Isolation of *Porphyromonas levii* from vaginal samples from cows in herds negative for bovine necrotic vulvovaginitis

S. BLUM, J. BRENNER, O. FRIEDGUT, Y. STRAM, O. KOREN, I. DAGONI, A. MUNBAZ, D. ELAD

BOVINE necrotic vulvovaginitis (BNVV) was first described in Israel in 2004 (Elad and others 2004). Outbreaks of the disease were observed between 2000 and 2007 on 12 Israeli dairy farms, consistently after the transfer of large numbers (tens to hundreds) of cows during mergers. The outbreaks were in most cases transitory, but the disease was observed for two or more years after the merger on two of the farms (D. Elad, unpublished data). The disease is characterised by the deterioration of normal vaginal injuries caused by calving to necrotic lesions, primarily in dairy heifers during the first week after calving. The clinical signs usually subside after a variable period of time (up to 10 weeks in extreme cases), but affected animals may develop complications such as metritis and peritonitis and die. The infection has been found to be resistant to various treatments with antibacterial or antibiotic compounds. In addition to direct economic losses, BNVV causes a decrease in fertility (Blum and others 2008).

*Porphyromonas levii*, a pigmented, Gram-negative anaerobic bacterium that is part of the ruminal microflora (Lev 1958), was found to be associated with the disease. Blum and others (2007) showed that there was significant association between the severity of the lesions and the number of colony-forming units (cfu) of *P levii* isolated from the lesions. Risk factors associated with the outbreaks have not been elucidated, but the location of the farms and bovine herpesvirus (BHV) were excluded in a previous study (Blum and others dated, but the location of the farms and bovine herpesvirus factors associated with the outbreaks have not been elucidated).

One risk factor may be the stresses associated with merging two or more farms. Stressors originating from the two merging farms were kept separated for two or more years after the merger on two of the farms (D. Elad, unpublished data). The disease is characterised by the deterioration of normal vaginal injuries caused by calving to necrotic lesions, primarily in dairy heifers during the first week after calving. The clinical signs usually subside after a variable period of time (up to 10 weeks in extreme cases), but affected animals may develop complications such as metritis and peritonitis and die. The infection has been found to be resistant to various treatments with antibacterial or antibiotic compounds. In addition to direct economic losses, BNVV causes a decrease in fertility (Blum and others 2008).

The study was conducted in three dairy herds located in the centre/south of Israel. They consisted of Israeli Holstein cows that were kept in ventilated covered sheds under a zero-grazing, loose-housing management system and were cooled by periodic showering. No outbreaks of BNVV were observed, possibly owing to the lower levels of stress (D. Elad, unpublished data). Alternatively, there may have been no outbreaks because there was no *P levii* on these farms. Another characteristic of BNVV is its much lower incidence in cows than first-calving heifers. This difference may be due either to the cows being more resistant to the infection or to the absence of the microorganism from their reproductive organs. This short communication describes a study aimed at elucidating these factors.

A retrospective study was conducted on farm C, which consisted of approximately 450 cows and 150 pregnant heifers. A total of 230 cows and 79 pregnant heifers had been transferred to farm C six months previously and were kept permanently separated from the original animals.

All the animals that calved during the survey period were sampled at weekly intervals for as long as any vaginal injuries were present or *P levii* was isolated. The sampling and laboratory examinations have been described in detail by Blum and others (2007). Briefly, vaginal swabs were taken after rinsing the perineum with soapy water. For the bacteriological examination, the swabs were suspended in transport medium (Venturi Transystem; Copan) and for the virological examination they were suspended in MEM vitamin solution (Sigma) supplemented with 4 per cent each of an antibiotic solution, horse serum and HEPES buffer (Biological Industries). The swabs were transported to the laboratory under refrigeration. The bacteriological samples were processed within two hours after sampling; virological samples were stored at –80°C until processing.

Aerobic and anaerobic cultures were prepared. Pigmented colonies that grew exclusively anaerobically were identified as *P levii* by selected reactions (negative indole, alpha-fucosidase and alpha-galactosidase, and positive beta-galactosidase and N-acetyl-beta-glucosaminidase) of the ID32A kit (bioMérieux). Attempts to isolate viruses were made on Madin-Darby bovine kidney cell monolayers, and PCR for BHV types 1, 2 and 4 was applied.

On farm A, six heifers were sampled on February 10, 16 and 23, 2006, starting from 256 days of pregnancy. On farm B, one heifer was sampled three weeks before calving, on February 3 and 10. On farm C, six heifers were sampled on February 10, 16 and 23, 2006, starting from 256 days of pregnancy. On farm A, 13 heifers were sampled on February 10, 16 and 23, 2006, starting from 256 days of pregnancy. On farm B, one heifer was sampled three weeks before calving, on February 3 and 10. *P levii* was isolated from this heifer, indicating that the microorganism was present on the farm; because the merger was about to take place no further animals were sampled.

Farms A and B merged during the last week of February. On the merged farm, 13 heifers were sampled between April 25 and May 22 during five weekly visits. Nine of the heifers were local and four had been transferred from farm A. The first samples were taken in the first week after the heifers had calved.

On farm C, six cows (one each in their second, third, sixth and seventh lactation, and two in their fourth lactation) and 10 heifers were sampled between January 6 and February 14, 2006, during seven weekly visits. Three of the heifers were...
sampled before calving and the other samples were taken starting in the first week after calving.

Normal postpartum vaginal injuries such as erythema, erosions and lacerations were observed in all the animals included in the survey. *P. levii* was isolated from three of the six heifers sampled on farm A, from the heifer on farm B, from three of the 13 heifers on the merged farm AB, and from six of the 10 heifers and three of the six cows on farm C (Table 1). No BHV was found in the samples.

The results indicate that *P. levii* was present in the heifers and multiparous cows on all the dairy farms sampled, in association with normal postcalving lesions. BNVV did not develop, suggesting that the presence of the bacterium by itself, probably as a result of faecal contamination of the lesions, is insufficient to cause the disease. The only apparent difference between merged farms with and without outbreaks of BNVV was that fact that precalving heifers were mixed in previous cases (D. Elad, unpublished data) but not in the present study (farms AB and C). It could be that stress resulting from social and hierarchical tensions plays a central role as a risk factor (Arave and others 1977), allowing *P. levii* to extensively colonise the postcalving lesions (hence the association between clinical signs and *P. levii* cfu), resulting in the degeneration of normal postcalving lesions to BNVV.

Following a merger, a large number of animals have to find their hierarchical status in the unified group; this may be a lengthy process, maybe taking months. However, the fact that postcalving heifers are the primary population at risk from BNVV, whereas postcalving cows are only rarely affected (Elad and others 2004), indicates that these tensions by themselves are insufficient to predispose the animals to the disease. Consequently, the authors hypothesise that it is the combined effect of periparturient immunosuppression, which has been reported to be more pronounced in heifers (Preisler and others 2000), and the stress caused by social tensions that results in the development of BNVV. While this could possibly explain the majority of transitory BNVV outbreaks, further studies are necessary to elucidate the risk factors, which are likely to be linked to management, that create conditions suitable for the disease to become endemic.

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**References**


