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Case Report

First confirmed diagnosis of Sheep-associated Malignant Catarrhal Fever in Bison in Argentina

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Abstract

In Argentina, cases of malignant catarrhal fever (MCF) are suspected to have occurred according to macro and microscopic lesions. However, none has been corroborated by molecular tests. We describe here the first laboratory confirmed case of MCF in Argentina occurring in American bison confined in the Buenos Aires Zoo.

Key Words: OvHV-2, bison, MCF, PCR

Introduction

Malignant catarrhal fever (MCF) is an acute generalized viral infection that affects different ruminant species such as domestic bovid, buffalo, American bison and deer. It has worldwide distribution and different morbidity and mortality according to the species (18).

Viruses associated with MCF belong to the *Rhanidnovirus* genus within the *Gammaherpesvirinae* subfamily and the *Herpesviridae* family (17).

The first one to be identified was the alcelaphine herpesvirus type 1 (AlHV-1) whose reservoir is the wildebeest . It causes the African version of the disease known as WA-MCF (wildebeest-associated malignant catarrhal fever). The second virus, known as ovine herpesvirus type 2 (OvHV-2), is the main cause of this disease around the world. It is asymptomatic and endemic in ovid and causes the SA-MCF (sheep-associated malignant catarrhal fever) in different ruminant and deer species (17). This disease is characterized by high fever, long viremia, profuse nasal discharge, corneal opacity, ophthalmia, generalized lymphoadenopathy, leukopenia, severe inflammation of mucous membranes (conjunctival, oral, nasal) with necrosis of the oral and nasal cavities, sometimes extended to esophagus, trachea (1) and skin (4,7).

Since many of the clinical signs are non-specific, diagnosis of this disease is traditionally made through anatomopathologic studies. The lack of isolation of the SA-MCF agent in tissue culture leads to diagnosis by alternative techniques such as ELISA and a polymerase chain reaction (PCR) with specific or degenerate primers (9).

In American bison this disease is lethal and usually occurs in animals that had contact with sheep, even for short periods of time (20).

In this work, it is described the first case of MCF confirmed by laboratory methods in Argentina.

Description of the outbreak

The outbreak occurred in American bison (*Bison bison*) confined in the Buenos Aires Zoo during the winter of 2007 which was particularly cold. It started with the

death of three Pere David's Deer (*Elaphurus davidianus)* in April 2007 with no clear clinical signs. These deer were located in proximity to a group of mouflon sheep (*Ovis orientalis musimon*) which in turn were neighbors to the bison.

During the same month, an American bison died with cough and decay. From April to July a total of 5 American bison from a group of 6 died with signs such as conjunctivitis, decay, nasal discharge and cough.

Necropsies were performed and tissue samples taken from all animals for histopathological studies. Tissue samples were fixed in 10 per cent buffered formol saline and processed in parafine wax. Four µm samples were cut and stained with haematoxylin and eosin. Anatomopathological studies were performed at the SENASA (Pathology Department) and INTA Castelar (Pathobiology Institute) facilities.

Bacteriologic tests were performed for the detection of common bovine pathogens such as *Leptospira* (by direct immunfluorescence with polyclonal serum) *and Brucella* (buffered antigen in plaque) following INTA and SENASA standard operating procedures (2,1,19).

PCR tests were performed for the diagnosis of bovine viral diarrhea virus (BVDV) and foot and mouth disease virus (FMDV). Inmunofluorescence tests were performed both on tissues and inoculated cells for the diagnosis of infectious bovine rhinotracheitis virus (BoHV-1), bovine parainfluenza virus type 3(BPIV-3), bovine respiratory syncytial virus (BRSV), bovine adenovirus types 3 and 5 (BAV-3, BAV-5), bovine rotavirus (BRV) and bovine coronavirus (BCV)(14). Tests also included BSE (Bovine Spongiform Encephalopathy) detection by the western blot method. All virological studies (except for the OvHV-2 PCR described below) and BSE tests were performed according to SENASA and INTA standard operating procedures (14). Search for helmints and oocysts was done by standard methods (22,13).

