

# Granulo-pustular vulvovaginitis (“Jackal bite”) an emerging disease: *Mycoplasma bovis* and *M. canadense* infection of dairy cattle in Israel

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## SUMMARY

A widespread outbreak of a syndrome in which white foci and granulo-pustular lesions were seen on the vaginal mucous membranes of Israeli Holstein cows occurred in seven large dairy herds, and *Mycoplasma bovis* and *M. canadense* were isolated from the lesions. Granulo-pustular vulvovaginitis (GPVV) thus appears to be an emerging disease of Israeli dairy cattle. We propose that *M. bovis* and *M. canadense* act as causative agents of GPVV. We also record other possible co-infectious factors, such as bovine enterovirus (BEV) and bovine herpes viruses (mainly BHV-4), which might act synergistically with or as triggers of mycoplasmas of the female genital canal. Similar syndromes have been documented previously by other authors, but none have described their extensive appearance in dairy herds.

## INTRODUCTION

Several syndromes affecting the bovine genitalia have been documented in Israel, these include bovine necrotic vulvovaginitis (BNVV) (1, 2), granulo-pustular vulvovaginitis (GPVV) (3), and suspected infectious bovine rhinotracheitis/infectious pustular-vulvovaginitis (IBR/IPV) (4). GPVV resembling lacerated vulvitis externally and pustular lesions internally was reported for the first time from South Africa in 1990 (5). These authors reported that *Mycoplasma bovis*, *M. canadense*, *M. bovis*, *Moraxella bovis*, and *Bovine herpesvirus 1* (BHV-1), were isolated from the affected cattle. Straub and Böhm, in 1964 (6) reported a case defined as “catarrhal vaginitis” characterized by rapid transmission for which the authors claimed that an enterovirus was responsible for this syndrome including young animals.

Illustrations of the lesions did not appear in the 1966 publication (7), but the clinical description of G(P)VV in English cattle was similar to the cases reported in the Israeli outbreaks of GPVV (3). Afshar et al., (7) noted a few buff-colored granules, 1 to 4 mm in diameter, around the vulvovaginal epithelium. These lesions tended to bleed after mere swabbing. The vaginal mucosa varied in color and appearance from the normal pale pink to markedly congested. In some cases there was a distinct demarcation between the congested area and the normal epithelium about 5 mm from the labia of the vulva. *M. bovis* was implicated as responsible since it was isolated from the lesions and transmitted by swabbing diseased heifers to healthy ones, leading to the appearance of similar

lesions (7).

This manuscript describes seven cases of GPVV reported so far to occur in Israel, with special given attention to the clinical and infectious pathogens involved in these outbreaks. The folkloristic name “wolf bite” describes the main characteristic lesion observed in all seven cases. Also the geographic location (north-east of Israel) of the affected herds and the traumatized vulva indicated a common causal agent. This might have also been a consequence of animal bites where wild or feral canidae attacks are very common, although such an extensive outbreak of GPVV has not been reported elsewhere. In addition this paper compares GPVV and BNVV as these two syndromes have been mainly reported in Israel, and are connected with bovine dairy cattle.

### Clinical definition of granular-papular vulvovaginitis (GPVV) in cattle

Usually only the final stage of GPVV attracts the farmers’ attention. This is the lesion that resembles “Jackal bites” and is visible on the external vaginal labia, presenting as an uncomplicated, lacerated traumatic-like vulvitis (Figure 1). Progression to a complicated vulvovaginitis seems to be common (Figure 2). One important aspect of GPVV is its rapid spread within farm premises, leading to the involvement of almost all the lactating cows irrespective of age and reproductive cycle stage within a few days (3). In two cases GPVV was reported also in heifers (Table 2).

As mentioned before, vulvar laceration is the predominant clinical manifestation. Vigilant clinical follow-up allows observation of a gradual progression of lesions. It begins with tumefaction of the external labia, which are soft to the touch at this stage, and when held firmly, ecchymotic spots appear immediately. The ventral part of the external labia, appear bulb-like in shape due to the accumulation of transuded fluid (Figure 3). This is the anatomical site where the rupture (“Jackal bite”) occurs (Figures 1 and 2). Examination of the internal vaginal mucosa revealed white focal spot lesions on the mucous membrane, in addition to the ripped external labia defining the general condition of the VV mucosa as an catarrhal inflammatory process (Figure 4). Body temperature of affected animals remained normal without general malaise or decrease in productivity (milk yield or fertility) being reported (3). If good managerial sanitary conditions are applied, the “Jackal bite” might disappear spontaneously. It is therefore feasible that the causal agents are propagated by iatrogenic means

and by contaminated foments especially during pre-milking udder washing and while the lactating cow stays in the milking parlor.

