

GRANULAR VULVOVAGINITIS (GVV) IN SHEEP EXPERIMENTALLY INDUCED WITH MYCOPLASMA OVINE/CAPRINE SEROGROUP 11

D. KUMAR, P. P. GUPTA, J. S. RANA and H. S. BANGA

Department of Veterinary Pathology,
Punjab Agricultural University, Ludhiana — 141 004, India

Received June 8, 1992

Abstract

Kumar D., P. P. Gupta, J. S. Rana, H. S. Banga: *Granular Vulvovaginitis (GVV) in Sheep Experimentally Induced with Mycoplasma Ovine/Caprine Serogroup 11*. Acta vet. Brno, 61, 1992: 241–249.

To have insight into the pathogenicity of *Mycoplasma ovine/caprine serogroup 11* for female genital system of sheep, 8 apparently healthy lambs were taken. Two ml of *M. ovine/caprine serogroup 11* (2-D) culture containing 6×10^6 CFU (colony forming units) ml^{-1} was inoculated into lightly scarified vulvovaginal canal of 7 lambs, while 2 ml of sterile mycoplasma broth was similarly inoculated in one lamb, which served as control. All the infected lambs exhibited swelling of vulva along with mucus discharge from next day post-inoculation (DPI), and this persisted up to 10–12 DPI. The experiment was of 70 d duration during which one lamb was sacrificed at every 10 d interval. The control lamb was also sacrificed on 70th d. Grossly, in the vulva and vagina, congestion, oedema and fine nodules were discernible by 10 DPI. Microscopically, all the infected lambs developed lymphocytic vulvitis, vaginitis, cervicitis, and endometritis, which aggravated with the increase in DPI. *M. ovine/caprine serogroup 11* could be re-isolated from vaginal swabs of all the infected lambs up to 70th DPI. The findings reported here indicate that *M. ovine/caprine serogroup 11* is pathogenic for female genital tract of sheep as it causes granular vulvovaginitis (GVV), and it is the first description of the pathologic features of GVV in sheep induced by this organism.

Vulva, vagina, cervix, uterus, lymphoid follicles, GVV, sheep

Mycoplasma ovine/caprine serogroup 11 was first isolated from an outbreak of granular vulvovaginitis (GVV) in sheep in Australia (Cottew et al. 1974). Subsequently, this organism was isolated from spontaneous cases of GVV in sheep in India (Tiwana 1982). Despite its association with spontaneous cases of vulvovaginitis in sheep, its pathogenicity to female genital tract and its role in producing GVV has not been proven experimentally. Therefore, the present work was undertaken to assess its pathogenicity and to study the sequential pathological changes in the female genital tract of sheep infected with this organism.

Materials and Methods**Experimental animals**

Eight female lambs aged 6–8 months were kept under observation for 1 week before starting the experiment. During this period, no mycoplasma organisms or any grossly visible vulvar/vaginal lesions were detected and all the animals were adjudged healthy.

Infectious agents

Forty eight hour old culture of *M. ovine/caprine serogroup 11* (2-D) at 3rd passage level, containing 6×10^6 colony forming units (CFU) per ml was used for inoculation.

Mode of infection

Two ml of this culture was inoculated into the vaginal canal of 7 lambs which was lightly scarified using sterilized dental brush, while 2 ml of sterilized mycoplasma broth was inoculated into the vaginal canal of a control lamb.

Experimental design

The experiment was continued for 70 d during which daily rectal temperature was recorded as well as, all the lambs were observed for any gross change in the appearance of vulva and abnormal discharge, if any. Haematological values of all lambs were estimated on 3rd, 6th and 10th day post-inoculation (DPI) and subsequently, on every 10th d up to 70th DPI. The control animal was also killed on 70th d of the experiment. The genital tract and other organs of all the lambs were examined grossly and microscopically. For microscopic examination, 5 μ m thick paraffin sections were cut and stained with haematoxylin and eosin. The vaginal swabs were taken and cultured on mycoplasma medium for *Mycoplasma* isolation by the method described by Banerjee et al. (1979).