Since all lesions observed were compatible with MCF, a PCR reaction was performed with DNA extracted from lymph nodes and spleen from all deceased animals with Dnazol (Invitrogen Corporation, GibcoBRL). For mouflon, domestic and Somalia sheep as well as for domestic goats, EDTA- anticoagulated blood samples were processed for DNA extraction using Dnazol BD (Invitrogen Corporation, GibcoBRL). PCR reactions were performed with 500 ng of DNA using a PCR protocol previously described which amplifies a region of 248 bp of the tegument protein of OvHV-2 (9). PCR was also attempted on paraffin embedded tissues from Père David's deer.

Results

Gross lesions consisted of emaciation, meningeal congestion, purple discoloration and frequent erosions of the lips, oral papillae, gums, soft palate, and sides of the tongue mucosae (Figure 1); severe congestion of the nasal and conjunctival mucosae with presence of catarrhal discharge; pulmonary edema and congestion- the lungs usually failed to collapse when the thoracic cavity was opened- showing edema, congestion, rib imprints and purple or whitish foci; mucosa of the pharynx, larynx and esophagus was congestive and hemorrhagic with longitudinal ulcerations (Figure 2); congestion in rumen, reticulum and omasum(Figure 3). There were small ulcerations and deep congestion of the mucous membrane in the abomasum (Figure 4). The small and large intestine as well as the caecum mucosa and contents showed patchy congestion and hemorrhages (Figures 5, 6). Changes could be seen in the rectum, consisting mainly of congestive lines along the mucous folds (Figure 7). Enlargement, hemorrhage and congestion of the external lymph nodes such as those from the head, neck and preescapular region were viewed. The liver was enlarged, congestive, softened with grayish mottling; epicardium and pericardium exhibited petecchiae and ecchimoses on them with sub-endocardical hemorrhages; slightly enlarged, congestive and grayish mottling of the liver; kidneys with small whitish foci in cortex; petechial hemorrhages in urinary bladder. There were no skin lesions.

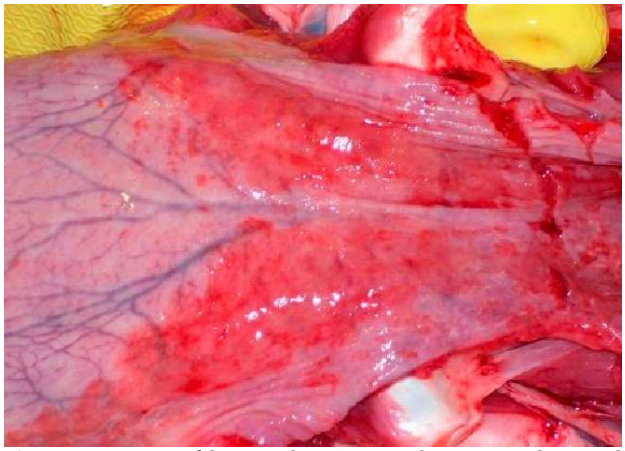

Figure 1 – Tongue of bison with MCF. Note hiperemia and mucosal erosions.

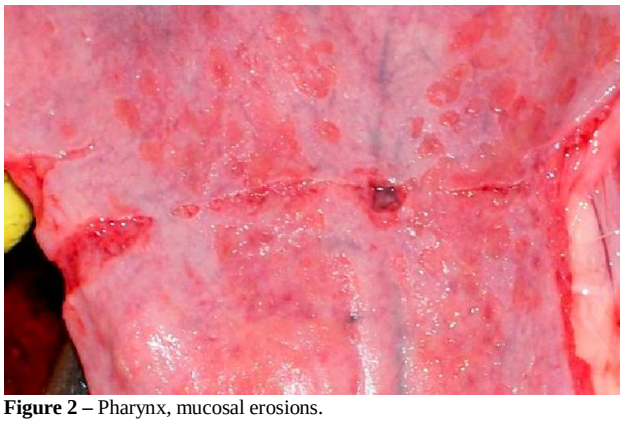

Figure 2 – Pharynx, mucosal erosions.

