

BOVINE PAPULAR STOMATITIS - ITS FIRST OCCURRENCE IN SERBIA

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During July and August 1996 we had the opportunity to investigate bovine papular stomatitis for the first time in Serbia. The disease was diagnosed while urgent efforts were being taken to control foot and mouth disease in Yugoslavia. A large number of animals was examined systematically; the animals originating from Kosovo and other parts of the Republic wherever there was a suspicion of the disease. Cattle of all categories were affected, marked by the presence of characteristic papules in the oral and perioral regions without any disturbances in their general health. The early lesion, in the form of erythematous macules, ranging from 2mm to 2cm diameter, occurred on the muzzle and lips. The central part of the erythematous macule occasionally was slightly raised as a papule. Larger circular lesions, with a necrotized centre and hyperaemic border, also occurred on the muzzle, lips, gingivae hard palate, floor of the mouth, ventral and lateral surface of the tongue and occasionally on the oesophagus and forestomach. The most characteristic symptom was that of ballooning degeneration of the stratum spinosum cells and the presence of dense eosinophilic inclusions in their cytoplasm. The described changes were detected in cattle of various ages, from calves barely 2 months old to adult cattle 7 or more years old. However, the disease was predominant in young cattle. The exact number of affected animals was never established, but in some villages almost all herds were affected, especially where there was a possibility of direct contact at pasture. Diagnosis was based on the clinical disease, patho-morphological findings, electron microscopical characterization of the causative virus and virus isolation.

Key words: bovine papular stomatitis, ballooning degeneration, eosinophilic inclusions, parapoxvirus, cattle

INTRODUCTION

Bovine papular stomatitis occurs worldwide. The report of its first appearance in Europe dates from 1884 (Aguilar-Setien et al., 1980). The disease has

been reported in the USA (Griesemer and Cole, 1960), Canada (Fraser and Savan, 1962), Africa (Plowright and Ferris, 1959), Australia (Snowdown and French, 1961) and Mexico (Aguilar-Setien et al., 1980). It is primarily a benign stomatitis accompanied by papules in the oral and perioral regions. Some reports (Sentsui et al., 1999) claim that Parapoxvirus causes a consistent infection in cattle accompanied by no clinical signs and that can be activated by stress factors inducing the modification of immune reactions. Bovine papular stomatitis must be differentiated from some other, more severe, conditions affecting the first segments of the alimentary tract and skin (Aguilar-Setien et al., 1980; Yeruham et al., 1994). It is caused by Parapoxvirus, which is very similar but not identical, to the virus that causes pseudocowpox (Menna et al., 1979; Wittek et al., 1979). Bovine papular stomatitis virus grows well in lung embryonic cells from calves and lambs, as well as in bovine kidneys (Aguilar-Setien et al., 1980; Pospischil and Bachmann, 1980). The detection and correct diagnosis of Parapoxvirus is made by polymerase chain reaction (PCR) (Inoshima et al., 2000). The virus can be identified by physicochemical methods and electron microscopy (Gassmann et al., 1985; Lard et al., 1991; Kuroda et al., 1999). The virus can be transmitted to humans, in which case small papules develop on the skin of the fingers and forearm and they can persist for several weeks (Lieberman and Jung., 1977; Schnurrenberger et al., 1980; Bowman et al., 1981; Shelley and Shelly, 1983; Smith et al., 1991; Mercer et al., 1997).

MATERIAL AND METHODS

During July and August 1996 we had the opportunity to deal with bovine papular stomatitis for the first time in Serbia. The disease was diagnosed while urgent efforts were being taken to control foot and mouth disease in Yugoslavia. A large number of animals was examined systematically; the animals originating from Kosovo and other parts of the Republic wherever there was a suspicion of the disease. Cattle of all categories were affected, marked by the presence of characteristic papules in the oral and perioral regions without any disturbances in their general health. The tissue samples of the oral mucosa, taken by biopsy from 20 head of cattle with typical lesions were examined histopathologically and microbiologically.

