's disease control, Prev. Vet. Med. (2002) 54:225-245.

Harwood, J. and Stokes, K., Coping with uncertainty in ecological advice: lessons from fisheries, Trends Ecol. Evol. (2003) 18:617-622.

Hyman, J. M., Li, J. I. A. and Ann Stanley, E., The initialization and sensitivity of multigroup models for the transmission of HIV, J. Theor. Biol. (2001) 208:227-249.

Keeling, M. J. and Rohani, P., Modelling infectious diseases in humans and animals, Princeton University Press, (2008), pp. 366.

Kruze, J., Salazar, F. J., Salgado, M. A., Pradenas, M. V., Rosas, A. V., Ramirez, L. A., Mella, A. F. and Alfaro, M. A., Effect of soil type and rainfall regime on the movement and survival of *Mycobacterium avium* subsp. *paratuberculosis* due to dairy slurry application, Proceedings of the 10th International Colloquium on Paratuberculosis, Minnepolis, Minnesota, USA, (2009), pp. 177.

Kudahl, A. B., Ostergaard, S., Sorensen, J. T. and Nielsen, S. S., A stochastic model simulating paratuberculosis in a dairy herd, Prev. Vet. Med. (2007a) 78:97-117.

Kudahl, A. B., Sorensen, J. T., Nielsen, S. S. and Ostergaard, S., Simulated economic effects of improving the sensitivity of a diagnostic test in paratuberculosis control, Prev. Vet. Med. (2007b) 78:118-129.

Lander, A., The edges of understanding, BMC Biology. (2010) 8:40.

Marcé, C., Beaudeau, F., Bareille, N., Seegers, H. and Fourichon, C., Higher non-return rate associated with *Mycobacterium avium* subspecies *paratuberculosis* infection at early stage in Holstein dairy cows, Theriogenology. (2009) 71:807-816.

Nielsen, S. S. and Toft, N., Management practices reducing the test-prevalence of paratuberculosis in Danish dairy herds, Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine, Nantes, France, (2010), pp. 189-200.

Pradhan, A. K., Kramer, A. J., Mitchell, R. M., Whitlock, R. H., Smith, J. M., Hovingh, E., Van Kessel, J. S., Karns, J. S. and Schukken, Y. H., Multilocus short sequence repeat analysis of *Mycobacterium avium* subsp. *paratuberculosis* isolates from dairy herds in Northeastern United States of a longitudinal study indicates low shedders are truly infected, Proceedings of the 10th International Colloquium on Paratuberculosis, Minneapolis, Minnesota, USA, (2009), pp. 30-33.

Rat-Aspert, O. and Fourichon, C., Modelling collective effectiveness of voluntary vaccination with and without incentives, Preventive Veterinary Medicine. (2010) 93:265-275.

Salgado, M. A., Alfaro, M. A., Salazar, F. J., Söderlund, R., Bölske, G., Pradenas, M. V., Mella, A. F. and Kruze, J., Detection and molecular confirmation of *Mycobacterium avium* subsp. *paratuberculosis* in drainage water and forage after application of dairy cattle manure on agricultural soils, Proceedings of the 10th International Colloquium on Paratuberculosis, Minnepolis, Minnesota, USA, (2009), pp. 176.

Shannon, R. E., Systems simulation, Englewood Cliffs, New Jersey, U.S.A., (1975), pp. 368.

Smith, R. L., Strawderman, R. L., Schukken, Y. H., Wells, S. J., Pradhan, A. K., Espejo, L. A., Whitlock, R. H., Van Kessel, J. S., Smith, J. M., Wolfgang, D. R. and Gröhn, Y. T., Effect of Johne's disease status on reproduction and culling in dairy cattle, J. Dairy Sci. (2010) 93:3513-3524.

Sorge, U., Kelton, D., Lissemore, K., Godkin, A., Hendrick, S. and Wells, S., Attitudes of Canadian dairy farmers toward a voluntary Johne's disease control program, J. Dairy Sci. (2010) 93:1491-1499.

Taisne, D., A control programme for paratuberculosis in infected dairy herds in the Ille-et-Vilaine county [in French], Doctorate in Veterinary Medicine Thesis, Nantes, (2009).

