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Short communication

Brucella abortus S19 vaccine protects dairy cattle against natural infection with *Brucella melitensis*

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ABSTRACT

Brucellosis is a zoonotic disease that can cause severe illness in humans and considerable economic loss in the livestock industry. Although small ruminants are the preferential host for *Brucella melitensis*, this pathogen has emerged as a cause for *Brucella* outbreaks in cattle. S19 vaccination is implemented in many countries where *B. abortus* is endemic but its effectiveness against *B. melitensis* has not been validated. Here we show that vaccine effectiveness in preventing disease transmission between vaccinated and unvaccinated cohorts, as determined by seroconversion, was 87.2% (95% CI 69.5–94.6%). Furthermore, vaccination was associated with a reduced risk for abortion. Together, our data emphasize the role S19 vaccination could play in preventing *B. melitensis* outbreaks in areas where this pathogen is prevalent in small ruminant populations.

1. Text

Brucellosis is a zoonotic disease that can cause severe disease in humans and considerable economic loss in the livestock industry. In Israel, *Brucella abortus* has been eradicated and has not been diagnosed since 1984 [1]. On the other hand, *Brucella melitensis* is endemic in the small ruminant population, mainly in the southern region of the country, and has recently caused an alarming number of cases in humans [2] as well as several outbreaks in the past in commercial dairy cattle farms [3]. Formerly, all heifer calves on all dairy farms in Israel were vaccinated at the age of 3–6 m, using a full dose ($4–6 \times 10^{10}$ CFU) of a commercial *B. abortus* S19 vaccine[1] administered subcutaneously as a 2 mL injection in the neck region. Following a risk assessment performed during 2013, the Israeli Veterinary Services decided to cease routine vaccination with S19. The decision leaned mainly on the fact that all previous *B. melitensis* outbreaks on dairy farms in Israel occurred in vaccinated herds, inferring a less protective value than has been suggested [4].

In October 2015, an outbreak of *B. melitensis* occurred on a large commercial dairy farm in Israel. At the time of the outbreak, a proportion of the pregnant heifer calves present on the farm had been vaccinated at the age of 3–6 m with the *B. abortus* S19 vaccine, whereas the remaining heifers had not been. These circumstances created the conditions for a natural experiment in which the effectiveness of the S19 vaccine against *B. melitensis* in dairy cattle could be assessed.

2. The study

We estimated the effectiveness of the *Brucella abortus* S19 vaccine following natural infection with *B. melitensis* in a large commercial dairy herd situated in the southern region of Israel. Prior to the outbreak, the herd comprised 1076 dairy cows, 943 replacement calves and 442 feedlot calves. In October 2015, the attending veterinarian became aware of an increase in the number of late term abortions in heifer calves and submitted a serum sample from one of these heifers to the National Reference Laboratory for Brucellosis at the Kimron Veterinary Institute (KVI). The sample tested positive for *Brucella* spp. in the complement fixation test (CFT) and microagglutination test (MAT). Following this finding, milk, colostrum and tissues from aborted fetuses and placentas from the farm were sent to the KVI and cultured on Tryptic soy agar plates supplemented with serum-dextrose and Farrel's selective plates, which yielded *B. melitensis* biovar 1. A test and cull policy was implemented and the entire herd was sampled and tested with CFT for the first time during October 2015. Seropositive animals (153) were found in all age groups (Fig. 1), indicating the disease was present in the herd for a considerable period of time. The risk

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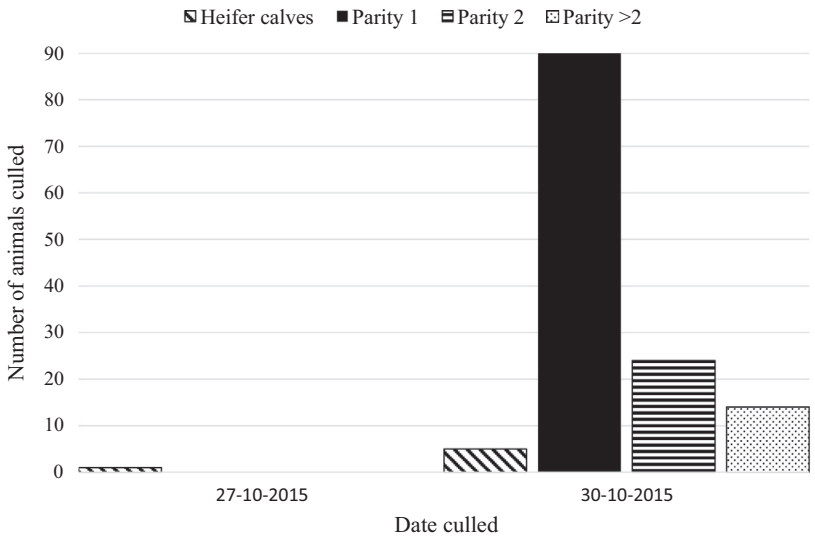

Fig. 1. Number of animals testing positive for *B. melitensis* and culled on the first herd test, 30th October 2015. The index case, a late term aborting null parity heifer, was diagnosed 3 d earlier.

to be seropositive was 0.49%, 19.78%, 8.49% and 4.67% in null parity, first parity, second parity and third and above parity cows, respectively. Data retrieved from the farm computer (NOA software, Israel Cattle Breeders Association) indicated that the outbreak most probably started in May 2015, with the late term abortion of three heifer calves. At this time, the risk to abort in this group was 1.72%, a 2.6 fold increase compared with the average risk in the 4 preceding months (0.66%). However, at the time no samples from the aborting heifers were submitted to the laboratory, and these heifers were kept in the herd as lactating animals. They subsequently tested positive at the first sampling of the entire herd in October 2015.

