

# ***Survey on paratuberculosis prevalence in dairy herds of the Lombardia Region (Italy)***

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391 dairy herds, stratified for size and province, were selected with the purpose of estimating paratuberculosis herd prevalence in the Lombardia region, a Northern Italian Region where 15.000 dairy herds and 45% of the national milk production are concentrated. The prevalence of infected herds was estimated by a commercial ELISA test (Institut Pourquier) on individual blood samples of 38.487 dairy cattle over 12 months. The percentage of herds showing at least one seropositive animal was 43.7%, while the percentage of seropositive cows was 2.6%. On the basis of the performances (sensitivity 45%, specificity 99%) of ELISA test on blood, the herds that didn't reach a herd specificity of 95% were tested for confirmation by fecal culture on the seropositive cows. The resulting estimated herd prevalence was 19.2%.

## **Introduction**

Paratuberculosis, an infectious and contagious disease caused by *Mycobacterium avium* subsp. *paratuberculosis* (Map), is reported all over the world. In Europe, the herd prevalence varies between 7 and 55% (FIL-IDF, 2001). Besides causing economic losses to dairy and beef herds, Map is regarded as a possible infectious agent of Crohn's Disease in man.

Given that the herd prevalence estimation is fundamental for planning a control programme, we have carried out a survey on an Italian northern region, Lombardia, in which 42% of the overall Italian milk production is concentrated.

## **Material and methods**

In the period between October 2003 and March 2005, we carried out a serological survey for the detection of Map antibodies in blood samples of 38,478 cows over one year of age, belonging to 391 dairy herds of the Lombardia Region; the tests for paratuberculosis were carried out on the samples collected by the Veterinary National Health Service for Brucellosis and Leukosis eradication programmes.

The 391 sampled herds, corresponding to nearly 3% of the 15,111 dairy herds of the Lombardia Region, were stratified for geographical distribution and size.

In the selected herds, the number of dairy cows over 12 months varied between 10 and 584.

The blood samples were submitted to a screening analysis using a commercial ELISA kit (Institut Pourquier, Montpellier, France); all the reactive (positive and inconclusive) samples were analysed using a verification ELISA test (Institut Pourquier, Montpellier, France).

It was established that the herds with a minimum number of seropositive animals (over a statistical threshold, see table 1) were considered as infected, taking into consideration the following parameters:

- Sensitivity 45%
- Specificity 99%
- Lower prevalence in infected herds 5%
- Confidence limits 95%
- Minimum herd-level specificity 95%

The threshold was related to the consistency of the herd and is reported in the following table.

Herd size (cows >1 year)	Threshold of ELISA positive cows
6-35	2
36-82	3
83-137	4
138-198	5
199-262	6
263-329	7
≥330	8

Table 1: Threshold of ELISA positive cows in relation to the herd size

Two different values of herd prevalence were calculated:

- *Apparent prevalence*: calculated by classifying a herd as positive if it contained at least one positive animal
- *Corrected prevalence*: calculated by classifying a herd as positive if it contained a number of seropositive animals over the threshold reported in table 1.

The seropositive cows belonging to the “inconclusive herds” (number of ELISA-positive animal below the threshold), were submitted to confirmation by faecal culture.

The confirmation by faecal culture, performed by a sedimentation method (Taddei e coll. 2004), was introduced as a further control to avoid the possible underestimation in herds with a very low prevalence.

## Results

Out of 391 tested herds, 171 registered at least one seropositive cow; consequently the *apparent prevalence* of infected herds was 43.7%, while the *corrected prevalence* was 16.1% (63 herds with a number of seropositive animals over the statistical threshold).

On the whole, the seropositive animals were 982 over 38,487 submitted to analysis (2.6%).

108 herds (27.6%) were classified as “inconclusive”; the seropositive animals belonging to 73 of these herds were submitted to faecal culture, while in the other 35 herds it was impossible, due to the immediate slaughtering of the reactors.

This further research, directed to minimize the underestimation of the corrected prevalence, can only partially reduce it, mainly because it was impossible to sample all the inconclusive herds, and also because the faecal culture has a low sensitivity (40%); for this reason it is possible that a percentage of infected seropositive animals, negative to faecal culture, were infected but shedding an undetectable number of Map in their faeces.

On the whole, 185 faecal samples were collected and submitted to culture, of which 43 tested positive.

Out of 73 inconclusive herds submitted to faecal culture, 12 turned out positive and 61 negative.

Taking into consideration these results, the number of infected herds went up to 75 and the corrected prevalence from 16.1% to 19.2% (c.i.95% 15.4%-23.4%).

It must be noted that in smaller herds (10-50 cows > 12 months) the corrected prevalence is 7.8%, while in medium sized herds (51-100 cows > 12 months) it went up to 20%, and to 37% in bigger herds (>100 cows > 12 months).

Number	10-50 cows	51-100 cows	>100 cows	Total
Tested	192	80	119	391
Positive	40	36	95	171
Infected	15	16	44	75
<b>Apparent prevalence</b>	20.8%	45.0%	79.8%	43.7%
<b>Corrected prevalence</b>	7.8%	20.0%	37.0%	19.2%

Tab.2: Prevalence related to herd size

Analysing the data of the “in-herd” prevalence, it appears clear that only 9.3% of the infected herds show a high prevalence (>15%), while respectively the 50.7% and the 40.0% of the infected herds are medium (5-15%) and low (<5%) prevalence herds.

### **Discussion and conclusions**

The results of this survey carried out in the Lombardia Region, if compared with the data reported by previous surveys carried out in Veneto (apparent prevalence 64.9%, corrected prevalence 26.7%, percentage of seropositive animals 3.5%) showed lower results.

Otherwise, the data observed in Lombardia are similar to that of the Lazio Region (Lillini et Al. 2004), where the apparent prevalence was 42% (corrected prevalence undetermined) and the percentage of seropositive animals 2.4%.

Furthermore, the risk of a herd being infected is directly related to the herd size, confirming the results of the survey carried out in Veneto; this could be due, apart from a higher probability of detecting a positive animal in a bigger herd, to a higher frequency of purchasing animals in big herds, or to the modern breeding technologies (free stalls) that enhance the risk of infection.

In general, the “in-herd” prevalence appears low (90.7% of herds show a sero-prevalence lower than 15%), and this should be regarded as favourable for the farmers that intend carrying out a control plan in their herds.

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