Sample preparation. The tissue samples from oral and perioral regions were fixed in 10% neutral formalin. After fixation processing was completed using an automatic tissue processor, involving graded alcohols, xylol and paraffin wax. Finally, 6µm thick paraffin sections were stained with HE (hematoxylin and eosin) and PAS (Periodic acid Schiff).

Microbiological examination. Tissue samples taken from the skin and affected mucosa were treated in the way appropriate for virological examinations. Prior to inoculation, the material was fragmented, homogenized with laboratory grit and centrifuged for 30 min at 3,500 rotations per minute. Filtrates and pure supernatants were used for inoculation into chicken embryos 10 days old. They were monitored for 5 to 7 days. All the embryos were alive at shell opening and

whitish changes in the form of thickenings and pox, were detected on the allantochorion. The material from the membranes, as well as those from the tissue samples from the affected animals, were inoculated into: young hamster kidney culture (BHK-21), chicken fibroblasts (CF) and bovine kidney cells (BKC).

RESULTS AND DISCUSSION

Macroscopic findings. The early lesion, in the form of erythematous macules, ranging from 2mm to 2cm diameter, occurred on the muzzle and lips (Figure 1). The central part of the erythematous macule occasionally was slightly raised as a papule. Larger circular lesions, with a necrotized centre and hyperae-



Figure 1. Calf: early lesions on the muzzle and lips

mic border, also occurred on the muzzle, lips, gingivae (Figure 2), hard palate, floor of the mouth, ventral and lateral surface of the tongue and occasionally on oesophagus and forestomach. The described changes were detected in cattle of various ages, from calves barely 2 months old to adult cattle 7 or more years old. However, the disease was predominant in young cattle. The exact number of



Figure 2. Bull: advanced lesions on the gingiva

affected animals was never established, but in some villages almost all herds were affected, especially where there was a possibility of direct contact at pasture. The observed symptoms, especially at the beginning, presented a lot of problems to clinicians attempting to make a correct diagnosis. Proliferative lesions of bovine papular stomatitis in the mouth may resemble early lesions of foot and mouth disease and vesicular stomatitis, whereas older lesions may resemble bovine viral diarrhea as well as infectious bovine rhinotracheitis (Plowright and Ferris, 1959; Anguilar-Setien et al., 1980).

Microscopic findings. The histological analysis of the damaged mucosa samples revealed focal intensive hyraemia and oedema in the lamina propria



Figure 3. Proliferation and vacuolisation in the cytoplasm of the oral epithelium cells (HE), 200X