Results

Clinically, the appetite of all the lambs remained unaffected during the experiment. However, their body temperature showed a mild rise of 1–2° F up to 3–4 DPI. From 2nd DPI, the vulvar lips of all the infected lambs were slightly swollen and mild mucus discharge was observed from their vulva up to 10–12 DPI. Haematological analysis of the infected animals revealed a marked increase in the average total leucocyte count (TLC) from the pre-inoculation basal value of $6.34 \times 10^3/\text{cmm}$ to $9.01 \times 10^3/\text{cmm}$ and $9.74 \times 10^3/\text{cmm}$ of blood on 3rd and 20th DPI, respectively. Thereafter, TLC started declining gradually but remained higher than the control ($6.10 \times 10^3/\text{cmm}$) as well as the pre-inoculation basal values ($6.34 \times 10^3/\text{cmm}$) till the end of the experiment. The differential leucocyte count (DLC) showed a progressive neutrophilia on 3rd DPI followed by a gradual lymphocytosis which peaked at 20th DPI. Other haematological components did not show any significant change. *M. ovine/caprino* serogroup 11 was re-isolated from the vaginal swabs of all the infected lambs up to 70th DPI.

Grossly, the mucosa of vulva and vagina of the lamb killed on 10th DPI was congested, thickened and rough due to scattered fine nodules raising the mucosa. The vulvovaginal mucosa of the lambs killed on 20th and 30th DPI showed pin head-sized translucent nodules arranged in a linear fashion giving it a corrugated appearance. The animals necropsied subsequently, showed similar but more severe changes in their vulva and vagina than those seen at earlier days. There was marked increase in the number and size of the nodules. Seventy days after infection, the GVV became very severe. The vaginal mucosa was completely occupied by relatively large sized translucent nodules which were raising the mucosa.

Microscopically, all the infected lambs showed lymphocytic vulvitis, vaginitis, cervicitis, endometritis and oophoritis but their extent and severity increased with increasing period after infection. The sections of vulva, vagina and cervix of the lamb killed on 10th DPI showed diffuse lymphocytic infiltration and lymphoid nodules in the sub-epithelial region of the mucosa (Figs. 1 & 2) as well as in the tunica muscularis. At places, the epithelium over the lymphoid nodules was exfoliated. There was also perivascular plasma cells and lymphocytic cuffing in the sub-mucosa as well as in the tunica serosa of these organs. The superficial epithelial cells lining the mucous membrane showed hydropic degeneration. Mild lymphocytic infiltration was also noticed below the endometrial epithelium, around the endometrial glands and also in the ovarian stroma. In the lamb killed

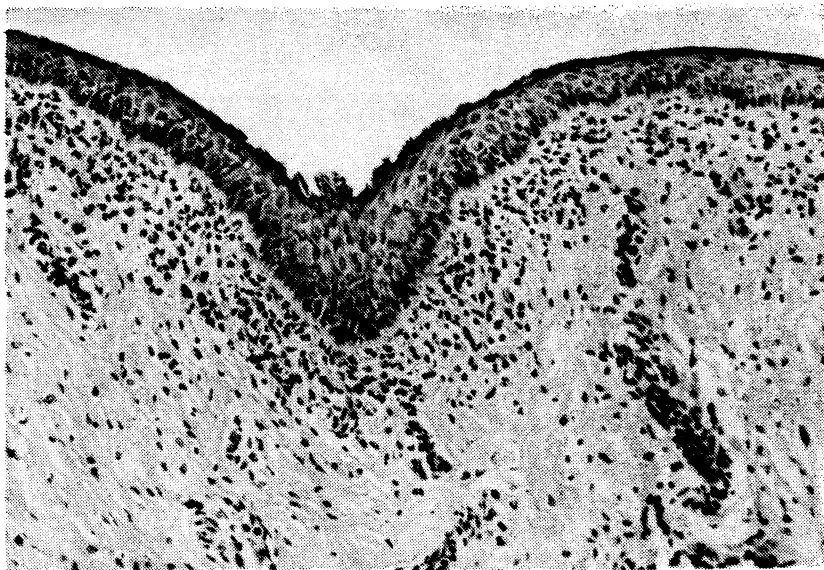


Fig. 1. Ten days post-inoculation; diffuse lymphocytic infiltration in the sub-epithelial region of vagina. H. E. x 150.

