

Detection of sheep-associated malignant catarrhal fever from clinical cases in Ethiopian cattle

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Summary

Mucoid nasal discharge, loss of weight, decreased milk production, diarrhoea, salivation, dyspnoea, fever, lacrimation, bilateral corneal opacity and bloody urine were observed in cattle located in the Arbe Gona district of southern Ethiopia. The disease was associated with a high case fatality rate: diseased cattle died within four to five days after showing clinical signs. The clinical presentation, gross pathological observations, histopathological findings and epidemiological data strongly suggested malignant catarrhal fever. Subsequently, the ovine herpesvirus type 2 (OvHV-2) DNA polymerase (UL30) gene was detected in pathological tissue samples using pan-herpesvirus nested polymerase chain reaction (PCR) and real-time PCR. As far as the authors are aware, this is the first report of a diagnostic investigation resulting in the detection of ovine OvHV-2 in cattle and confirming the existence of sheep-associated malignant catarrhal fever in Ethiopia.

Keywords

Cattle – Ethiopia – Malignant catarrhal fever – Ovine herpesvirus type 2 – Pan-herpesvirus nested polymerase chain reaction – Real-time polymerase chain reaction.

Introduction

Malignant catarrhal fever (MCF) is a lethal infection of many species of Bovidae and Cervidae caused by a member of the *Macavirus* genus in the *Gammaherpesvirinae* subfamily (1, 2, 3). Alcelaphine herpesvirus type 1 (ALHV-1) and ovine herpesvirus type 2 (OvHV-2) are the major causative agents responsible for wildebeest-associated MCF and sheep-associated MCF, respectively (1, 3). The ALHV-1 is endemic in wildebeest, in which it causes a subclinical infection (3). Domestic and wild sheep are reservoirs for OvHV-2 (3, 4).

Malignant catarrhal fever occurs in clinically susceptible hosts, such as cattle, bison, deer and pigs, when a sufficient dose of malignant catarrhal fever virus (MCFV) is transmitted from a reservoir host. The disease usually has an acute onset and involves a spectrum of clinical signs that may include corneal opacity, profuse ocular and nasal discharge, diarrhoea, enlarged lymph nodes, fever and anorexia. The distribution of lesions differs slightly depending upon the

species affected, but the basic pathological features are consistent and include widespread lymphoproliferation, vasculitis and epithelial necrosis (1, 3, 5). The transmitted viral dose does not affect lesion severity once clinical MCF has developed; however, the transmitted viral dose is significantly correlated with the incubation period and the timing of first viral DNA detection by polymerase chain reaction (PCR) in peripheral blood leucocytes (PBL) (6). Both the clinical presentation and the pathological features are of significant diagnostic value (5). Detection of viral DNA by PCR in PBL and tissues can support the diagnosis of disease caused by MCFV (7).

The epidemiology of MCF, with respect to the pattern of virus transmission from reservoir hosts to clinically susceptible hosts, has been relatively well defined for ALHV-1 and OvHV-2 (4). Both viruses are shed into the environment through nasal, and perhaps ocular, secretions from their reservoir hosts (8). Clinically susceptible species acquire the virus through inhalation, although ingestion of virus-laden secretions from contaminated feed or water has

also been suggested as a route of transmission (8). Efficient transmission via infected secretions is enhanced by close contact and by a cool moist environment; however, long-distance transmission has also been documented (9). The MCFV is not transmitted by natural means from one clinically susceptible host to another; thus, affected animals are dead-end hosts (10).

The objective of this paper is to describe the molecular diagnostic detection of OvHV-2 in pathological tissue samples, and to document the existence of sheep-associated malignant catarrhal fever in Ethiopian cattle.

Materials and methods

Study area

The cattle disease problem occurred in the Arbe Gona district of southern Ethiopia (Fig. 1). The population depends on agricultural activities, mainly livestock production.