#### Descriptions of individual cases

**Case 1 - Geshur** This herd was the first to report on clinical manifestations compatible with GPVV. This case was ascertained clinically as GPVV retrospectively by observing photographs of the lesions and by collecting clinical data that were similar to those which had been investigated in case 2 and 3 (Table 1, 2). The attending veterinarian and the farmers suspected that BNVV was the cause (1) and therefore did not report it to the KVI diagnostic laboratories. They did notify the KVI when the primary laboratories' diagnosis of cases 1 and 2 became available. This was the reason for the delay and lack of laboratory confirmation. Moreover, it was later erroneously claimed to be a case of IPV

**Case 2 - Meitzar** This dairy herd comprising about 550 lactating cows and an additional 400 replacement heifers, had suffered from a large-scale outbreak of the BNVV syndrome 5 years previously (1). This was the first case of GPVV that KVI personnel attended and the site from where the first laboratory data were recorded. The presence of "traumatic" lesions in of all ages of lactating cows only was observed, most of them presented with uncomplicated lesions (Figure 1) but secondary complications appeared later in some cases (Figure 2). Initially, this case was suspected also as being IPV. This diagnosis was resolved when BHV-1 was not isolated, and was negative serologically (Table 1). Alternatively, bovine enterovirus infection may have acted as the trigger for the mycoplasmal infection and the subsequent clinical manifestations (3) (Table 1).

**Case 3 Even-Itzhack** – This herd, comprising approximately 300 milking cows and an equal number of heifers, differed from case 2 as no previous history of BNVV had been observed. Upon clinical examination this case suspected as "Jackal bite" syndrome, revealed internal vaginal mucosa dotted with white focal spot lesions on the mucous membranes, in addition to torn external labia. About 50% of the lactating cows were affected. In this herd BHV-4 infection was identified and therefore may have constituted the trigger for the mycoplasma infection and the accompanying clinical Figure (3) (Table 1).

**Case 4 Nachshonim** – Twelve fecal samples from diarrheic lactating cows in a large dairy herd were examined by EM at KVI. All of them yielded on negative-staining small round, non-enveloped, viral particles about 25 nm in diameter, characteristic of enteroviruses. KVI investigators suspected that BEV was an indicator for an additional pathogen. Indeed during the subsequent visit the "Jackal bite" appearance was observed in the external genitalia *M. bovis genitalium*, *M. canadense* and BEV were found in swabs taken from the vagina of affected cows. This herd suffered from GPVV for about 3 months. In additional fecal samples that were submitted 4 months after KVI investigators visited the farm, did not yield viruses. BNVV syndrome was not previously recorded in this herd.

**Case 5 - Natur** This herd was affected 6 years previously with BNVV (1). The "new" clinical cases were therefore initially suspected as being a recrudescence of BNVV in the farm with some cases involving the pathogens associated with "Jackal bite". But the multiple cases appeared within a very short time in yearling heifers and later in heifers prior to artificial insemination, and lactating cows, seemed to rule out BNVV. This was the first case of GPVV where all the groups of cattle in the herd were affected clinically and the syndrome was observed in all the sheds and in all the sub-populations. *M. bovis genitalium* and *M. canadense* were demonstrated by PCR (3) in all the swab samples taken from all the affected sites (n=7). Two BHV-1 isolates were made from the nine swabs examined.

**Case 6 – Tefen** The attending veterinarian reported retrospectively on GPVV only in heifers (six cases), and only did so after being informed about other cases that occurred in the same area where he practiced. He was shown figures that were similar to others already diagnosed as "Jackal bite". By applying a sanitary regime the case resolved spontaneously and the syndrome did not spread further.