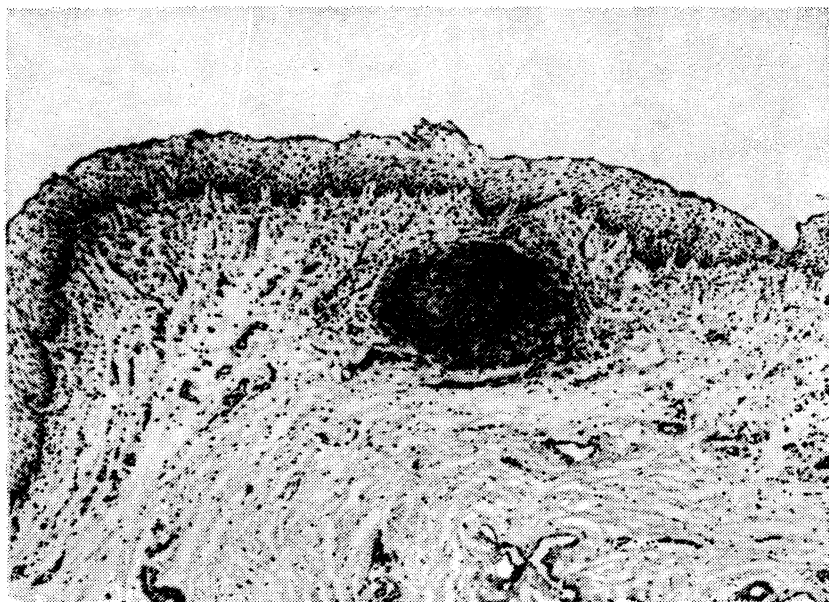


Fig. 2. Ten days post-inoculation; small lymphoid nodule below the epithelium of vulva. H. E. x 75.

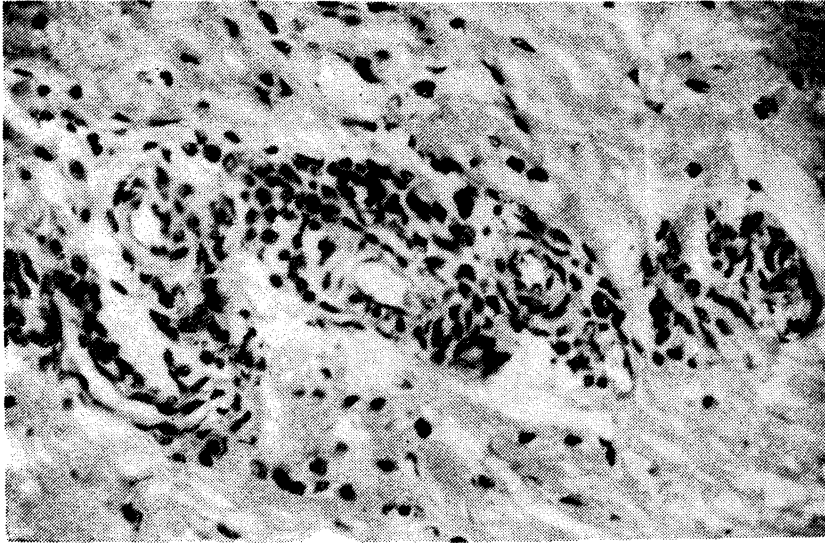


Fig. 3. Twenty days post-inoculation; perivascular plasma cells and lymphocytic cuffing in the sub-mucosa of vagina. H. E. x 300.

