VETERINARSKI ARHIV 75 (5), 391-397, 2005

Severe bovine papillomatosis: detection of bovine papillomavirus in tumour tissue and efficacy of treatment using autogenous vaccine and parammunity inducer

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ABSTRACT

Two cases of severe bovine papillomatosis occurred in northwest Croatia during 2000 are described. Diagnosis was based on presented clinical signs, histopathological findings, and detection of the papillomavirus by transmission electron microscopy. Animals successfully recovered following treatment with the autogenous (non-purified) vaccine and a parammunity inducer. For the first time in Croatia we report the detection of papillomavirus by electron microscopy.

Key words: bovine papillomavirus, transmission electron microscopy, autogenous vaccine, parammunity inducer

Introduction

Bovine papillomatosis is a common viral disease of the skin, mostly of young cattle, manifested as benign tumours or warts, caused by bovine papillomavirus (BPV) that has

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ISSN 0372-5480
Printed in Croatia

six serotypes hitherto described (OLSON, 1990). These neoplasms most often regress spontaneously, occasionally persist, and, in the presence of additional critical genetic or environmental factors, can progress to cancer (CAMPO, 1987). It is thought to be a multistep affair (KOLLER and OLSON, 1972; LANCASTER and OLSON, 1982). Furthermore, papillomavirus infection in cattle could be connected with serious disorders of the metabolism (mainly mineral, energetic and nitrous) probably caused by damage of the liver and kidney with mutagenic, carcinogenic and immunosuppressive cadmium, arsenic and lead, observed in the serum of tested animals (LESNIK et al., 1999). Infection by BPV occurs as a result of the virus exposure to single or multiple lesions of the epithelium. Papillomaviral infection, transformation and multiplication of basal cells, lead to wart formation, but most warts are benign and do not proliferate indefinitely (SHAH and HOWLEY, 1996). Different methods have been used to treat bovine papillomas. A formalinized suspension of bovine warts with inactivated virus provides a vaccine for effective treatment and prophylaxis of bovine papillomatosis (BARTHOLD et al., 1976; HUNT, 1984; LESNIK et al., 1999; SÜVEGES and SCHMIDT, 2003). Intra-lesional immunotherapy by *Corynebacterium parvum* has also been reported (HALL et al., 1994). There are no reports about combined therapy of bovine papillomatosis using the autogenous vaccine and a parammunity inducer.

Although numerous cases of bovine papillomatosis have occurred in Croatia over past years, written data regarding diagnosis and treatment are still scarce. Thus, in order to obtain a better insight into bovine papillomatosis, we report here on severe cases recently observed in Croatia, detection of BPV by transmission electron microscopy, treatment with the autogenous vaccine and a parammunity inducer, and clinical recovery of animals.

Materials and methods

Animals. Two cases of severe bovine papillomatosis occurred in northwest Croatia during 2000 are described. The first case was registered in a 15-month-old Holstein heifer suffering from severe generalized papillomatosis with multiple papillomas, from 0.5 to 50 mm in diameter, disseminated on the ears, head, neck, shoulders, abdomen, udder and perigenitaly. The warts on the abdomen had a cauliflower-like appearance, strongly attached to the dermis (Fig. 3). The second case was registered in a 4-year-old dairy Simmental cow with multiple papillomas (from 0.5 to 30 mm) on the head, neck, shoulder and udder.

The diagnosis of bovine papillomatosis was arrived at on the basis of presented clinical signs, since the structure of the papillomas on the skin was easily observed and identified. However, a few papillomas from each animal were surgically removed in order to confirm the diagnosis by histopathology, and for the preparation of an autogenous vaccine. Furthermore, the tumours were taken in order to detect BPV by transmission electron microscopy in supernatant of dissected homogenized tissue.

Histopathology. Surgically obtained tissue samples were immediately immersed in 10% formalin at room temperature for at least 48 h before processing. Fixed specimens were dehydrated through graded alcohols and embedded in paraffin wax; 6 μm-thick serial sections were cut, stained with haematoxylin and eosin (HE), and examined with a light microscope equipped with an ocular micrometer (Nikon Eclipse E600, Japan).