Van Genugten, F., Mitchell, R. M., Stehman, S. M. and Schukken, Y. H., Birth clusters of animals infected with *Mycobacterium avium* subspecies *paratuberculosis* in a New York State dairy herd, Proceedings of the 10th International Colloquium on Paratuberculosis, Minneapolis, Minnesota, USA, (2009), pp. 155-158.

Van Roermund, H. J. W., Bakker, D., Willemsen, P. T. J. and De Jong, M. C. M., Horizontal transmission of *Mycobacterium avium* subsp. *paratuberculosis* in cattle in an experimental setting: Calves can transmit the infection to other calves, Vet. Microbiol. (2007) 122:270-279.

Whitlock, R. H., Fyock, T., Schukken, Y., van Kessel, J., Karns, J., Hovingh, E. and Smith, J., Passive MAP fecal shedding in dairy cattle, Proceedings of the 10th International Colloquium on Paratuberculosis, Minneapolis, Minnesota, USA, (2009), pp. 196.

Winston, W. L., Operations Research: Application and algorithms, Boston, Massachusetts, U.S.A., (1987), pp. 1392.

General Conclusion

In the research project of this thesis, we aimed at increasing, thanks to the use of stochastic modelling, the understanding of the epidemiological and economic consequences of selected paratuberculosis control measures in infected dairy herds. Motivated by the review of the literature and current knowledge on paratuberculosis, a new dynamic epidemiological model has been built. In a baseline scenario for transmission parameters, herd management, and housing facilities, fadeout was frequent. After several years, persistent infection was established and never followed anymore by fadeout, in the absence of control measures. The cumulated number of clinically affected animals appeared to be a good indicator of the progression of *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) infection dynamics towards persistent infection, and it furthermore is very easy to use in the field. A threshold of 2 clinically affected animals seemed adequate to trigger control measures in a herd. The evaluation of control measures limiting the early exposure of calves to any environment contaminated by other calf or adult faeces was possible since epidemiological and zootechnical aspects were taken into account. Classifying the different routes of transmission in terms of their relative contribution to the infection within a dairy herd was also possible. In persistently infected herds, the two main routes of transmission were transmission via the environment of the whole farm (contaminated by adult faeces) and *in utero* transmission. Calf-to-calf transmission was minor as well as other known routes of transmission. As a priority, exposure of calves to any environment contaminated by adult faeces should be reduced, particularly at and just after birth when calves are the most susceptible. Culling rapidly clinically affected animals (shedding a large amount of *Map*) should also be targeted. This model has then been coupled to an existing bioeconomic model to compare the effectiveness of implementing a systematic test-and-cull programme from the introduction of an infected heifer in an initially susceptible dairy herd or the same test-and-cull programme for which the implementation is based on clinical surveillance. Limiting the persistence of *Mycobacterium avium* subsp. *paratuberculosis* infection and the prevalence of infectious animals at reasonable costs was targeted. Implementing a test-and-cull programme always improved the situation from an epidemiological perspective. From an economic perspective, it improved the situation for systematic test-and-cull but neither improved nor impaired the situation for standard herds when clinical surveillance was performed. However, economic improvement could be noticed when the level of hygiene of the herd decreased. A systematic test-and-cull programme limited the persistence of *Map* infection and maintained lower within-herd prevalence at a similar cost than doing nothing. If nothing was done, the prevalence reached a high level. There was no value added of culling the last two calves of test-positive cows. The impact of postponing the culling once an animal is detected positive is null on *Map* prevalence and persistence and negative on gross margins. Whatever the programme implemented, obtaining both better epidemiological and better economic results took time. With the

flexible tool constructed here, it is possible to take into account new knowledge, to test hypothesis in relation to infection dynamics, or to study other control scenarios.