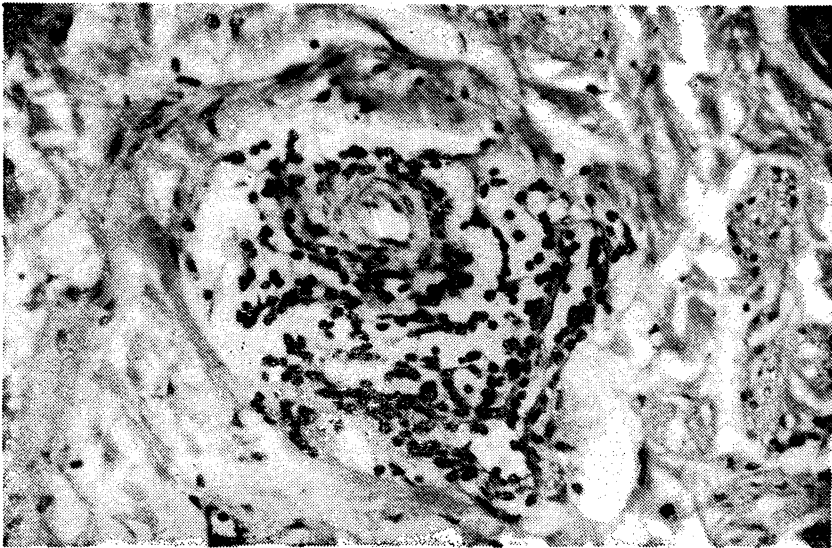


Fig. 4. Thirty days post-inoculation; perivascular lymphocytic cuffing in the tunica muscularis of cervix. H. E. x 300.

on 20th DPI, the microscopic changes in the vulva, vagina, cervix, uterus and ovary were similar but more pronounced than those seen on 10th DPI. The perivascular plasma cells and lymphocytic cuffing (Fig. 3) as well as hydropic degeneration in the mucosal epithelium of the vulva and vagina were more severe than those seen on 10th DPI. On 30th DPI, the cervix showed diffuse lymphocytic and plasma cells infiltration below the epithelium lining its lumen and in its muscle coat. The muscle coat of cervix also revealed intense perivascular lymphocytic cuffing (Fig. 4). The lymphoid nodules in the lamina propria of vulva and vagina increased in size and number (Fig. 5). The uterus and ovary showed almost similar changes as seen on 20th DPI. In the lambs killed on 40th and 50th DPI, the vulva and vagina revealed large lymphoid follicles in their lamina propria (Figure 6). At this stage, the cervix also showed formation of small lymphoid nodules besides diffuse lymphocytic infiltration below the epithelium lining the cervical canal (Fig. 7). The uterine changes were similar to those described earlier but the ovarian stroma also showed small lymphoid aggregates at a few places. On 60th DPI, the vulva and vagina showed increased number of very large lymphoid follicles in the lamina propria (Fig. 8) and also in the tunica muscularis. At this stage, cervix also revealed large lymphoid follicles below the epithelium lining its lumen. The uterus and ovary showed almost similar histopathological changes as seen earlier. The genitalia of the lamb killed on 70th DPI showed almost similar changes as seen on 60th DPI.

The genitalia of the control animal killed on 70th d of the experiment did not show any gross or microscopic pathological change. Also no pathological change was detected in other organs of the control as well as the infected lambs.