**Case 7 - Yonatan** While this manuscript was being prepared the "Yonatan case" was an incomplete ongoing outbreak. This case differed from the rest of the cases, as it is the only case in which two syndromes co-existed, BNVV and GPVV, in the dairy farm. This particular clinico-epidemiological situation poses a diagnostic dilemma, and the possibility of the co-existence of BNVV and GPVV syndromes in the same cow became real. This herd has been suffering from BNVV for the last 9 years while GPVV appeared only in August 2009.

## DISCUSSION

*M. bovis genitalium* and *M. canadense* were consistently isolated or demonstrated in five of the seven clinical cases, suspected as being the GPVV syndrome, or "Jackal bite" in Israel (Table 1). The involvement of *M. bovis genitalium* and *M. canadense* in the pathology of the bovine genital tract has already been published (5, 8-12), but no such extensive syndromes have been reported in the veterinary literature; thus, the Israeli Veterinary Authorities might consider GPVV as an emerging disease in this area.

It is conceivable that other primary infectious agents might act as triggering factor under conditions in which bacteria might act as secondary opportunistic agents. The best candidates would be the viruses that are most often found in the bovine genital tract, such as BHV-1 (13), BHV-4 (1, 2) and BEV (6). BHV-1 as a primary cause of GPVV could not be considered until proved otherwise (Lysnyansky 2009, Table 1). Surprisingly, BEV that are more "adapted" to the alimentary bovine tract (14) than to the genital tract are often found in female genitalia (Straub and Böhm) (6), Abraham (15), Lysnyansky (3) and found also in our studies (Table 1). Moreover, in human medicine, enteroviruses have been implicated as a probable pathogen in the premenarcheal child (16, 17) indicating their probable role

in the pathogenesis of female genital tract infections of other species.

Our clinical experience suggests that the softness and fragility of the distal ventral vulva is the probable reason for the labial rupture, resulting from merely touching the vulva, and corroborates Gilbert and Oettlé (5) observations of tumefaction that preceded the lacerated lesion in those cases where *M. bovigentalium* and *M. canadense* were isolated. Petit and others (11, 18) reported that *M. bovigentalium* was found more often in cattle that had aborted than in those that had not. Therefore, we propose that *M. bovigentalium* alone or *M. bovigentalium* with *M. canadense* can be considered as essential for the establishment of VV inflammation. This hypothesis is supported by the findings of Saed and Aubaidi (9), who inseminated 12 heifers during oestrus with semen spiked with pathogenic *M. bovigentalium*, and all the inseminated heifers developed GVV. Saed and Aubaidi (9) studies might provide additional clues to the possible primary agent of GPVV; conceivably hormonally dependent, which could partially explain the tumefaction phenomenon (Figure 3). In Table 2, we have compared the two emerging syndromes BNVV (1) and GPVV (3). The clinical appearances generally constitute a sufficient basis to discern between these two syndromes. Table 2 shows the clinical findings of which the various stages of lesion progression are the best to consider. Only in GPVV is it possible to observe a wide range of external lesions, whereas in BNVV some of the affected cows cannot not be diagnosed if the veterinarian does not expose the vulval labia. The repugnant smell of necrotic tissue in affected cows with BNVV is associated with this syndrome. Additional findings used as a basis for clinical differential diagnosis are the epidemiological differences (Table 2). BNVV is associated with parturition and animal movement and appears in lactating cows only approximately one week post-parturition, especially in primiparous animals (1), while GPVV might be present in all ages and is not connected with parturition or animal movement (3) (Table 2). The rapid inter-herd propagation is characteristic of GPVV syndrome that can affect most of the animals before it recedes. BNVV presents a chronic inter-herd feature, which affects only the parturient population, and therefore only a few affected cows will be observed at any given time. Differential diagnosis should also consider the normal microscopic traumatic lesions that appear post-parturition. These lesions are always linked with the physiology of parturition and may mislead owners and veterinarians when BNVV and PVV appear in the same herd.

Laboratory findings provide the most convincing data for differentiating between BNVV and GPVV. *Porphyromonas levii* is the bacteria most associated with BNVV (1, 2) while *M. bovigentalium* alone or *M. bovigentalium* with *M. canadense*. In only one case these two syndromes have been suspected exceptionally, and in general these two syndromes do not appear concurrently (Table 2). This does not rule out that such events might not occur in the some herds in the future.