Summary in French / Résumé Substantiel en Français

La paratuberculose est une maladie inflammatoire chronique et progressive des intestins des ruminants due à *Mycobacterium avium* subsp. *paratuberculosis (Map)*. Elle est présente dans la plupart des régions d'élevage. Il n'y a actuellement aucun traitement. La paratuberculose entraîne des pertes économiques dans les exploitations atteintes. La production laitière est effectivement limitée par la diminution de l'absorption des protéines et cela avant qu'un animal infecté ne puisse être détecté avec les tests disponibles actuellement. Les signes cliniques caractéristiques sont des diarrhées profuses et un amaigrissement pouvant conduire à la mort si l'animal n'est pas réformé avant. Dans les élevages infectés avec signes cliniques, les pertes peuvent être si grandes qu'un élevage productif ne peut plus être maintenu. Il y a donc un réel besoin de mettre en place des actions de maîtrise de l'infection par *Map*.

La modélisation épidémiologique est une voie de recherche qui permet d'étudier la dynamique d'infection d'un troupeau, sous différents scénarios de maîtrise. Une approche par modélisation est adaptée à l'étude de la transmission de *Map* au sein d'un troupeau et à l'étude de l'impact de programmes de maîtrise. Il est effectivement difficile de mettre en place des études de terrain évaluant la transmission de *Map* du fait du développement progressif de cette maladie. Seuls les bovins de moins de un an semblent sensibles alors que les signes cliniques n'apparaissent qu'au bout de quelques années (2 à 12 ans). Les études de terrain sont d'autant plus difficiles que les tests de diagnostiques actuellement disponibles présentent une très faible sensibilité (entre 0.13 et 0.94 pour les tests indirects) et ne permettent peu ou pas de détecter les jeunes animaux infectés. Par ailleurs, les signes cliniques ne sont pas spécifiques de la paratuberculose et un diagnostic différentiel doit être établit. Enfin, plusieurs actions de maîtrise peuvent être combinées : évaluer l'effet de ces différentes stratégies nécessiterait de comparer de multiples situations. La modélisation permet au contraire de réaliser une telle comparaison pour un coût et un temps raisonnables. Une telle approche peut être utile pour les prises de décisions lors du développement de programmes de maîtrise.

De façon à répondre à la demande des consommateurs en terme de produits sains à un prix bas, l'organisation du programme de maîtrise le plus rentable semble fondamentale dans notre industrie. Dans ce contexte, l'objectif de ma thèse est d'évaluer l'efficacité épidémiologique et la rentabilité économique d'un nombre restreint d'actions et programmes de maîtrises de la paratuberculose en troupeau bovin laitier infectés. L'effet sur la persistance de l'infection et le niveau de prévalence d'un programme de tests suivis de réformes appliqué systématiquement dès l'achat d'un animal infecté dans un troupeau initialement sensible est particulièrement étudié grâce à une approche par modélisation.

Une revue des différents modèles de transmission intra-troupeau de *Map* a d'abord été réalisée de façon à évaluer s'il était nécessaire de construire un nouveau modèle pour atteindre notre objectif (Chapitre 1). De façon à représenter la transmission indirecte de *Map* via l'environnement et la transmission veau à veau, un modèle épidémiologique a ensuite été construit (Chapitre 2). Ce modèle a été utilisé pour mieux comprendre comment *Map* se transmet au sein d'un troupeau bovin laitier, notamment en terme de contribution relative des différentes voies de transmission et de persistance versus extinction de l'infection lorsque aucune action de maîtrise n'est mise en place au sein d'un troupeau. Dans ce modèle épidémiologique, la dynamique de population d'un troupeau bovin laitier est représentée. Il est effectivement nécessaire de considérer précisément la structure de contact lorsqu'une transmission indirecte via l'environnement existe. En troupeau laitier, les veaux et les adultes sont élevés dans des logements séparés, entraînant ainsi une séparation des animaux sensibles (veaux) et des principaux excréteurs (adultes). Les systèmes de logement des veaux laitiers les plus couramment rencontrés en Europe ont été évalués grâce à un questionnaire (Chapitre 3). Cette étude nous a permis de valider les choix réalisés dans le modèle épidémiologique en terme de logement des veaux. L'impact de la structure de contact sur la transmission de *Map* a été évalué grâce au modèle épidémiologique précédemment construit (Chapitre 4). Engin, un modèle bioéconomique a été construit à partir d'un simulateur économique de troupeau pré-existant et le modèle épidémiologique construit lors de ma thèse (chapitre 5). Ce modèle a été

utilisé pour évaluer la rentabilité économique et l'efficacité épidémiologique d'actions de maîtrise sélectionnées.