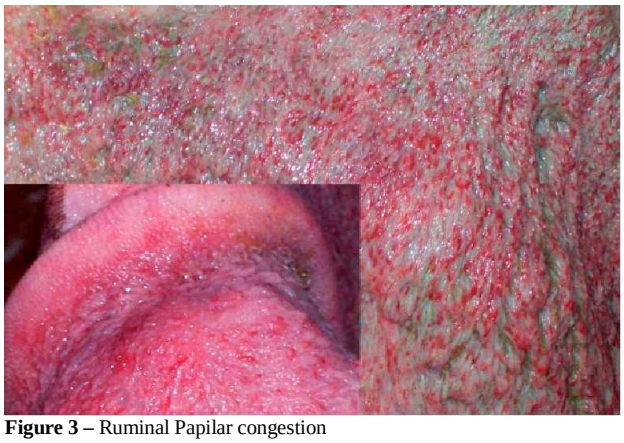

Figure 3 – Ruminal Papilar congestion

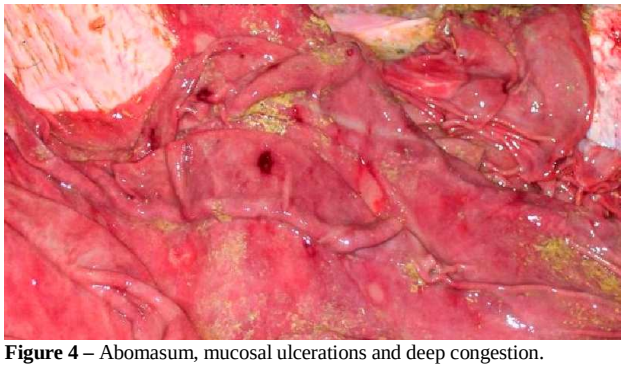

Figure 4 – Abomasum, mucosal ulcerations and deep congestion.

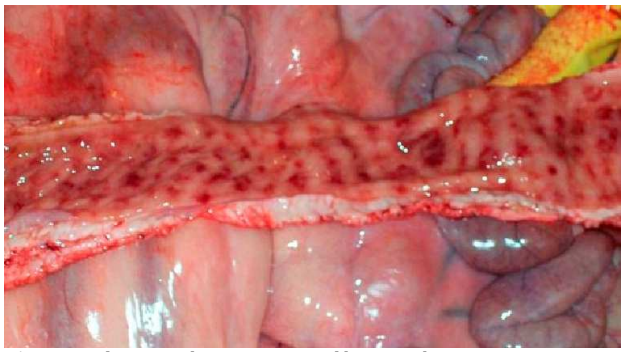

Figure 5 – Ileum, patchy congestion and hemorraghes.