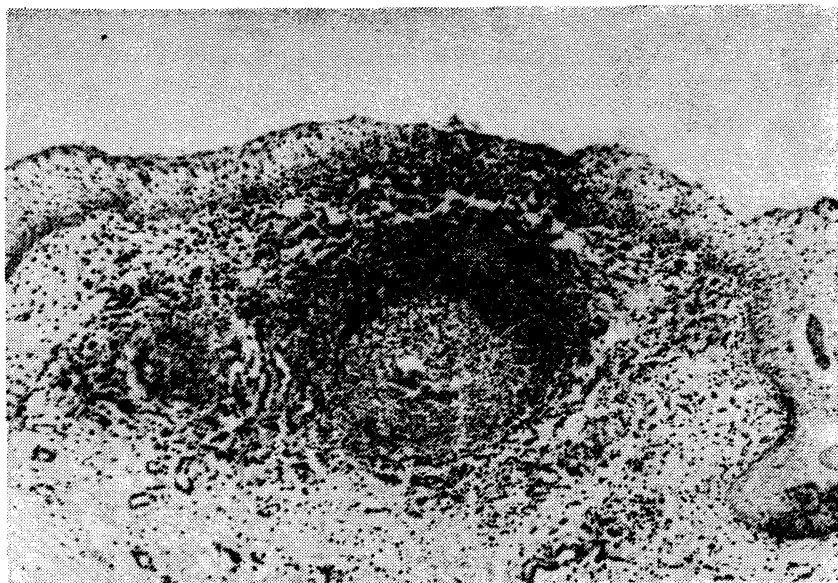


Fig. 5. Thirty days post-inoculation; large lymphoid nodule in the lamina propria of vagina. H. E. x 75.

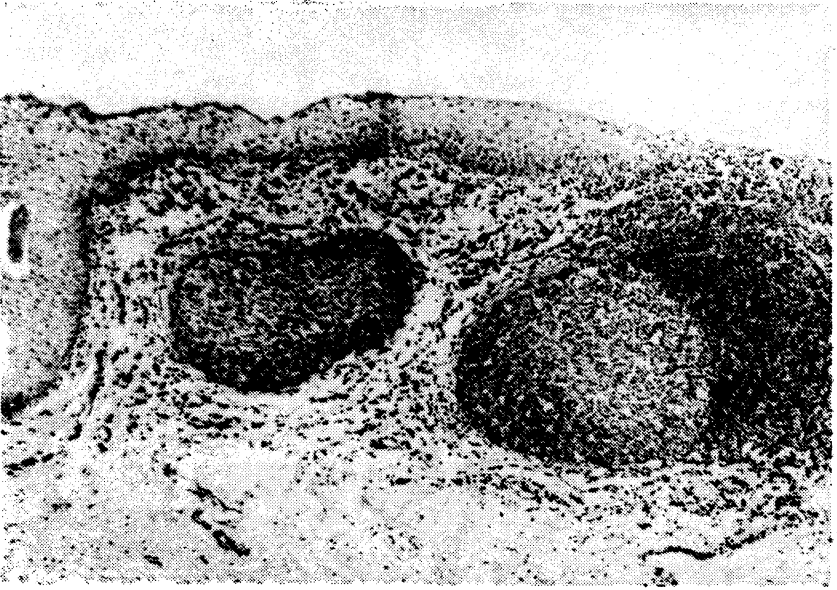


Fig. 6. Forty days post-inoculation; large lymphoid follicles in the lamina propria of vagina. H. E. x 75.