Deux nouveaux modèles sont maintenant disponibles pour étudier la transmission de *Map* en troupeau bovin laitier et la rentabilité économique de programmes de contrôle. La revue de la littérature a révélé le besoin de construire un nouveau modèle épidémiologique de transmission de *Map* prenant en compte la résistance de *Map* dans l'environnement et la structure de contact entre les animaux d'un troupeau. L'étude grâce au modèle épidémiologique construit lors de ma thèse de la transmission de *Map* en troupeau bovin laitier a révélé qu'en l'absence d'actions de maîtrise, une extinction de l'infection survient dans 70% des cas sur un horizon de simulation de 25 ans. Quand au moins deux animaux infectés cliniques ont été présents successivement ou simultanément en 5 ans, l'infection devient endémique dans 96% des cas, alors qu'en l'absence de cas cliniques, elle le devient dans seulement 5% des cas. Dans les troupeaux infectés persistants, la transmission *in utero* et via l'environnement contaminé de l'élevage sont les deux principales routes de transmission. Empêcher le contact entre veaux lors des premières semaines n'a pas d'impact sur la transmission de *Map*. Il est recommandé de limiter ou retarder l'exposition des veaux aux adultes et de réformer rapidement les animaux cliniquement infectés pour réduire la prévalence de l'infection. Selon l'objectif ciblé en terme de contrôle de l'infection et le niveau d'hygiène du troupeau, différentes stratégies de tests et réformes peuvent être recommandées. L'utilisation de tests puis réformes semble rentable s'il est mis en place de façon systématique dès l'introduction d'un animal infecté dans un troupeau initialement sensible.

Les deux modèles présentés dans cette thèse peuvent maintenant être utilisés pour des objectifs à courts et longs termes que ce soit pour des travaux de recherche fondamentale ou appliquée. Ces outils flexibles permettront effectivement d'étudier d'autres actions de maîtrise en troupeau bovin laitier.

Appendix

List of publications

Paratuberculosis refereed scientific papers

Accepted

Marcé, C., Beaudeau, F., Bareille, N., Seegers, H. and Fourichon, C. Higher non-return rate associated with *Mycobacterium avium* subspecies *paratuberculosis* infection at early stage in Holstein dairy cows, Theriogenology (2009) 71:807-816.

Marcé, C., Guatteo, R., Bareille, N., Fourichon, C. Dairy calf housing systems across Europe and risk for calf infectious diseases, Animal (2010) 4:1588-1596.

Marcé, C., Ezanno, P., Seegers, H., Pfeiffer, D. U., Fourichon, C. Modelling within-herd transmission of *Mycobacterium avium paratuberculosis* in cattle: a review, J. Dairy Sci. (2010) 93:4455-4470.

Nielsen, S. S., Weber, M. F., Kudahl, A. B., Marcé, C. and Toft, N. Stochastic models to simulate paratuberculosis in dairy herds, OIE Scientific and Technical Review (2011) 30(1).

Submitted

Marcé, C., Ezanno, P., Seegers, H., Pfeiffer, D. U., Fourichon, C. Within-herd contact structure and spread of *Mycobacterium avium* subsp. *paratuberculosis* in a persistently infected dairy herd, Manuscript submitted to Prev. Vet. Med., 24 December 2009. Under review.

Marcé, C., Ezanno, P., Seegers, H., Pfeiffer, D. U., Fourichon, C. Modelling the spread of *Mycobacterium avium* subsp. *paratuberculosis* towards fadeout or endemic infection in a dairy herd, Manuscript submitted to Vet. Res., 30 June 2010. Under review.

In preparation

Guatteo, R., Marcé, C., Lehebel, A., Vermesse, R., LeDréan, E., Fourichon, C. Paratuberculosis in cattle: proportion of not detected infected animals in infected herds, In preparation, 2010.

