Mycoplasma bovis Seroprevalence in Israeli Dairy Herds, Feedlots and Imported Cattle

Lysnyansky, I.,[1*] Mikula, I.,[1] Ozeri, R.,[2] Bellaiche, M.,[2] Nicholas, R.A.J.[3] and van Straten, M.[4]

[1] Mycoplasma unit, Division of Avian and Aquatic Diseases, Kimron Veterinary Institute PO Box 12, Beit Dagan 50250, Israel.
[2] Israeli Veterinary Services, PO Box 12, Beit Dagan 50250, Israel.
[3] The Oaks, Upper Hale, Farnham, Surrey GU9 0HG, UK.
[4] Hachaklait Veterinary Services Ltd., PO Box 3039 Caesarea, Israel.

*** Corresponding author:** Dr.Inna Lysnyansky, Tel: +972-3-9681689, Fax: +972-3-9681739. Email:innal@moag.gov.il

ABSTRACT

A total of 2079 sera were tested between 2010 and 2011 for antibodies to *Mycoplasma bovis* by BIO K162 ELISA kit. At the recommended cut-off "≥+", animal seroprevalence was 89% for local cows, 94% for local beef calves and 68%, 74% and 94% for calves imported from Lithuania, Hungary and Australia, respectively, with herd/shipment-prevalence of 100% for all groups. These values changed to 30% and 59% for cows and feedlot calves, respectively, and to 31%, 38% and 77% for calves imported from Lithuania, Hungary and Australia, respectively when the cut-off was increased to "≥++". The herd prevalence, however, was still high (83-100%). ELISA values found in local dairy cows were significantly lower ($p < 0.001$) than those in calves. Notably, 42% of Australian calves showed high "≥+++" ELISA values.

Keywords: Cattle; *Mycoplasma bovis*; ELISA; Seroprevelence; Cattle Importation.

INTRODUCTION

Mycoplasma bovis is a bovine pathogen which can cause mastitis, pneumonia, arthritis, otitis, genital disorders and keratoconjunctivitis (1). It is also recognized as a key pathogen associated with bovine respiratory disease complex (BRDC). Since *M. bovis*-associated disease frequently results in chronic infection and persistence in the herds, there is an adverse impact on the welfare of cattle livestock in addition to major economic losses. In Israel, despite the detection of *M. bovis* from 26-65% of BRDC cases submitted to Kimron Veterinary Institute during 2005-2008 (Lysnyansky et al., unpublished results), the prevalence of *M. bovis* in the general cattle population is unknown. During the period of 2008-2014 Israel imported about106,000 calves annually from several European countries in particular from Lithuania, Hungary, and Australia (annual reports, Israeli veterinary services; http://www.vetserv.moag.gov.il/vet). Trade in live animals can lead to the introduction of new *M. bovis* strains. In addition, imported calves naïve to *M. bovis* may become infected with endemic strains leading to substantial economic losses. Therefore, the aim of this study was to investigate the seroprevalence of *M. bovis* in: (i) dairy cows; (ii) calves raised for meat (feedlots); and (iii) calves imported into Israel. This will enable the development of future prevention and management strategies.

MATERIALS AND METHODS

Samples

The survey was conducted between January 2010 and September 2011. Total 2,079 sera were collected from 32 dairy herds (n=696 cows) and 24 feedlots (n=419 calves),

which were randomly selected from all over the country as well as from 19 shipments of calves imported from Lithuania (n=366 animals), 16 from Hungary (n=303) and 13 from Australia (n=295). The sera from the imported calves were taken while in quarantine before their transfer to farms.

The median number of samples per herd/shipment was 20 (range 7-41). Calves from local feedlots were 3-7 months old, from Australia were 6-9 months old, from Lithuania and Hungary were 3-4 months old and dairy cows were more than two years old.

Serology

Blood samples (about 5 ml per animal) were aseptically collected and sera were tested for antibodies to *M. bovis* by using a commercial enzyme-linked immunosorbent assay (ELISA; BIO K162 or (Bio-X Diagnostics, Jamelle, Belgium)). The test was done according to the manufacturer's instructions.