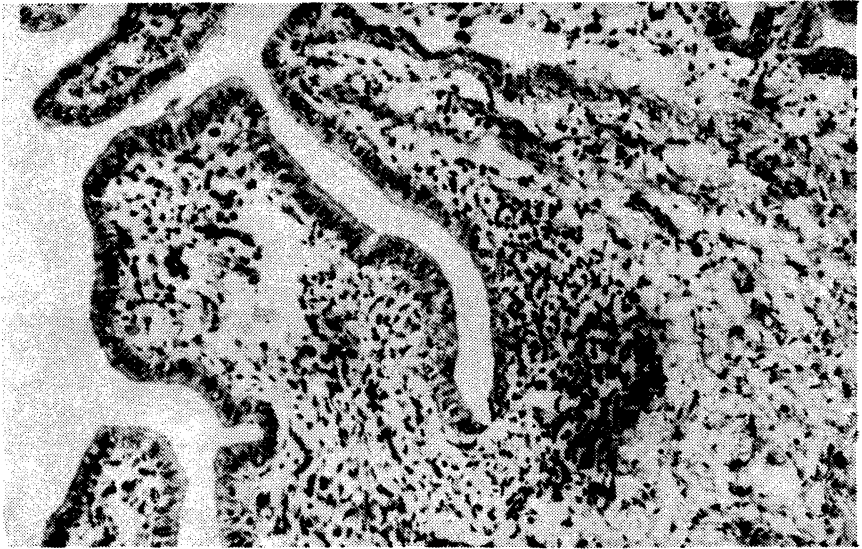


Fig. 7. Forty days post-inoculation; lymphoid aggregate below the epithelium lining the lumen of cervix. H. E. x 150.

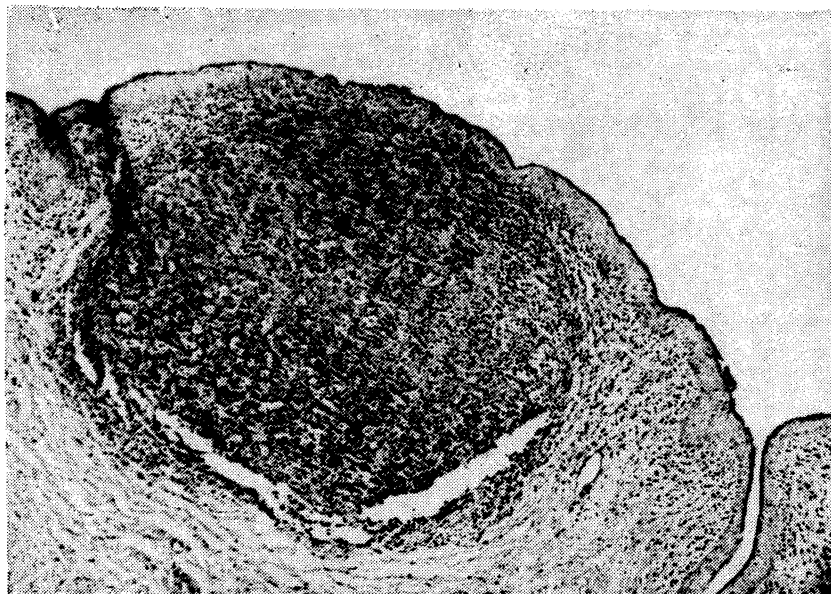


Fig. 8. Sixty days post-inoculation; very large lymphoid follicle in the vagina, raising the overlying epithelium and extending downwards into the sub-mucosa. H. E. x 75.

Discussion

The clinical observations in the present study are in accordance with those reported by other workers in spontaneous cases of GVV in sheep due to ureaplasmas, *Mycoplasma* sp. and *Acholeplasma laidlawii* (Doig and Ruhnke 1977; McCaughey and Ball 1985) and in goats due to *M. ovine/caprine* serogroup 11 and *A. oculi* (Tiwana and Singh 1982). The findings of the present study are also in conformity with experimental GVV in sheep due to ureaplasmas (Doig and Ruhnke 1977; McCaughey and Ball 1985). The haematological finding of neutrophilic leucocytosis in the initial stages is in accordance with finding of Hartman et al. (1964) during experimental intrauterine inoculation of *M. bovis* in heifers. Neutrophilia occurs as a result of systemic response to tissue injury and acute inflammation due to mycoplasma invasion. This systemic response is mediated by interleukin-1 (IL-1) which is released by macrophages following exposure to the stimulus of invading mycoplasma and also due to damaged or inflamed tissue. IL-1 is chemotactic for neutrophils and acts directly on bone marrow to stimulate the release of neutrophils into the circulation, causing neutrophilia (Tizard 1987). After 3rd DPI, gradual increase in blood lymphocytes might be due to the fact that after completing their short life span, the neutrophils degenerate and release chemotactic factors which attract large number of lymphocytes from bone marrow.