Marcé, C., Ezanno, P., Pfeiffer, D. U., Fourichon, C., Seegers, H. Cost-effectiveness of control strategies for *Mycobacterium avium* subspecies *paratuberculosis* in dairy herds based on clinical *versus* active surveillance, In preparation, 2010.

Other refereed scientific papers

Haddad, N., Marcé, C., Magras, C., Cappelier, J. M. An overview of methods used to clarify pathogenesis mechanisms of *Campylobacter jejuni*, J. Food Prot. (2010) 73(4):786-802.

Conference papers and presentations in scientific conferences

Marcé C., Beaudeau F., Bareille N. Seegers H., Fourichon C. Effects of infection by *Mycobacterium avium paratuberculosis* on fertility of dairy cows, 9th International Colloquium on Paratuberculosis (2007) Tsukuba, Japan, 29 October – 2 November (oral presentation and poster)

Marcé C., Fourichon C., Pfeiffer D. Evaluation of the effectiveness of control measures for prevention of calf infection by *Mycobacterium avium paratuberculosis*: a modelling approach, Society of Veterinary Epidemiology and Preventive Medicine annual meeting (2008) Liverpool, Royaume-Uni, 26-28 march (poster).

Marcé C., Ezanno P., Weber M.F., Seegers H., Pfeiffer D. U., Fourichon C. Transmission assumptions in paratuberculosis models. Society of Veterinary Epidemiology and Preventive Medicine annual meeting (2009) London, UK, 1-3 April (poster prize).

Marcé C., Ezanno P., Fourichon C. Représentation dans les modèles épidémiologiques des hypothèses biologiques de transmission de *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) en troupeau bovin, Journées d'Animation Scientifique du Département Santé Animale (2009) Port d'Albret, France, 25-28 may (oral presentation).

Marcé C., Ezanno P., Seegers H., Pfeiffer D. U., Fourichon C. Influence of between-calves contacts on *Mycobacterium avium paratuberculosis* (*Map*) transmission in a dairy herd, 10th International Colloquium on Paratuberculosis (2009) Minneapolis, United-States, 9-14 August (oral presentation – travel award).

Marcé C., Billon D., Seegers H., Pfeiffer D. U., Fourichon C. Evaluation of the epidemiological and economic effectiveness of paratuberculosis control programmes in infected dairy cattle herds, Workshop on Animal Health Economics (2010) Nantes, France, 14-15 January (oral presentation).

Marcé C., Ezanno P., Seegers H., Pfeiffer D.U., Fourichon C. The influence of contact structure on disease transmission in a dairy herd using paratuberculosis as an example, Society of Veterinary Epidemiology and Preventive Medicine annual meeting (2010) Nantes, France, 24-26 March (oral presentation).

Professional papers and presentations

Marcé, C. Focus : Actualités sur le diagnostic de la paratuberculose [in French], Le Point Vet. (2008) n°287.

Marcé, C. Focus : L'impact de la paratuberculose et sa transmission sont mieux cernés [in French], Le Point Vet. (2008) n°288.

Marcé, C. Question de lecteur : La paratuberculose a-t-elle potentiel zoonotique ? [in French], Le Point Vet. (2008) n°288.

Marcé, C. Focus : Aperçu mondial sur les mesures de maîtrise de la paratuberculose [in French], Le Point Vet. (2008) n°289.

Kasemsuwan, S., Poolkhet, C., Patanasatienkul, T., Buameetoop, N., Watanakul, M., Chanachai, K., Wongsathapornchai, K., Metras, R., Marcé, C., Prakarnkamanant, A., Pfeiffer, D. Qualitative Risk Assessment of the Risk of Introduction and Transmission of H5N1 HPAI Virus for 1-km Buffer Zones surrounding Compartmentalised Poultry Farms in Thailand, Pro-Poor HPAI Risk Reduction. (2009) pp. 46.

Marcé C. Overview of quantitative modelling methods in the context of risk assessment, First workshop as part of project 'Risk assessment for HPAI in the Mekong Region (Part 1)' under the DFID-funded Pro-poor HPAI Risk Reduction Strategies Research Project (Module 2, Risk Assessment) (2008), Bangkok, Thailand, 28-29 April 2008 (oral presentation).