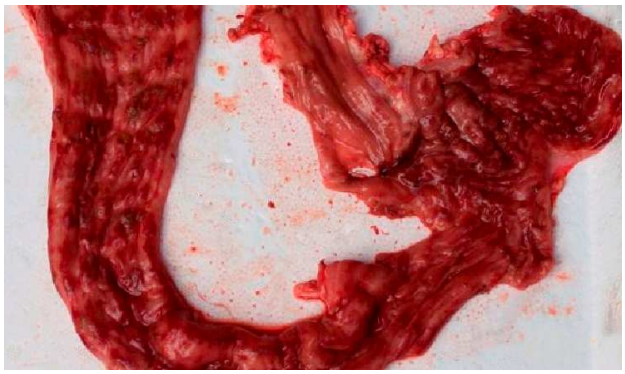

Figure 6 – Ileocecal Valve, congestion and hemorraghes

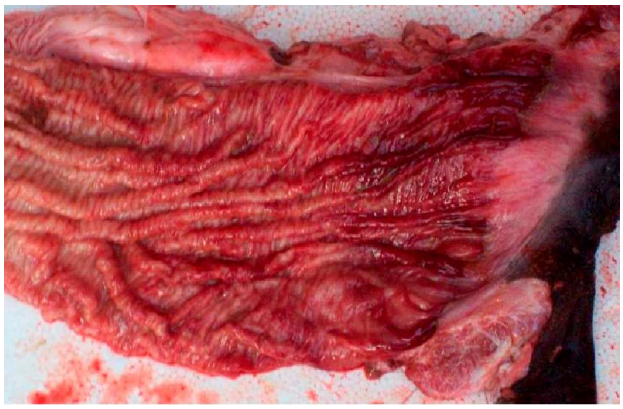

Figure 7 – Congestive lines along the mucous folds

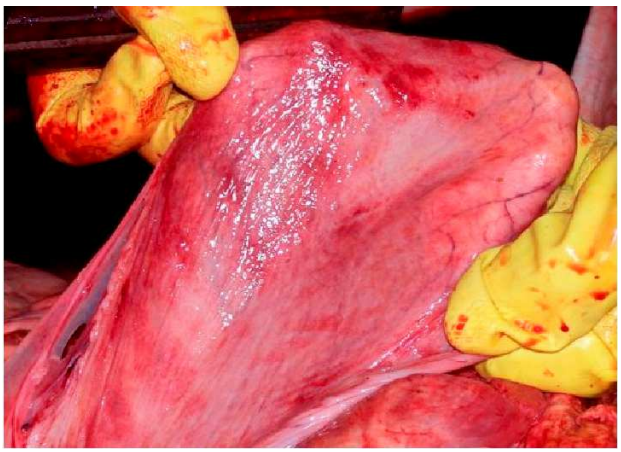

Figure 8 – Urinary Bladder, congestion, erosions and petechial hemorraghes

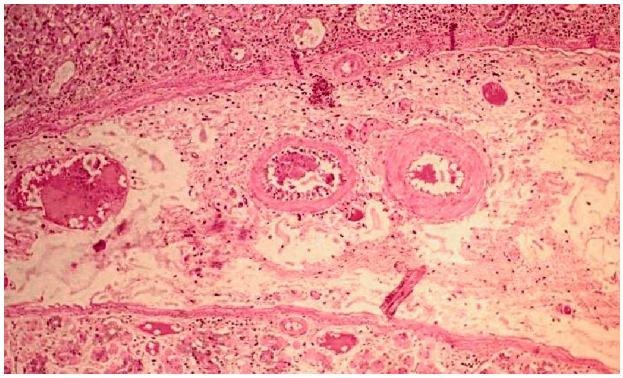

Figure 9 – Abomasum, vasculitis

In one bison, both whitish and purple infarctions were viewed on the diaphragmatic hepatic surface. The renal cortex had whitish mottling. Petechial hemorrhages were observed on the urinary bladder (Figure 8) which contained pink turbid urine. In one animal, minute ulcers with adhered crusts were spread on the bladder mucosa. The spleen was softened and deeply congestive, with black discoloration.

Microscopic findings included: generalized vasculitis of small and medium caliber arteries with

fibrinoid necrosis between the intima and media layers; and accumulation of mononuclear cells in the adventitia layer were observed in the brain, superficial lymph nodes, thyroid glands, adrenal glands, omasum, abomasum, jejunum, ileum, liver, kidneys and urinary bladder (Figure 9). Blood vessel lesions were often associated to edema, hyperemia, hemorrhage, disseminated intravascular coagulation (DIC) and infarction in many of the above mentioned sites. Interstitial lymphohistiocytic infiltrates were evident in most of these tissues as well as in the myocardium and rumen. There was fibrinohemorrhagic lymphadenitis of the superficial lymph nodes. The mucosal membrane of the digestive tract exhibited erosions and ulcerations, frequently associated with infarction. The mucosa of the urinary bladder was ulcerated. There was interstitial pneumonia with proliferation of both alveolar epithelium and bronchial-associated lymphoid tissue (BALT). Membranous glomerulopathy and glomerulosclerosis could also be seen.

Bacteriologic, parasitologic and BSE tests were negative. All attempts for viral isolation and immunofluorescence tests for viral agents were also negative. PCR tests for BVDV and FMDV did not show evidence of these viruses. On the contrary, PCR for OvHV-2 was positive in all bison samples tested.

Before confirmation by PCR and due to the possibility that the disease was in fact MCF (a disease of mandatory notification) and to prevent the infection of other susceptible species, the National Animal Health Service (SENASA) decided to close down the facilities after the death of the fourth bison.

After PCR confirmation of OvHV-2 in all the samples (lymph nodes and spleen of all affected bison), an epidemiologic study was performed in search of potential sources of the virus in the form of asymptomatic carriers. At the zoo, bison were housed at 20 meters from eleven mouflon sheep (*Ovis orientalis musimom*) and at 150 meters from thirty-two domestic sheep (*Ovis aries aries)*, eleven sheep from Somalia (*Ovis aries sleatopigas)* and eleven domestic goats. (*Capra aegragus hircus)*. EDTA-anticoagulated blood samples from these animals were sent to the Faculty of Veterinary Science, University of Buenos Aires where PCR tests were performed. PCR results proved that mouflon sheep as well as the domestic sheep were positive whereas the sheep from Somalia and the goats were negative. DNA extracted from Père David's deer paraffin embedded tissues was also negative.