The gross and microscopic lesions in the vulva, vagina, cervix and uterus were almost similar but different in severity to those observed by other workers in

experimentally induced GVV in sheep due to ureaplasmas (Doig and Ruhnke 1977), and in goats due to *M. agalactiae* (Singh et al. 1975), *A. laidlawii* (Gupta et al. 1990) and *A. oculi* (Sharma et al. 1991). However, in the present study, lymphocytic infiltration was also seen in the ovarian stroma.

The hydropic degeneration of the epithelial cells lining the mucosa of vulva and vagina, observed in this study, indicates that *M. ovine/caprine* serogroup 11 causes damage to the epithelial cells. Mycoplasmas have special affinity for secretory epithelial surfaces, where they get intimately attached to sialic acid receptors and the receptors other than sialic acid present on host cells, thereby causing damage to host cells by various mechanisms (Razin 1978).

The infiltration of large number of lymphocytes and plasma cells in the epithelial, sub-epithelial, muscular and serosal layers and also around the blood vessels in the genital tract of the infected lambs indicates that strong cell-mediated responses are directed against the invading *M. ovine/caprine* serogroup 11 organisms.

M. ovine/caprine serogroup 11 was re-isolated from the vaginal swabs of the infected lambs and not from the blood, indicating that local infection with this organism remains confined to the genital tract and does not cause systemic reaction. Barile (1979) also stated that most *Mycoplasma* pathogens were not highly invasive, confined themselves to epithelial surfaces and produced localized infections.

The observations made in the present study lead to the conclusion that *M. ovine/caprine* serogroup 11 is pathogenic for female genital tract of sheep and it produces granular vulvovaginitis, lymphocytic cervicitis, endometritis and oophoritis.

Acknowledgements

We thank Dr. R. H. Leach, Director, Mycoplasma Reference Facility, National Collection of Type Cultures, Central Public Health Laboratory, London, U. K. for supplying *Mycoplasma ovine/caprine* serogroup 11 (2-D) culture.

Granulární vulvovaginitis (GVV) u ovcí experimentálně vyvolaná *Mycoplasma ovis/caprae* sérologické skupiny 11

K objasnění patogenity *Mycoplasma ovis/caprae* sérologické skupiny 11 pro samičí reprodukční orgány ovcí bylo použito 8 zdravých jehňat. Do lehce skarifikované sliznice vulvy a vagíny 7 jehňat byly inokulovány 2 ml kultury *M. ovis/caprae*, sérologická skupina 11 (2-D) s obsahem 6×10^2 CFU (colony forming units). Sterilní půda ke kultivaci mykoplasmat byla podobným způsobem inokulována osměmu, kontrolnímu jehněti. U všech infikovaných zvířat se do druhého dne objevil edém vulvy s mukózním výtokem, který přetrvával do 10. až 12. dne po infekci. Pokus trval 70 dní a jehňata byla utrácena vždy po jednom v 10denních intervalech. Kontrolní jehně bylo utráceno 70. dne. Vulva a vagina byla u pokusných zvířat prokrvená, edematózní s drobnými uzlíky od 10. dne. Mikroskopický nález u všech jehňat tvořila lymfocytární infiltrace vulvy, vagíny, krčku děložního a endometria. *M. ovis/caprae* sérologické skupiny 11 byla reizolována z vaginálních výtěrů všech zvířat až do 70. dne po infekci. Z nálezů vyplývá, že *M. ovis/caprae* sérologická skupina 11 je patogenní pro samičí pohlavní aparát u ovce, neboť u nich vyvolává granulární vulvovaginitis (GVV). Prezentován je první popis symptomů GVV u ovcí vyvolaných tímto zárodkem.

