Effects of infection by *Mycobacterium avium paratuberculosis* (*Map*) on fertility of dairy cows

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ABSTRACT

This study aimed at quantifying the variation in fertility of dairy cows according to their *Map*-infection status. The hypothesis of an indirect effect of the infection on fertility was set. Fertility was measured by the non-return rate at first and second services. A non-return was defined as the absence of another artificial insemination (AI) after the first one while the cow was still present. Three different statuses were defined based on both individual and herd test results: positive cow, negative cow in a negative herd and negative cow in a positive herd. 237,612 AI from 72,135 cows in 1,470 herds were studied by logistic regression after adjusting on known factors influencing reproduction. Non-return rate was higher for infected cows compared to negative cows from negative herds (OR of 1.14, or +3.2 point of % of non-return rate). This increase was higher for parity 1 cows (OR of 1.20, or +4.4 point of % of non-return rate) than for other parities. The effects were lower when comparing positive cows to negative cows in the positive herds. Looking at these observations, the hypothesis of *Map*-effect based on the relation between *Map*-infection, production and reproduction is formulated. Due to the lack of protein absorption in the intestine, the milk production is reduced. In the early stages of the infection, this could lead to a lower negative energy balance that could be associated with improved fertility.

INTRODUCTION

In affected herds, Johne’s disease can lead to reduced milk yield, lower slaughter value of clinically infected cows and mortality or premature culling of sick animals. A precise appraisal of the production losses attributable to the infection is needed to evaluate the economic losses in dairy herds. Estimates of reduced milk yield associated with *Map*-infection are abundant (Beaudeau et al., 2007; Gonda et al., 2007; Johnson et al., 2001; Kudahl et al., 2004) with a decrease of mean milk yield estimated between 500 and 1400 kg per cow in the lactation when the infection is detected. A decrease is sometimes noticed as soon as the first lactation (Nielsen et al., 2006). Conversely, there are only few studies analysing the effect of *Map*-infection on fertility and their results are contradictory (Haddad et al., 2003; Johnson-Ifearulundu et al., 2000; Kostoulas et al., 2006). It is all the more difficult to estimate the effect of the infection that the diagnostic of the disease is not easy: first of all because of the low sensitivity of the tests but also because even if the infection occurs early, the diagnostic is always postponed in time.

The objective of this study was to assess if there is an effect of *Map* infection on fertility and more precisely to quantify the difference of fertility between infected and non-infected dairy cows. Since the infection develops with age, we studied the effect by parity in order to see if there is an effect at early stage of infection.

MATERIAL AND METHODS

A retrospective study was performed to compare individual reproductive performance of *Map*-infected or non-infected dairy cows. The data were obtained between January 1, 1999 and February 28, 2007 from herds located in western France that were monitored for *Map*-infection, enrolled in the official Milk Recording scheme and used artificial insemination (AI).

Assessment of fertility

Fertility was assessed by the outcome variable « non-return-to-service » opposite to « return-to-service ». A non-return was defined as the absence of another artificial insemination after a given service while the cow was still present in the herd. Only returns after first and second
services were taken into account. A new insemination after a third service or more is indeed dependent upon the farmer decision in France because in the region of the study, farmer generally has to pay for a fourth service whereas he does not for the second or third.

Map infection status

Map-status can be defined at individual and herd levels based on individual tests. At the herd level, a herd was considered positive as soon as one test was positive. A herd was considered negative when all the tests were negative. At the individual level, a positive cow was a cow with at least one positive test and a negative cow had all its tests negative independently of the type and number of tests performed (ELISA on serum, PCR, Faecal culture, Ziehl-Neelsen staining). Finally, cows were classified into 3 groups: positive cows, negative cows in positive herds and negative cows in negative herds.

Selection of data

Heifers were excluded because their fertility differs from that of cows. In order to limit classification bias for infection, cows vaccinated against paratuberculosis were excluded. Several exclusions were done in order to get rid of cows or lactations with missing information or to get rid of special cases. We also chose to exclude cows culled early after an insemination (less than 200 days after an AI) in order to limit the risk of misclassifying a cow as non-return when she is culled early without knowing if she is pregnant or not (assuming that when kept more than 200 days and in absence of AI, cows were likely to be pregnant). Another reason to exclude cows that were culled less than 200 days following AI is because farmers in France must cull positive cattle quickly following a positive test result if they wish to receive some compensation for her. Our final data set was composed of 237,612 AI from 72,825 cows in 1,472 herds.

Modelling

The effect of the Map-infection cow status on fertility was studied by logistic regression after adjusting on several independent variables described as risk factors for fertility traits in the literature. The confounding factors taken into account were calving-to-service interval, rank of service within lactation, month and year of service, lactation number, herd, inseminator and bull. Diseases, feeding and reproduction management were not recorded and were assumed to be taken into account in the herd effect.