In this herd, routine vaccination of the heifer calves aged 3–6 m with S19 was conducted in compliance with the national policy until September 2013, the time the program was ceased by the Israeli Veterinary Services. Based on data obtained from the farm management software, the last heifer calf vaccinated was born on the 19th of June, 2013. Accordingly, two cohorts of heifer calves were chosen for the study: those born in the 3 m prior to (and including) this date (i.e. vaccinated group) and those born in the 3 m after this date (i.e. unvaccinated group). The cohorts were chosen to allow for a sufficient number of animals in each group, while limiting the maximum age difference between the oldest vaccinated calf and youngest unvaccinated calf to 6 m.

Vaccine effectiveness was calculated using the following formula:

$$(1 - \mathrm{RR}) * 100\%,$$

where RR is the relative risk for a given outcome. RR was calculated as:

$$\mathrm{RR} = \left(\mathrm{risk}_{(\mathrm{vaccinated})}\right) / \left(\mathrm{risk}_{(\mathrm{unvaccinated})}\right) * 100\%,$$

where $\mathrm{risk}_{(\mathrm{vaccinated})}$ represents the risk of a given outcome in the vaccinated group, and $\mathrm{risk}_{(\mathrm{unvaccinated})}$ represents the corresponding risk in the unvaccinated group. In this study we chose 2 outcomes: the risk to be diagnosed as seropositive for *Brucella* and subsequently to be culled, and the risk to abort, which was defined as the loss of a confirmed pregnancy up to 260 d of gestation. However, in the case of abortion the RR and vaccine effectiveness could not be estimated due to the fact that no vaccinated heifers aborted. Therefore, the association between vaccination and abortion was assessed using Fisher's exact test. The 95% confidence interval (95% CI) for the RR was calculated using the exact method. The upper and lower values of the vaccine effectiveness 95% CI were then calculated as $1 - \mathrm{RR}_{(\mathrm{lower\ value})}$ and $1 - \mathrm{RR}_{(\mathrm{upper\ value})}$, respectively. Data used for the study were retrieved from the farm computer (NOA software) and were analyzed using SAS 9.4 [5].

A total of 162 heifers were included in the study. Ninety-five (59%) were not vaccinated and 67 (41%) were vaccinated with S19 between 3 and 6 m of age. The risk of being diagnosed with *B. melitensis* and consequently being culled was 57.9% and 7.5% in the unvaccinated and vaccinated heifers, respectively (Table 1). RR was 0.128 (95% CI 0.054–0.305). Vaccine effectiveness was 87.2% (95% CI 69.5–94.6%). Nine (9.5%) of the 95 non-vaccinated heifers aborted, whereas none of the 67 vaccinated heifers aborted ($P = 0.011$) (Table 2).

Table 1
Number and proportion of heifers diagnosed with *B. melitensis* and subsequently culled in unvaccinated heifer calves and in those vaccinated with S19 at the age of 3–6 m.

Vaccinated with S19	Serologically diagnosed with *Brucella* and culled		Total
	No	Yes	
No	40	55	95
%	42.1	57.9	
Yes	62	5	67
%	92.5	7.5	
Total	102	60	162
%	63.0	37.0	

Table 2
Number and proportion of heifers that aborted among unvaccinated heifer calves and in those vaccinated with S19 at the age of 3–6 m.

Vaccinated with S19	Abortion		Total
	No	Yes	
No	86	9	95
%	90.5	9.5	
Yes	67	0	67
%	100	0	
Total	153	9	162
%	94.4	5.6	

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3. Conclusions

We demonstrate that the *B. abortus* S19 vaccine was effective in preventing disease transmission and clinical symptoms such as abortion and reduced gestation length (data not shown) following natural challenge with *B. melitensis*. To the best of our knowledge, cross protection of a *B. abortus* vaccine in dairy cattle naturally infected with *B. melitensis* has not been demonstrated previously. Our findings emphasize the possible role *B. abortus* vaccines could play in programs aimed to control and prevent *B. melitensis* outbreaks in dairy herds. Although the evidence for our findings was quite conclusive, it must be stressed that our study was not designed as a randomized, controlled trial. Therefore, the presence of a cohort effect cannot be excluded. Such an effect, if present, could introduce bias if the exposure to *B. melitensis* was not of the same magnitude or varied over time between the vaccinated and unvaccinated heifers. We aimed to reduce the probability of a cohort effect by choosing the groups in such a way that the maximum age difference between 2 heifers from different cohorts was 6 m. This way the cohorts most likely shared pens or were housed in adjacent pens at the time of exposure.

Conflict of interest

None.

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