BULK MILK CONTAMINATION BY *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS* AND RELATED RISK FACTORS

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52 dairy herds, infected with *Mycobacterium avium* subsp. *paratuberculosis* (Map), were submitted to repeated bulk milk sampling. On 183 samples tested by culture and nested-PCR, 20 (11%) resulted positive for Map. In the risk factors analysis for milk contamination, both the paratuberculosis herd prevalence and the hygienic measures to control the fecal contamination were taken into consideration; the risk of milk contamination appears directly related both to the infection prevalence in the herd and to udder hygiene. In contrast, neither the ideal hygienic measures in routine milking, nor proper milk filtration were effective in preventing the presence of Map in milk.

Introduction

Mycobacterium avium subsp. *paratuberculosis* (Map) is considered one of the possible infectious agents of Crohn's disease in man. The aim of the present paper is to evaluate, in dairy cattle infected herd, the resultant risk of contamination of bulk milk by Map.

Materials and methods

This study was carried out on 52 dairy herds, previously classified as positive for Map infection by ELISA test (Institut Pourquier), performed on individual blood samples of cows over one year of age. When the number of seropositive animals was below a statistical threshold, the positivity was confirmed by fecal culture (Arrigoni *et al.* 2006).
On the basis of the prevalence of seropositive animals, the herds were classified as:

- "low prevalence" herds (<5% seroprevalence),
- "moderate prevalence" herds (5-15%),
- "high prevalence" herds (>15%).

The bulk milk of the infected herds was repeatedly sampled, each farm being submitted to an average of 3.5 samples, at a frequency interval of at least 30 days with the aim of sampling all the lactating cows, resulting in a total of 183 bulk milk samples being collected.
The culture was performed following the method suggested by Dundee *et al.* (2001). PCR tests were performed, after magnetic separation (Adiapure kit, Adiagene), by a nested "home made" protocol. An anamnestic questionnaire was offered to each farmer, to evaluate the risk factors for bulk milk contamination by Map.

Results

Of a total of 183 samples, 176 were submitted to analysis, the remaining 7 were not because the milk curdled (tab. 1). On the whole, there were 20 positive samples, one of which was positive by both culture and PCR (tab. 2).

Prevalence	***Number of herds***	***Number of samples***
Low	21	68
Moderate	27	93
High	4	15
total	**52**	**176**

Table 1. Characteristics of the sampling programme.

	Culture +	***Culture -***	***Contaminated cultures***	***Total PCR***
PCR +	1 (0.6%)	10 (5.7%)	8 (4.5%)	19 (10.8%)
PCR -	1 (0.6%)	135 (76.7%)	21 (11.9%)	157 (89.2%)
Total cultured	**2 (1.1%)**	**145 (82.4%)**	**29 (16.5%)**	**176 (100%)**

Table 2. Analysis results on the 176 samples examined.

The 20 positive samples were distributed as follows:
- 3 samples (4.4%) of 68 coming from “low prevalence” herds,
- 10 samples (10.8%) of 93 coming from “moderate prevalence” herds,
- 7 samples (46.7%) of 15 coming from “high prevalence” herds.

On the whole, taking into consideration the herds, 11 herds of 52 controlled (21.2%) registered at least one positive sample, and were distributed as follows:
- 3/21 (14.3%) “low prevalence” herds,
- 5/27 (18.6%) “moderate prevalence” herds,
- 3/4 (75.0%) “high prevalence” herds.

All the herds whose milk tested positive in culture, tested positive at least once by PCR.
Among the 11 herds producing contaminated milk,
- 6 herds, tested positive on a single sample;
- 4 herds, on more than one sample, but not on all the samples;
- 1 herd, on all the samples.

Farm data in relation to milk contamination

On a whole, 49 anamnestic questionnaires were collected from infected farms, of which 11 were producers of contaminated milk; in 3 cases it was not possible to obtain the data.

Herd size

On the basis of the number of cattle >12 months of age, the herds examined were classified as: small (<100 heads); mid (101-200 heads), and large size (>200 heads). Map was detected on bulk milk in 5 out of 11 mid size herds (45.5%), in 4 out of 20 large herds (20.0%), and 2 out of 18 small herds (11.1%).

Incidence of clinical cases

In 42.1% of infected farms producing uncontaminated milk and in 72.7% of infected farms producing contaminated milk, clinical cases of Paratuberculosis were recorded. Moreover, while the disease had a low incidence in the first case (lower than 2% in 97.4% of cases), in farms producing contaminated milk the incidence was over 2% in 54.6% of cases. All the farms in which the incidence of clinical cases was over 5%, in addition to those in which clinical cases in heifers were registered, produced contaminated milk.

Bedding conditions

In general, the hygienic conditions of bedding was **good** or **quite good**. Relevant differences between farms producing contaminated and uncontaminated milk were not registered.

Udder hygiene

In 87% of infected farms producing uncontaminated milk, udders were **free of dirt** or **only slightly dirty**, while 13% were **moderately dirty**, but never **very dirty**. Among farms producing contaminated milk, the percentage with **dirty udders** (from **moderately** to **very dirty**) was 36%.

Milking system employed

Milking in tie stall barns, being carried out in the same environment where the cows live, is generally considered a risk factor for fecal contamination of milk. From our study, 18% of infected farms adopted this kind of milking. In spite of this, no farms producing contaminated milk belonged to this category. On the contrary, all the farms producing

contaminated milk had milking parlours, 54% of which were fishbone parlours, which in theory should give the best safeguard against fecal contamination of milk.