In brief, the kit was an indirect ELISA, where the plate's odd columns contained the recombinant protein whereas the even columns contained a negative control antigen. The calculation of the samples' values was done by subtracting the signal reading of the negative control microwell from that of the positive microwell sensitized by the bacteria [Val = (OD sample – OD negative control)/(OD positive control – OD negative control) × 100%]. Comparison of the received values with those given in the quality control data sheet enabled quantification of unknown serum antibodies. The ranges of the values correspond to a scale ranging from 0 to +++++. The signal value numerical limits were as follows: for BIO K162 kit - Val_0< 8.87 < Val_+ < 33.85 < Val_{++} < 58.82 < Val_{+++} < 83.80 < Val_{++++} < 108.78 < Val_{+++++}. As recommended by the kit manufacturer, a sample was considered to be positive if the result was "≥ +". A herd was deemed positive for *M. bovis* if it included at least one positive animal.

Statistical analysis

Statistical analysis was performed using SAS 9.4 (SAS/STAT Software. Release 9.2. SAS Inst. Inc. Cary, NC). For binomial proportions, exact confidence intervals were calculated using the EXACT option in PROC FREQ. The association between testing positive for *M. bovis* (dependent variable) and geographic area and farm type (independent variables) was quantified using a simple logistic regression model (PROC LOGISTIC). Associations were expressed as odd ratios.

RESULTS AND DISCUSSION

High individual animal and herd seroprevalence was found in this study. Indeed, interpretation according to kit instructions revealed animal seroprevalence of 89% for local milking cows, 94% for local beef calves and 68%, 74% and 94% for calves imported from Lithuania, Hungary and Australia, respectively. The herd/shipment-prevalence was 100% for all the groups (Table 1), while the average number of positive animals in herds/shipments ranged from 69% to 96% (data not shown). Similar high animal (76.7%) and herd (80.9%) *M. bovis* seroprevalence was reported in Polish cattle using the same ELISA BIO K162 kit (2). The authors mentioned that when the lower value ("≥+") was discounted from the ELISA interpretation, the prevalence was reduced to 28.2%, reflecting the levels reported in other European countries. In addition, recently the performance of the ELISA kit (BIO K302) for herd-level diagnostic of *M. bovis* in bulk tank milk was evaluated by comparison to a PCR test (Patho-Proof

Table 1: Seroprevalence of *Mycoplasma bovis* in Israeli dairy herds, feedlots and calves imported from Lithuania, Hungary and Australia

Samples		Between-herds					Within-herds				
		Cut-off "≥ +"			Cut-off "≥ ++"		Cut-off "≥ +"			Cut-off "≥ ++"	
		No. herds tested	No. positive	Prevalence (%)	No. positive	Prevalence (%)	No. animals tested	No. positive	Prevalence (%) (CI 95%)	No. positive	Prevalence (%) (CI 95%)
Farm type (local cattle)	Dairy	32	32	100	27	85	696	621	89 (87-91)	211	30 (27-34)
	Feedlots	24	24	100	20	83	419	393	94 (91-96)	249	59 (55-64)
Beef calves (import)	LU	19	19	100	17	89	366	250	68 (63-73)	114	31 (26-36)
	H	16	16	100	15	94	303	225	74 (69-79)	115	38 (32-44)
	AU	13	13	100	13	100	295	279	94 (94-97)	226	77 (71-81)

CI = confidence interval.
LU=Lithuania; H= Hungary; AU=Australia.

Mastitis Major-3 kit, Thermo Fisher Scientific, Helsinki, Finland) (3). The authors showed that the ELISA would have more favorable specificity and continue to have a better sensitivity, compared to the PCR, if the cut-off was increased.

In our study, using a higher threshold for the test (i.e "≥++") as a cut-off for positivity revealed an animal-seroprevalence of 30% and 59% for dairy cows and local feedlot calves, respectively, and of 31%, 38% and 77% for calves imported from Lithuania, Hungary and Australia, respectively. The herd prevalence, however, was still high, 83-100%. (Table 1).

In infected herds, the average number of animals showing "≥++" per herd was 30% and 53% on the dairy farms and feedlots, respectively, and 31%, 36% and 79% per shipment arriving from Lithuania, Hungary and Australia (data not shown). The odds of testing positive for *M. bovis* in local calves were 2.2 ($p = 0.002$) times greater when compared with those in dairy cattle. In addition, the odds of testing positive for *M. bovis* were associated with geographic area ($p < 0.001$) (data not shown).

