

SEROPREVALENCE OF PARATUBERCULOSIS IN DAIRY HERDS IN NORTH WESTERN ITALY (Piedmont Region)*

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SUMMARY

Paratuberculosis (PTB) is a major cause of economic losses in the cattle industry due to reduced milk production, reproductive disorders poor feed conversion, shortened production age and increased predisposition to other disease etc. PTB occurs worldwide with a high frequency. Control programs have been implemented in many countries at regional and even at national level. To estimate PTB prevalence in dairy herds in the Piedmont Region a survey was carried out between January 2007 and June 2008. Random samples from 1531 dairy herd representing 63% of the target population were selected and bulk tank milk samples were tested for the presence of PTB antibodies using a commercially available absorbed ELISA test kit, 3 times a year. Estimated seroprevalence in dairy herds corrected for sensitivity and specificity was found to be 21.75% (CI95%: 19.3%-23.3%). These results underlined the need for a control plan for PTB in the Piedmont Region.

Keyword: *Mycobacterium avium subsp. paratuberculosis*, Bulk-tank-milk, Seroprevalence.

RÉSUMÉ

Les programmes de contrôle de la paratuberculose en Europe ont déjà commencé. En Italie, il n'existe aucun programme de contrôle de la maladie, alors qu'on estime la prévalence entre 10% et 50%. Dans la région du Piémont, pour évaluer la prévalence de l'infection, nous avons testé 1 531 troupeaux échantillons de lait de mélange avec la méthode ELISA (Pourquieser screening/Verification) trois fois par an. La prévalence a été estimée avec @Risk, tenant en compte de la sensibilité et de la spécificité du test.

Nous avons considéré positif un élevage qui a été trouvé positif au moins à un des trois tests. La séroprévalence correcte a été évaluée à 21,75% des troupeaux testés.

Mots-clés : lait de mélange, *Mycobacterium avium subsp. paratuberculosis*, séroprévalence.



I - INTRODUCTION

Paratuberculosis (PTB) is a contagious bacterial disease caused by *Mycobacterium avium* subsp. *Paratuberculosis* (MAP),

responsible for a chronic inflammation of the small and large intestine primarily in ruminants.

* Article reçu le 20 juin 2012, accepté le 10 novembre 2012

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Major reasons for monitoring PTB are economic losses [Ott *et al.*, 1999 ; Nielsen *et Toft*, 2009], in dairy herds, due to reduced milk production, reproductive disorders, poor feed conversion, shortened production age and increased predisposition to other diseases. Moreover, MAP infection has also been suspected to be associated with Crohn's disease in humans [Greenstein *et Collins*, 2004].

PTB diagnosis is usually performed by MAP detection in faecal culture (FC) or by specific antibody ELISAs detection in milk or serum samples.

The disease has been described around the world [Lombard, 2011] in most of livestock production systems. To reduce MAP occurrence several countries have experienced PTB control programmes at regional or national level. In order to set an efficient and effective PTB control program data about PTB estimated prevalence are needed. To obtain information about the prevalence of PTB in a country or in a large

region bulk milk tank (BMT) testing is a convenient and cost effective screening of dairy herds [Nielsen *et al.*, 2000].

In Europe, herd level prevalence has been estimated to be between 30 and 50% [Arrigoni *et al.*, 2007], but probably the prevalence of MAP infection is higher than 50% in most countries especially in dairy herds [Nielsen *et Toft*, 2009].

In Italy, herd-level prevalence estimates are limited to some regions: Veneto [Robbi *et al.*, 2002] Lazio [Lillini *et al.*, 2005], Lombardia [Arrigoni *et al.*, 2007], and Umbria [Cenci Goga *et al.*, 2008]. Recent studies conducted in different areas have indicated a high prevalence of MAP positive herds ranging from 10% to over 70% in Lombardy and Veneto Regions [Pozzato *et al.*, 2011]. In Piedmont reliable estimates of the PTB dairy herd-prevalence are not available.

The purpose of this work was to describe the seroprevalence of PTB in a random sample of dairy cattle herds by BMT in North-Western Italy (Piedmont Region).

II - MATERIAL AND METHODS

To estimate PTB herd prevalence in dairy herds in the Piedmont Region a survey for the detection of MAP antibodies in BMT samples was carried out between January 2007 and June 2008.

Dairy herds were randomly selected from the regional database used for the identification and registration of the Piedmont cattle population. The number of herds to be sampled was set considering an expected prevalence of 50%, 99% Confidence interval and an absolute error of 3%. A total of 1531 dairy herds representing 63% of the targeted population were randomly selected using SAS® proc survey. Sampling design was simple with replacement. Herds were controlled three times a year to increase the sensitivity of the study. One herd was classified positive if at least one positive outcome occurred during the three trials. BMT was collected by the Veterinary National Health Service from cows older than 24 months.

Bulk milk samples were tested for the presence of antibodies against MAP using an indirect enzyme-linked immunosorbent assay

(ELISA). In particular, a commercially available adsorbed ELISA test kit (Pourquier-Screening/Verification) was used. All samples were tested according to the manufacturer's instructions. The specificity of the test is 100%. Test sensitivity is 18% [Rubinetti *et al.*, 2008] calculated on 30 dairy herds where mean herd size was 200. When a test is performed three times per herd a year sensitivity increases until 53% considering parallel interpretation and the « OR rule » according to Weinstein (2005) as the formula is:

$$Se_1 + Se_2 + Se_3 - (Se_1 \times Se_2 \times Se_3)$$

True prevalence was estimated by frequentist approach (Equation 1) using the formula of Rogan and Gladen [1978] where p^* is true prevalence, $\hat{p}$ is apparent prevalence, α is specificity, and β is sensitivity:

$$\hat{p} = \frac{\hat{p} + \beta - 1}{\alpha + \beta - 1}$$

Equation 1

A herd was considered "infected" if at least one BMT positive sample was detected.