Milking machine and bulk cooling tank hygiene

In 91% of farms producing contaminated milk, the bulk bacterial count (BBC) was <50,000 cfu/ml and in 100% BBC was <100,000 cfu/ml. Therefore the BBC cannot be considered a significant indicator for milk contamination by Map, as presumably, given that the BBC is generally a consequence of milking machine and bulk cooling tank hygiene, as well as rapid and proper refrigeration. The BBC can only be minimally influenced by milking routine hygiene and by udder hygiene, which are directly related to fecal contamination of milk.

Milking hygiene

Among farms producing contaminated milk, 27.3% did not clean the udder properly, while 72.7% did, 45.5% of which did so in an excellent way (cleaning with disinfecting towels and wiping with individual paper towels); the hygienic measures adopted in these herds were equal or superior in comparison to farms producing uncontaminated milk.

Milk filtration

In more than 90% of farms overall milk was filtered, with the filters being changed at least daily.

Risk factors analysis

To assess the risk of milk contamination by Map, odds ratios (ORs) were estimated using multiple logistic regression. The findings are presented on Figure 1 as ORs with 95% confidence intervals (CI). After exploratory analysis, only occurrence indicators (seroprevalence, and clinical cases incidence), herd size, and udder hygiene could be included in the logistic model. Because the low number of observations, only high clinical cases incidence (>2%), and number of adult cattle between 101 and 200 resulted significatively associated to Map contamination in milk.

Logit estimates — Number of obs = 49; LR chi2(8) = 31.35; Prob > chi2 = 0.0001; Log likelihood = -10.421108; Pseudo R2 = 0.6006

		Odds Ratio	Std. Err.	z	P>z	[95% Conf.	Interval]
Clinical cases incidence	No cases	1.00	-	-	-	-	-
	<2%	1.708	3.088	0.30	0.767	0.049	59.025
	>2%	236.052	549.850	2.35	0.019	2.456	22686.02
Udder hygiene	Free of dirt	1.00	-	-	-	-	-
	Slightly dirty	0.082	0.141	-1.46	0.145	0.003	2.364
	Dirty	6.738	12.847	1.00	0.317	0.160	282.837
Sero-prevalence	Low	1.00	-	-	-	-	-
	Moderate	1.001	1.367	0.00	0.999	0.069	14.558
	High	49.699	135.149	1.44	0.151	0.241	10258.03
No. Cattle >12 months	1-100	1.00	-	-	-	-	-
	101-200	374.844	1017.147	2.18	0.029	1.837	76489.42
	>200	14.476	29.675	1.30	0.192	0.260	804.574

Fig. 1 – Factors associated to the presence of Map in bulk milk in infected herds

Discussion and conclusions

Culture of bulk milk samples, although producing promising sensitivity results under experimental conditions, proved problematical due to frequent contamination by environmental microorganisms, *M. porcinum* in particular. These organisms have been shown to inhibit the growth of Map, giving rise to false negative results which impacts on the sensitivity of the diagnostic method (Taddei *et al.* 2005).

It is likely that the difficulties encountered in collecting milk from the whole large area of the Lombardia Region and the prolonged storing of the samples, before transporting them to the laboratory, negatively influenced the sample quality and therefore the results.
If performed on bulk milk, PCR appears more rapid and sensitive than culture, because it revealed milk contamination by Map more efficiently than culture (PCR 10.8% vs. culture 1.1%) in samples from proven infected herds. In total, 21.2% (C.I.95%: 11.1% - 34.7%) of infected farms tested positive by culture and/or PCR.
The presence of Map in milk is related to seroprevalence: a higher percentage of milk samples from high seroprevalence herds tested positive (46.7%) compared to those exhibiting moderate or low seroprevalence (10.7 and 4.4% respectively). It should be noted that repeated sampling greatly increased the percentage of positivity.
The contamination of bulk milk by Map is related to both endogenous and exogenous contamination; the former by lymph-haematogenous spreading and the latter as a result of fecal contamination of udder skin which occurs particularly in high prevalence herds which practice poor hygiene (Arrigoni et al. 2004).
From the anamnestic data collected, the risk of milk contamination appears directly related both to the infection occurrence in the herd (seroprevalence, and high clinical case incidence, particularly in young animals), and to udder hygiene. Aniway, neither the ideal hygienic measures in routine milking, nor proper milk filtration were effective in preventing the presence of Map in milk. The mid size of the herd (101-200 cattle >12 months) seems to be another risk factor; there are probably other management practices (e.g. delivery hygiene, feeding, etc.), not covered by the questionnaire, associated with presence of Map in bulk milk.
Therefore, in order to reduce the milk contamination risk by Map, suitable actions should be taken into consideration: on the one hand the infected cows should be culled, in particular the “heavy shedders”, and on the other the hygienic, sanitary and managerial measures should be implemented to reduce the fecal contamination of udders.
As recommended by several European Sanitary Authorities, it is necessary to start to take suitable measures to reduce the herd prevalence, with the aim of limiting the food chain contamination and hence the risk to man through exposure to this potential pathogen. The problem appears particularly urgent for farms that sell raw milk; in those cases it is necessary for them to introduce, in their “own health checks”, sanitary parameters which would limit contamination of milk by Map as well as other pathogens.

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