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**TABLES**

**Table 1:** Summary of seven cases of Granulo-pustular vulvovaginitis (GPVV) reported in Israel, 2007-2009

	Farm (case)	Mycoplasma detection	Viral involvement	Sub-population involved
1	Geshur	Not tested	Not tested	Lactating
2	Even-Yizchak	<i>M. bovis genitalium</i> + <i>M canadense</i>	BHV-4	Lactating
3	Meizar	<i>M. bovis genitalium</i> + <i>M canadense</i>	Enterovirus	Lactating
4	Nachshonim	<i>M. bovis genitalium</i> + <i>M canadense</i>	Enterovirus	Lactating, heifers?
5	Natur	<i>M. bovis genitalium</i> + <i>M canadense</i>	BHV-1?	Lactating, heifer, yearling, calves, weaning calves
6	Tefen	Not tested	Not tested	Heifers only
7	Yonatan	<i>M. bovis genitalium</i>	Unidentified viruses	Lactating, heifer, yearling, calves, weaning calves

**Table 2:** Clinical, etiological and epidemiological comparison of bovine necrotic vulvovaginitis (BNVV) and Granulo-pustular vulvovaginitis (GPVV)

Factors and variables	GPVV (Figures 1-4 )	BNVV (Figure 6)
Essential pathogens	<i>Mycoplasma bovis genitalium</i> & <i>M canadense</i>	<i>Porphyromonas levii</i>
Necessary pathogens	Enterovirus and or BHV-4 and or others not yet identified (estrogen ref)	
Lesions and lesions progression	Progression from catarrhal to edema, disseminate miliary small vesicle, labial rupture. Secondary bacterial superinfection with pus production frequent.	Mucosal necrosis. Pus may appear in rare chronic cases.
Dermal-mucosal junction involvement	Yes +	Yes+
External labia involvement	Yes +++	Yes+
Vulval involvement	Yes +++	Yes +++
Vaginal involvement	Yes +	Yes +++
Smell	No smell	Repugnant smell
Connected with parturition	No, might be noted in animals of all ages and lactation phases (see table 1)	Yes, appears during first week after parturition, almost exclusively in heifers
Propagation in herd	High morbidity, many animals present clinical manifestations at the same time	Variable from sporadic to outbreaks
Primary stressor(s)	Unknown (estrogen?) (9)	Transport, transfer, hierarchy struggles

+ rarely observed, ++ often observed, +++ almost always observed,

## FIGURES



**Fig. 1:** Uncomplicated lacerated vulvitis.



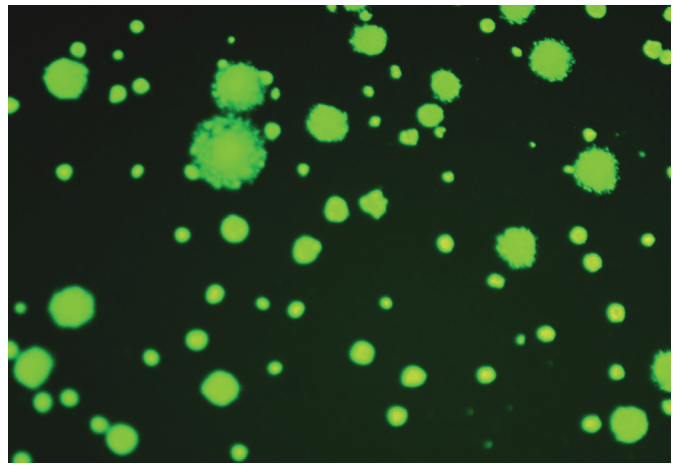
**Fig. 2:** Complicated lacerated vulvitis.



**Fig. 3:** Bulb-like shape of the ventral part of labia and the initial rupture process.



**Fig. 4:** Catarrhal inflammatory process of the mucosa in which also ecchymotic spots appear.



**Fig. 5:** Immunofluorescence of cultured *Mycoplasma bovis* isolates from clinical cases.



**Fig. 6:** This figure shows the characteristic image of bovine necrotic vulvovaginitis. Note the vast necrotic areas which are located deeper than those described for granulo-pustular vulvovaginitis.