Гранулярный вульвовагинит (ГВВ) овец, экспериментально вызванный *Mycoplasma ovis/caprae* серологической группы 11

С целью объяснения патогенеза *Mycoplasma ovis/caprae* серологической группы 11 для органов размножения самок овец использовали 8 зпоровых ягнят. В слегка скарифицированную слизистую вульвы и влагалища 7 ягнят вводили 2 мл культуры *M. ovis/caprae* серологической группы 11 (2 -), содержащей 6×10^7 CFU (colony forming units). Стерильную среду для культивирования микоплазм аналогичным способом вводили восьмому ягненку - контрольному. У всех инфицированных животных в течение двух суток появилась отечность вульвы со слизистыми выделениями, продолжающаяся 10 - 12 суток после инфекции. Эксперимент длился 70 суток и ягнят умерщвляли всегда по одному в интервале 10 суток. Контрольного ягненка умерщвили на 70 сутки. Вульва и влагалище подопытных животных были кровенаполнены, отечны с мелкими узелками начиная с 10 суток. Микроскопические исследования у всех ягнят выявили лимфоцитарную инфильтрацию вульвы, влагалища, шейки матки и эндометрия. *M. ovis/caprae* серологической группы 11 реизолировали из вагинальных мазков всех животных до 70 суток после инфекции. Из результатов исследования вытекает, что *M. ovis/caprae* - серологическая группа 11 - является патогенной для полового аппарата самок овец, так как вызывает у них гранулярный вульвовагинит (ГВВ). Представлено первое описание симптомов ГВВ овец, вызванных данным зародышем.

References

- BANERJEE, M. - SINGH, N. - GUPTA, P. P.: Isolation of mycoplasmas and acholeplasmas from pneumonic lesion in sheep and goats in India. Zbl. Vet. Med. B., **26**, 1979: 689 - 695.
- BARILE, M. F.: Mycoplasma-tissue cell interactions. In: The Mycoplasmas, Vol. II. Human and Animal Mycoplasmas. Edited by Tully, J. G. and Whitcomb, R. F., Academic Press, New York, 1979: 425 - 464.
- COTTEW, G. S. - LLOYD, L. C. - PARSONSON, I. M. - HORE, D. E.: Isolation of *Mycoplasma* from vulvovaginitis in sheep. Aust. Vet. J., **50**, 1974: 576 - 577.
- DOIG, P. A. - RUHNKE, H. L.: Isolation of ureaplasma from sheep with granular vulvitis. Vet. Rec., **100**, 1977: 179 - 180.
- GUPTA, P. P. - SINGH, A. - BANGA, H. S. - SINGH, S. P.: Pathology of genital tract of goats experimentally infected with *A. laidlawii*. Indian Vet. J., **67**, 1990: 871 - 872.
- HARTMAN, H. A. - TOURTELLOTTE, M. E. - NIELSEN, S. W. - PLASTRIDGE, W. N.: Experimental bovine uterine mycoplasmosis. Res. Vet. Sci., **5**, 1964: 303.
- McCAUGHEY, W. J. - BALL, H. J.: The physical appearance of the vulva in ureaplasma infected ewes. Vet. Res. Communications., **9**, 1985: 123 - 125.
- RAZIN, S.: The Mycoplasmas. Microbiol. Rev., **42**, 1978, 414 - 470.
- SHARMA, C. J. - GUPTA, P. P. - BANGA, H. S.: Pathogenicity of *Acholeplasma oculi* for female genital tract of goats. Acta vet. Brno, **60**, 1991: 289 - 295.
- SINGH, N. - RAJYA, B. S. - MOHANTY, G. C.: Pathology of *Mycoplasma agalactiae* induced granular vulvovaginitis (GVV) in goats. Cornell Vet. **65**, 1975: 363 - 373.
- TIWANA, J. S.: Pathology of female genital system of sheep and goats with special reference to mycoplasma infection. M. V. Sc. thesis submitted to Punjab Agric. Univ., Ludhiana, India, 1982.
- TIWANA, J. S. - SINGH, N.: Isolation of *Acholeplasma oculi* from genital lesions in sheep. Vet. Rec. **111**, 1982: 417.
- TIZARD, I.: Trapping and Processing of foreign material; inflammation. In: Veterinary Immunology: An Introduction. 3rd ed. W. B. Saunders Co., Philadelphia, 1987: P 11-23, 263 - 279.