Electron microscopy. Tissue samples were prepared for electron microscopy by the negative staining technique. Pelleted viral particles were resuspended in distilled water and a drop of viral suspension was placed on a Petri dish. A Formvar-coated electron microscopy (EM) grid was placed Formvar side down on top of the virus drop for approximately 1-3 minutes. The grid was removed, blotted with filter paper and placed onto a drop of 2.0% phosphotungstic acid (PTA), pH 7.0, for one minute. The excess PTA was removed, and the EM grid was ready for viewing in the Zeiss EM 10A transmission electronic microscope.

Autogenous vaccine preparing and treatment of animals. The autogenous vaccine was prepared according to laboratory protocol reported previously by HUNT (1984). The animals were treated by autogenous vaccine administered in doses of 0.5 ml i/c and 10 ml s/c, and twice revaccinated at 10-day intervals. Prior to vaccination, as a parammunity inducer, on each occasion the immunomodulator (Baypamun®, Bayer Pharma, Leverkusen, Germany) was administered in doses of 2 ml s/c.

Results

Diagnosis was based on presented clinical signs, histopathological findings, and detection of the papillomavirus by transmission electron microscopy. Grossly, tumour tissue was composed of hyperplastic epidermis supported by thin, inconspicuous dermal stalks. In both cases histopathology revealed fibropapillomatosis with acanthosis, hyperkeratosis and down-growth of rete ridges. The virus appears to infect the basal cells of the epithelium, causing hyperplasia with hydropic ballooning of their cytoplasms, large eosinophilic keratohyaline granules and vesicular nuclei. Some cells degenerated, while others were stimulated to excessive growth and formation of warts (Fig. 1).

Electron microscopy examination of tumour tissue by the negative staining technique demonstrated virions that were very similar to BPV (Fig. 2). The diameter of the virions measured approximately 60 nm. The typical papillomavirus virions are composed of capsomeres arranged in icosaedral symmetry of the capside. Only sporadic virions were found in the hyperkeratotic surface layer, while large clusters were formed in the deep layers. Both solitary destroyed and corelless particles with electrondense core space were present in the aggregates.

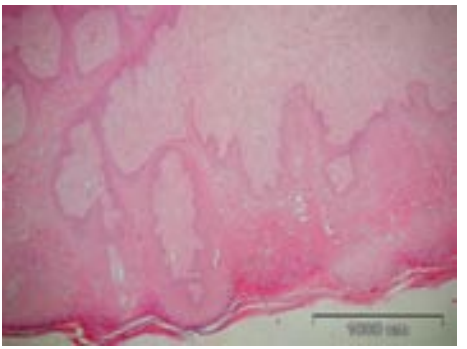

Fig. 1. Histopathological section of a wart tissue stained with HE

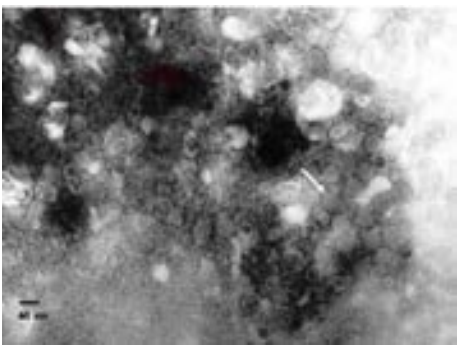

Fig. 2. Transmission electron micrograph of negatively stained tissue homogenizatum shows cluster of BPV. Arrow shows one virion measuring approximately 60 nm.

Fig. 3. Multiple papillomas in Holstein heifer disseminated on the abdomen, measuring from 0.5 to 50 mm.

Fig. 4. Complete regression of papillomas 6 weeks after the beginning of treatment.

The animals were treated successfully by autogenous vaccine and an immunomodulator. Regression (Fig. 3 and Fig. 4) of papillomas in both cases occurred about 3 weeks after the beginning of treatment, and within 6 weeks all warts spontaneously disappeared and animals completely recovered. No recurrence of papillomas has been observed in treated cows.