Preventive measures were implemented: removal of sheep and mouflon sheep as well as sheep of Somalia, goats and African goats with their offspring; implementation of a pediluvium; bison, farm animals and giraffe caretakers were instructed not to visit other sites considered as hazardous; areas where possible carriers or potentially susceptible animals lived, were cleaned with chlorine and usual tools (scoops, brooms, etc) assigned only for those areas.

In June 2008 the ban declared by SENASA was lifted. Interestingly, a female bison born to a positive dead bison during the time of the outbreak (2007) was PCR tested for OvHV-2 in 2008 resulting negative. Other bison that was part of the outbreak but survived in spite of developing disease, has yet to be tested again.

Discussion

In this work we describe an outbreak of MCF in bison at the Buenos Aires Zoo. This was the first PCR confirmed case of MCF in exotic zoo animal species from Argentina. Bison are known to be a very susceptible species as described in several feedlot outbreaks (15,3,11). It is also known that the proximity to sheep is crucial for these animals to get infected existing a very strong correlation between the distance sheep-bison and the mortality rate which can be as high as 50 per cent as described in numerous outbreaks (10,21,16,12). To date, few MCF outbreaks have been reported in bison in captivity. In 1964 there was a MCF outbreak at the Munich Zoo affecting Indian gauer (*Bos gaurus gaurus*) and Javan banteng (*Bos javanicus javanicus*) (8). This outbreak, which killed all affected animals, was followed years later by cases of the head-and-eye-form of MCF in European and American bison (*Bison bonasus, Bison bison bison, Bison bison athabascae*), elk (*Alces alces*), red deer (*Cervus elaphus*), Père David's deer (*Elaphurus davidianus*) and again in gaur and banteng. In 2001-2002 a wildlife park in North Carolina experienced an acute outbreak of morbidity and mortality in Pere David's deer, axis deer (*Axis axis*), blackbuck antelope (*Antelope cervicapra*), white-tailed deer (*Odocoileus virginianus*), and Rocky Mountain elk (*Cervus elaphus nelsoni) (*6). Laboratory tests proved MCF as the cause of the outbreak.

In the Buenos Aires Zoo outbreak, the Père David's deer which were also close to sheep and mouflon sheep (*Ovis orientalis musimon)* could have been the first affected species although we were unsuccessful amplifying OvHV-2 sequences from paraffin embedded tissues from these animals. It is known that herpesvirus sequences may be difficult to amplify from this material and thus, we cannot exclude the Père David's deer were also infected with OvHV-2, specially considering that necropsy and microscopic findings resulted compatible with MCF (23). Furthermore, this species has been described as highly susceptible to OvHV-2 (6).

More recently, a single adult American bison was affected by MCF in a zoo in southern Italy (5). Like in the Buenos Aires Zoo outbreak, this bison was in close habitation with a group of domestic sheep (*Ovis aries aries*) which later proved to be positive for OvHV-2 PCR. This result suggested this species could be one of the possible sources of the infection. The positive PCR results obtained with domestic sheep and some mouflon sheep would put forward these two species as the potential sources of the virus in the Buenos Aires Zoo outbreak

since both (and specially mouflon sheep) were in proximity to the bison. Domestic sheep are known virus carriers and are often involved in these outbreaks (15, 3, 12). However, there have been recent descriptions of MCF in captive ruminants where mouflon sheep were the most possible source of the infection (6). Furthermore, in the Buenos Aires zoo, the caretaker for the bison was also taking care of the mouflon suggesting a possible transmission through this individual.

In any case, removal of the ovine species, like done in the Buenos Aires Zoo, has been a successful measure to reduce the number of affected animals (6). In addition to the possible sources of virus, another important factor which probably contributed to stress the animals was the cold weather. The 2007 winter was particularly cold in Buenos Aires where there was snow fall after almost one hundred years. Stressed ovine species could have shed more virus and this virus, with cold conditions, could have survived longer periods in nasal secretions. Birds have been considered a possible carrier of these secretions turning them into another potential component of the epidemiologic chain (12).

To the authors' knowledge, this has been the first MCF case in captive ruminants in Argentina and the first time the disease was confirmed by molecular techniques.

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