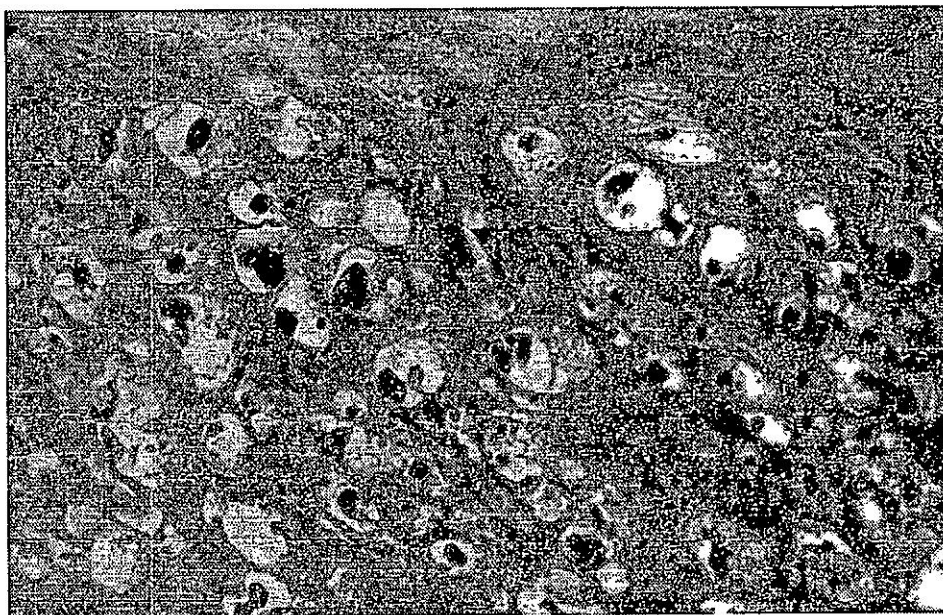


Figure 4. Ballooning degeneration and eosinophilic inclusions in the cytoplasm of the stratum spinosum cells, (HE), 400X

papilla accompanied by an aggregation of a small amount of mononuclear cells. In some samples the epithelium was twice as thick as that of the unaffected one. Early lesions were perceived in the stratum spinosum of the oral epithelium marked by the presence of proliferation, enlargement and vacuolization of epithelial cell cytoplasm (Figure 3). The most characteristic symptom was that of ballooning degeneration of the stratum spinosum cells and the presence of dense eosinophilic inclusions in their cytoplasm (Figure 4). This finding is in agreement with the reports of other investigators (Plowright and Ferris, 1959; Angular-Setien et al., 1980).

Cell vaculization and proliferation, accompanied by inclusions, were perceived also in the allantochorion cells used for the inoculation of the supernatant tissue originating from affected animals (Figure 5). Although patho-morphological



Figure 5. Proliferation, vacuolisation and inclusions in the allantochorion cells, (HE), 200X

changes in bovine papular stomatitis are characteristic enough, isolation of the virus is require for a correct diagnosis. Inoculation of the infective material from the oral mucosa into cell culture media (BHK-21, BKC, CF) induced cytopathogenic effects and changes on chicken embryos. Although it has been stated in the literature that parapoxvirus is not easy to cultivate in chicken embryo allanto-

chorion, in our work, with a passage of the material through embryos, we achieved a typical cytopathogenic effect and identical changes in the pathohistological picture as those on the oral mucosa of the primary host. Electron microscopy helped us to detect the presence of cytoplasmic structures typical for parapoxvirus in the oral mucosa with ulcerative lesions and in allantochorion cells.

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PAPULOZNI STOMATITIS GOVEDA - PRVA POJAVA BOLESTI U SRBIJI

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SADRŽAJ

U radu je opisana prva pojava papuloznog stomatitisa u goveda na teritoriji Srbije. Bolest se pojavila tokom jula i avgusta 1996 godine. Inicijalne lezije su bile izražene u vidu eritematozne makule veličine od 2mm do 2 sm u prečniku na njušci i usnama. Centralni deo eritematozne makule ponekad je bio blago izdignut kao papula. Šire lezije oblika novčića sa nekrotizovanim centrom i hiperemičnim rubom takođe su se javljale na njušci, usnama, zubnim jastučićima, tvrdom nepcu, podu usne šupljine, ventralnoj i lateralnoj površini jezika i povremeno u ezofagusu i predželucima. Najkarakterističniji histopatološki nalaz je predstavljala balonska degeneracija ćelija stratuma spinošuma i prisustvo gustih eozinofilnih inkluzija u njihovoj citoplazmi. Opisane promene ustanovljene su u goveda različitog uzrasta, počev od teladi starih dva meseca pa do goveda starih 7 i više godina. Bolest je međutim, dominirala kod mlađih grla. Tačan broj obolelih životinja nije ustanovljen ali u pojedinim selima bila su obolela gotovo sva stada, naročito tamo gde je postajala mogućnost kontakta na zajedničkoj ispaši. Dijagnoza je postavljena na osnovu kliničke slike, makroskopskog i mikroskopskog nalaza, elektronsko mikroskopske karakterizacije parapoksvirusa i izolacije virusa.