Marcé C., Beaudeau F., Fourichon, C. Effets de la paratuberculose sur la production et sur les performances de reproduction des vaches laitières, Colloque Grand-Ouest (2010) Rennes, France, 27 April 2010 (oral presentation).

Marcé C., Ezanno P., Seegers H., Fourichon C. Apports de la modélisation pour la compréhension des mécanismes de transmission intra-cheptel de la paratuberculose et pour le choix de mesures de maîtrise, Colloque Grand-Ouest (2010) Rennes, France, 27 April 2010 (oral presentation).

Abstract

Paratuberculosis is a worldwide incurable disease of ruminants resulting in a decrease in milk production and slaughter value. The aim of this thesis was to evaluate the epidemiological and economic effectiveness of selected paratuberculosis control programmes in infected dairy herds. A stochastic simulation model has been developed to represent both the population dynamics within a dairy herd and the indirect transmission of *Mycobacterium avium* subsp. *paratuberculosis* (*Map*). It has been coupled to an existing bioeconomic model. The spontaneous within-herd progression of *Map* infection after the introduction of one infected cattle in an initially susceptible herd was studied in the absence of control measure. The effect of within-herd contacts on *Map* spread in a persistently infected herd was investigated. The cost-effectiveness of test-and-cull strategies to control *Map* infection in dairy herds was assessed. Simulation outcomes put forward that, even when no control measure is implemented, fadeout can occur if less than two clinically affected animals are present. In persistently infected herds, the two main transmission routes are transmission via the environment of the farm and *in utero* transmission. Isolating calves from their herd mates during the first weeks of age has no significant impact on *Map* transmission. Limiting or delaying calf exposure to adults and early culling of clinically affected adults are thus recommended to decrease *Map* prevalence in infected dairy herds. Depending on the targeted objective in terms of infection control and the level of hygiene implemented in the herd, test-and-cull strategies can be prioritized. Systematic test-and-cull appears cost-effective both in epidemiological and economic terms if implemented from the day one infected cattle is introduced in an initially fully susceptible herd. The tool designed here is flexible and enables studying other control options within a dairy herd.

Résumé

La paratuberculose est une maladie incurable des ruminants présente à travers le monde entraînant une diminution de la production laitière et de la valeur de carcasse des animaux infectés. L'objectif de ma thèse est d'évaluer l'efficacité épidémiologique et économique d'actions de maîtrise de la paratuberculose en troupeaux bovins laitiers infectés. Un modèle de simulation stochastique représentant la dynamique de population d'un troupeau bovin laitier et la transmission indirecte de *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) a été élaboré puis couplé à un simulateur bioéconomique existant. L'évolution spontanée de l'infection après l'introduction d'un animal infecté dans un troupeau initialement sensible est étudiée en l'absence d'action de maîtrise. L'effet de la structure de contact sur la transmission de *Map* est exploré. La rentabilité de stratégies de maîtrise de l'infection est évaluée. Il en résulte qu'une extinction peut survenir lorsque moins de deux animaux cliniquement infectés sont présents successivement ou simultanément en cinq ans, même en l'absence d'action de maîtrise. Dans les troupeaux infectés persistants, la transmission *in utero* et via l'environnement contaminé de l'élevage sont les deux principales routes de transmission. Empêcher le contact entre veaux lors des premières semaines n'a pas d'impact sur la transmission de *Map*. Il est recommandé de limiter ou retarder l'exposition des veaux aux adultes et de réformer rapidement les animaux cliniquement infectés pour réduire la prévalence de l'infection. Selon l'objectif ciblé en terme de contrôle de l'infection et le niveau d'hygiène du troupeau, différentes stratégies de tests et réformes peuvent être recommandées. L'utilisation de tests suivis de réformes mis en place de façon systématique dès l'introduction d'un animal infecté dans un troupeau initialement sensible semble rentable. L'outil développé ici est flexible et permettra d'étudier d'autres actions de maîtrise en troupeau laitier.