Overall, the ELISA values observed in dairy cows were significantly lower ($p < 0.001$) than the values observed in local feedlot calves. Indeed, 70% of cows were negative or had low ("+") values in comparison to 41% of feedlot calves having the same values (Fig. 1), suggesting the lower susceptibility of adult animals to infection or/and that the infection may not be very active in adults. There was the possibility that some *M. bovis* positive sera obtained from calves might be result from residual maternal antibodies. However, the half-life of maternal antibodies against *M. bovis* is reported to be about 20 days (4) so the values are predicted to be nominal by a few months of age. The higher *M. bovis* seroprevalence in feedlot calves may be explained also by stress due to transportation, changes in diet, social changes, co-mingling of animals from different farms over the country as well as introducing imported calves, high turnover of animals and presence of different age groups (5, 6). In contrast, most of dairy herds in Israel maintain a 'closed herd' policy. The more stringent biosecurity practices on dairies may limit the exposure to *M. bovis*.

When ELISA values were compared among groups of imported calves, 69%, 62% and 23% of calves from Lithuania, Hungary and Australia, respectively were negative or had low ("≥ +") ELISA value (Fig. 1). Notably, 42% of calves imported from Australia showed high "≥ +++" ELISA values (Fig. 1). Australian calves are imported to Israel by sea voyage and are confined together for at least 3-4 weeks, conditions

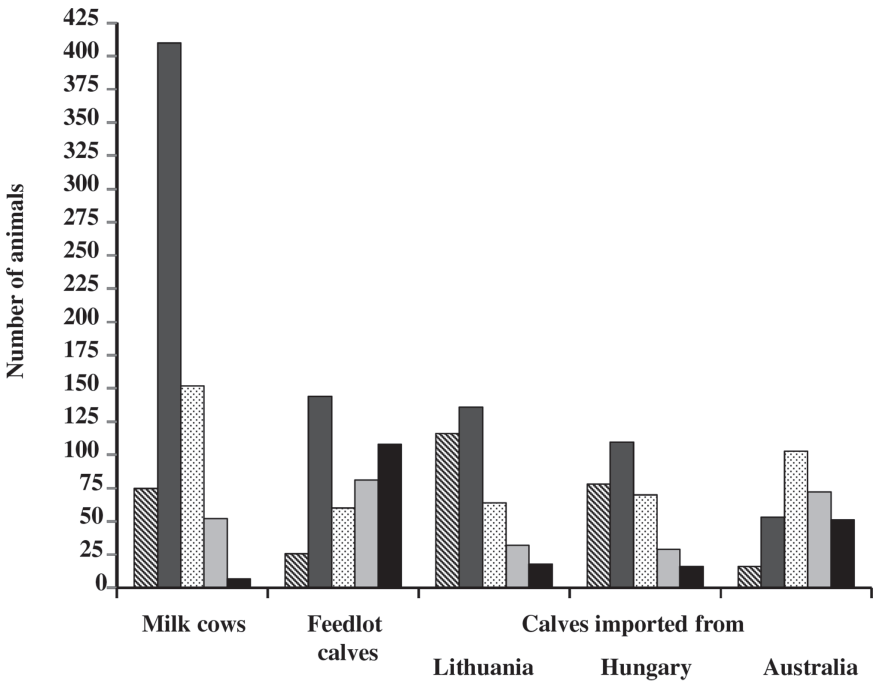

Figure 1: Distribution of ELISA values among sera samples tested. Distribution of ELISA values among animal cohorts is shown on axis X; the number of animals showing different ELISA values (defined as "0"; - "+"; - "++"; - "+++"; - "≥++++") is shown on axis Y.

that may increase spread of *M. bovis* infection among the calves resulting in higher rates of antibody titres. Recently, Moore *et al.* (7) showed that the main cause of death in cattle on 20 long-haul live export voyages from Australia (including 3 voyages to Israel) was respiratory disease (59.4%) and *M. bovis* was identified in 60% of the lungs with histological evidence of respiratory disease.

Several serological studies testing the presence of antibodies against *M. bovis* have been performed so far (2, 8-12), but comparison among the results should be done with caution as study populations and sampling years vary and different serological assays with different performance were used.

In conclusion, this study shows high animal- and herd-seroprevalence of *M. bovis* among local and imported animals even after applying a higher threshold for interpretation of ELISA sensitivity. Data on the prevalence of *M. bovis* in different cattle cohorts will enable the development of future prevention and management strategies. Testing of herds by exporting countries to ensure *M. bovis*-free cattle may be suggested to minimize the introduction of new *M. bovis* strains.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no financial or other relationships that might lead to a conflict of interest. All authors have seen and approved the manuscript.

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