Discussion

Although bovine papillomatosis is a self-limiting disease the animals in our study have long lasting multiple papillomas without any sign of regression within months. The clinical diagnosis was confirmed by histopathology findings and detection of BPV in tumour tissue

by transmission electron microscopy. For the first time in Croatia, we report the detection of papillomavirus by electron microscopy.

The autogenous vaccine and a parammunity inducer (Baypamun®, Bayer Pharma, Leverkusen, Germany) were used for treatment of animals. Reports of bovine papillomatosis treatment with vaccine produced from formalinized suspension of wart tissue indicate variable results. LESNIK et al. (1999) reported that treatment with vaccine showed 93.5% efficiency with no difference in the used vaccine after 105 days of vaccination. SÜVEGES and SCHMIDT (2003) showed autogenous vaccination made from sterile homogenized tumour tissue and, performed twice, prevented new cases and with sick animals recovering after vaccination. On the contrary, treatment with autogenous wart vaccine sometimes failed (SMITH, 1990). Commercial vaccines for cattle rarely seem to effectively promote regression of existing warts or to prevent malignant progression, although they may be capable of preventing the development of new lesions if the same strain is involved (SMITH, 1990; CAMPO, 1991; SCOTT and ANDERSON, 1992). As shown in several *in vivo* studies, the stimulation of endogenous, non-antigen related defence mechanisms by parapoxvirus-based immunomodulators opens up new possibilities for the control and treatment of infectious diseases in domestic animals (STRUBE et al., 1989; ZIEBELL et al., 1997; CASTRUCCI et al., 1998; KYRIAKIS et al., 1998; GLITZ, 2002). Inactivated parapox ovis viruses, due to their complex genetic structure and thereby their strong immunogenic properties, induce host immune reaction. There is growing evidence that such immune reactions may result in more than elimination of the virus (FACHINGER et al., 2000). Our results showed the efficacy of bovine papillomatosis treatment with the autogenous vaccine and a parammunity inducer in the manner of earlier regression of papillomas. Data reported previously based on the treatment only with autogenous vaccine showed a longer period necessary for animal recovery then we obtained (SCOT and ANDERSON, 1992; LESNIK et al., 1999). Additionally, we consider that our treatment could be appropriate in the early stage of disease (growing stage of warts) when surgical intervention is contraindicated due to the possibility of recurrence of papillomas. Although we treated only two animals in that manner, we believe that a parammunity inducer also shows a beneficial effect in additional treatment of bovine papillomatosis. However, our hypothesis needs to be clarified and proved in further studies. We consider that it is important to compare reports of new cases to obtain a better insight into the aetiology, pathogenesis and different methods of treatment. Furthermore, molecular epizootiology of BPV viruses circulating in Croatia remains to be investigated in the future.

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Received: 15 December 2004
Accepted: 7 September 2005

TURK, N., Ž. ŽUPANČIĆ, V. STAREŠINA, S. KOVAČ, T. BABIĆ, M. KRESZINGER, S. ĆURIĆ, LJ. BARBIĆ, Z. MILAS: Teški oblik papilomatoze goveda: nalaz papilomavirusa u tumorskom tkivu i učinkovitost liječenja autogenom vakcinom i imunomodulatorom. Vet. arhiv 75, 391-397, 2005.

SAŽETAK

U radu su opisana dva slučaja generalizirane papilomatoze u goveda ustanovljena u sjeveroistočnom području Republike Hrvatske tijekom 2000. godine. U oba slučaja dijagnoza je postavljena na osnovi kliničke slike, histopatološke pretrage tumorskog tkiva i nalaza papilomavirusa goveda u homogenizatu tumorskog tkiva elektronskom mikroskopijom. Životinje su liječene autogenom vakcinom i imunomodulatorom (Baypamun®, Bayer Pharma, Leverkusen, Germany), a tumorozne promjene u potpunosti su iščezle nakon 6 tjedana od početka liječenja. U radu je po prvi put u Republici Hrvatskoj opisan nalaz papilomavirusa goveda u tumorskom tkivu elektronskom mikroskopijom.

Ključne riječi: papilomavirus goveda, elektronska mikroskopija, autogena vakcina, imunomodulator