The association between occurrence of PTB and the following factors (herd size, grazing, geographic position, presence of goats/sheep in the farm) was evaluated by Pearson Chi-square test. Herd size was reduced to categorical variable based median value. The variables found statistically significant ($p < .05$)

at bivariate analysis were entered into a multivariate logistic regression model to control for confounding. Odds ratio were estimated. Statistical analysis was carried out with SAS® software v9.2 [SAS Institute Inc., Cary, NC, USA]. Spatial distribution of sampled dairy herds was represented with ARCGIS® (ESRI).

III - RESULTS

A total of 4,718 BMT tests were performed. The dairy herds were 1531, however only 1406 were tested three times. 125 herds were tested four times as few cows were present during the third sampling. Positive samples were $n=203$, positive dairy herds were $n=145$. Apparent herd prevalence was 9.48% (CI95%: 8%-11%). True herd prevalence was estimated at

21.75% (CI95%: 19.3%-23.3%). Distribution of sampled dairy herds is shown on Figure 1.

Results of bivariate analysis are shown in Table 1. The logist regression confirmed results of bivariate analysis as grazing pattern and herd size were related to PTB positivity (Table 2).

Figure 1
Geographic distribution of dairy selected herds

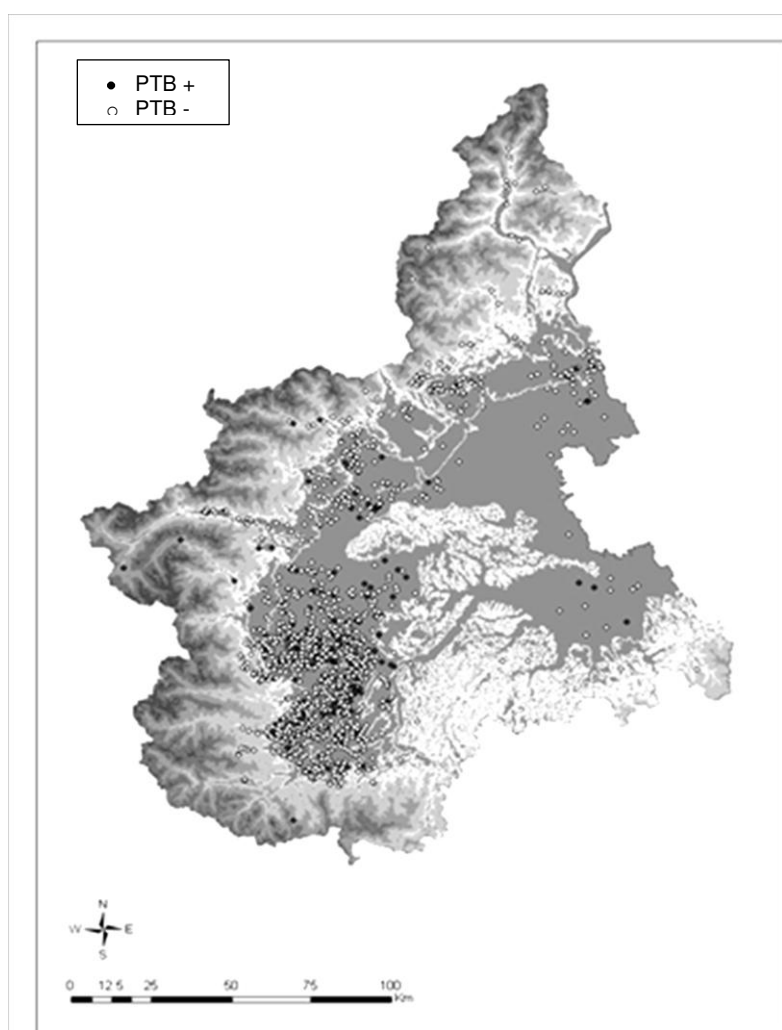


Table 1
Results of bivariate analysis

Factors	Level	reference	Chi-square	OR	CI95%	Pr >
Presence of goats/sheep in the farm	Not/yes	yes	0.68	-	-	- 0.41
Geographic distribution	South Piedmont (Cuneo, Asti, Alessandria provinces)	South	0.09	-	-	-
	North Piedmont (Turin, Novara, Vercelli, Biella and Verbania provinces)					
Herds size	< 129 heads / > 129 herds	< 129 heads	8.9731	1.3	1.1	1.5 0.0027
Grazing pattern	Not/Yes	Not	4.8541	1.7	1.1	2.6 0.0276

Table 2
OR estimate calculated by regression model

Factors	Level	reference	OR	CI95%	
Herds size	< 129 heads / > 129 herds	< 129 heads	1.7	1.2	2.4
Grazing pattern	Not/Yes	Not	1.7	1.1	2.6

IV - DISCUSSION

Estimated herd prevalence of PTB (21.75%; 19.3%-23.3%) in dairy herds corrected by sensitivity and specificity of the test used was lower than those reported by Pozzato [2011] in Veneto (71%) and Lombardia (70%). The difference could be explained as BMT will only provide a positive result consistently in a herd with a high level of infection [Nielsen et al, 2000]. Hence a negative result does not exclude PTB infection within the herd and further testing of individual animals may be required. Results of bivariate analysis are similar to those reported from other studies as

large herds are associated with the occurrence of PTB, and the probability of MAP detecting is depending on the size of the herd. Grazing was also associated with PTB, the herds that performed grazing being more likely to be PTB positive.

Geographic distribution was not related to PTB as herd prevalence was not different between the North and the South of the Region checked in this study.

Results highlight the need of a control plan for PTB in the Piedmont Region.

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