Two models were run. Positive cows were first compared to negative cows in negative herds and then to negative cows in positive herds. For each variable, and each class, the logistic regression model provides odds-ratio (OR) adjusted for other explanatory variables. ORs were converted into relative risks (RR) using Beaudeau and Fourichon’s method (Beaudeau and Fourichon, 1998) and the effects in % of non-return rate were calculated from RRs estimates.

RESULTS

The results reported here are the ones for the model excluding early culling and adjusting for milk yield.

Whatever the parity-group, Map positive status was significantly (p<0.05) associated with non-return-to first and second services (Fig. 1). Non-return rate was significantly increased in positive-testing cows (OR = 1.14 or +3.2 point of % of non-return rate) compared to negative cows from negative herds. A similar trend of lower magnitude was found when comparing positive cows to negative cows from positive herds (OR = 1.11).

For positive cows in first lactation compared to negative cows from negative herds, the effect was greater in first lactation and tended to decrease with each successive lactation (OR of 1.20; 1.09 and 1.05 or +4.4; +2.1 and +1.2 point of % for lactation 1, 2 and 3 and more respectively). When comparing cows in positive herds, the ORs were always lower (even if above 1) than when comparing positive cows to negative cows of negative herds. The association between Map status and non-return-to-service was not significant for positive cows in 3rd lactation or more compared to negative cows of the same herds.
All the adjustment factors had an effect on non-return-to-service as described in literature. Results were comparable for all parities.

**DISCUSSION AND CONCLUSION**

*Map* positive status as here defined was associated with a higher non-return rate after first and second services. The effect of *Map* positive status on fertility varied with age. The non-return rate was higher for young cows and decreased for older cows, suggesting that the effect of infection which results in improved fertility starts early. These results were not expected, as fertility barely improves when concurrent disease happens for diseases frequently investigated.

The effect was lower, but still positive, when comparing positive cows to negative cows of the same herds. This is consistent with the assumption that the bias of misclassifying a cow as negative is higher in a positive herd. In order to prevent the selection bias from the farmers, all the AI were taken into account as soon as the first lactation, before the *Map*-infection status was known.

Milk production is known to influence the fertility of cows. A relation between production and reproduction functions could participate in the biological mechanism relating paratuberculosis to fertility, as both functions depend on the nutrition of cows. *Map* infection influences milk production and milk production influences reproduction. Including simultaneously the two variables could drive to over-adjustment. The model was therefore studied with and without the milk production variable as a confounding factor. The effect was higher when not adjusting on milk production (results not shown).

Hypotheses on possible biological mechanisms underlying the positive effect of *Map* infection on fertility are made. Present knowledge on *Map* does not support a direct effect of the infection on the reproductive tract or on embryo or foetus viability. Tropism for the reproductive tract, or lesions in calves born from infected dams are indeed not reported. A likely possible biological mechanism connecting infection to reproduction could be indirect and bring into play the effect of *Map*-infection, production and reproduction. At early stage of the disease, *Map*-infection causes lesions on the intestine where proteins absorption takes place. The infection could then provoke a decrease of protein absorption (Patterson and Baret, 1968) that could lead to a decrease of milk yield particularly in first lactation. This decrease in yield is associated with a fall of energy use bringing to a reduction of the negative energy balance (normally increasing in early lactation). This lower negative energy balance could then possibly be associated with a better fertility, particularly in first lactation.
The link between Map-infection, milk yield and fertility could evolve with animal age since paratuberculosis develops with time. In young animals, intestinal lesions are small. Energy absorption is likely not influenced at first by Map infection since energy absorption takes place in the rumen while Map lesions are on the intestine. Furthermore, feed intake seems not to be affected by Map infection at least in early stages of the disease contrary to other health disorders. As infection get in a more advanced stage, general status could be affected because of the greater decrease of nutrient absorption. Finally, acute granulomatous enteritis and thickening of intestinal mucous lead to a significant digestive dysfunction. In the long term, significant decrease of nutrient absorption and concurrent diseases could then impair fertility.

The study was mainly performed on herds involved in control programs. The majority of the cows were then subclinically infected and generally culled before symptoms appear. This could explain why fertility was always improved in positive cows of the present study. This study demonstrates that Map infection may have an effect on fertility in dairy herds varying with age, which could occur in the absence of any clinical sign attributable to paratuberculosis. It seems now important to study the presence of a potential link between infection and negative energy balance in order to validate the proposed hypothesis.

ACKNOWLEDGEMENTS
The authors gratefully acknowledge the Centre de Traitement de l’Information Génétique (INRA, Jouy-en-Josas, France) and the Groupements de Défense Sanitaire des Côtes-d’Armor, du Finistère, du Morbihan, de Seine Maritime (France) for providing the data and the French State Veterinary Services for funding the first author’s PhD.

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