Outbreak history

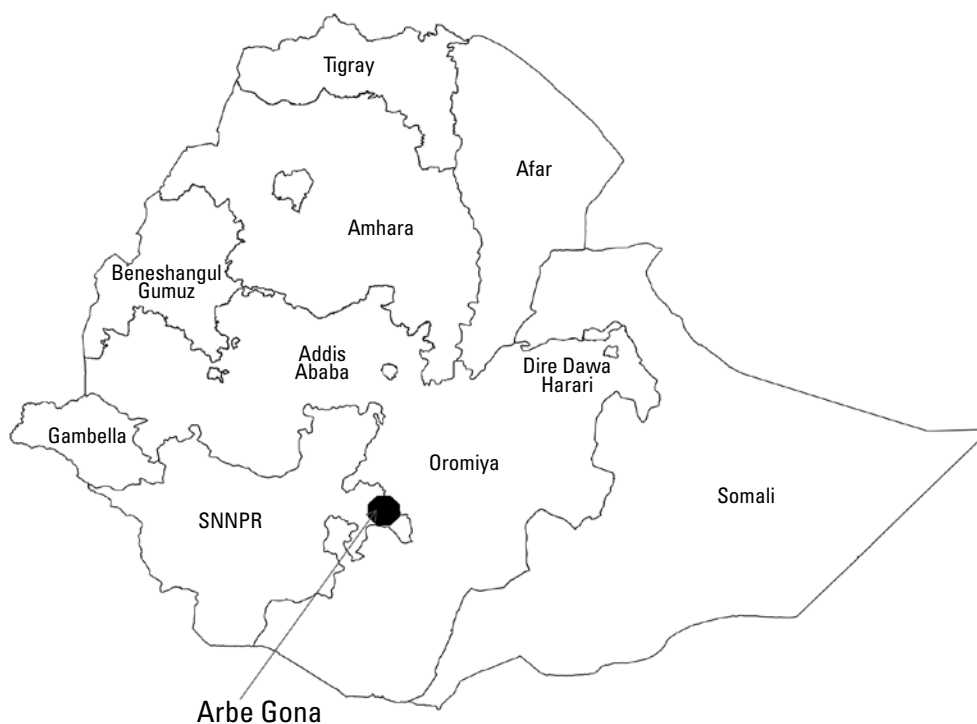
The disease affected only cattle and started in August 2009. The early clinical signs were lacrimation, serous nasal

discharge, a dry muzzle and elevated body temperature (41.5°C). Mucoid oculonasal discharge, loss of weight and decreased milk production, diarrhoea, salivation, difficulty in breathing/dyspnoea, bilateral corneal opacity and bloody urine were observed later. Animals died within four to five days of showing clinical signs. Eighteen animals died during the outbreak (two calves, five heifers, four bulls, three pregnant cows and four lactating cows that had given birth recently). All animals that showed prominent clinical signs died. The disease had no age or sex predilection.

The disease was manifested only in cattle owned by one farmer in a single village, and no animals were affected in the neighbouring herd. The cattle that showed clinical signs were treated with broad-spectrum antibiotics (short-acting oxytetracycline and penicillin–streptomycin; Norbrook), but without any improvement in the clinical signs.

Sample collection for virus detection

Tissue samples were collected from the lungs, heart muscle with carotid artery, brain (cerebrum, cerebellum, midbrain), lymph nodes (at various sites), tonsil, liver, kidneys and adrenal glands from recently deceased animals and kept in sterile universal media containing antibiotics and an antifungal agent. The samples were transported under cold-chain conditions to the laboratory.



SNNPR: Southern Nations, Nationalities and People's Region

Fig. 1
Map of Ethiopia showing the outbreak area

Virus isolation

A 10% tissue homogenate (weight/volume) was prepared using sterile phosphate-buffered saline (PBS). Subsequently, 0.5 ml of the homogenate supernatant was inoculated into confluent monolayers of African green monkey kidney (Vero) and baby hamster kidney (BHK21) cells grown in 25 cm² plastic tissue culture flasks. After incubation for 1 h at 37°C to allow adsorption, the inoculating suspension was discarded. The cell culture flasks were washed with 10 ml sterile PBS and refilled with 10 ml of Glasgow minimal essential medium (GMEM; Sigma-Aldrich) supplemented with 5% fetal calf serum, 100 IU/ml penicillin G and 100 µg/ml streptomycin (Invitrogen, Eugene, Oregon, United States of America [USA]) and incubated at 37°C. The cultures were observed daily for the development of cytopathic effect (CPE). Subculture was performed by harvesting 1 ml of cell suspension after repeated freeze and thaw cycles. When CPE was not observed in three blind passages the sample was considered negative for virus isolation.

Nucleic acid extraction

The DNA was extracted from 200 µl of tissue homogenate using a DNeasy® mini kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. The DNA was eluted with 50 µl nuclease-free water into a new clean microcentrifuge tube, labelled and stored at -80°C until analysis.

Polymerase chain reaction

The DNA extracted from 16 pathological tissue samples (liver, lung, spleen, prescapular lymph node and mesenteric lymph node) collected from three dead cattle was tested by real-time PCR for the presence of OvHV-2 DNA and also by degenerate nested pan-herpesvirus PCR, as described previously (11, 12). The amplicons generated by the pan-herpesvirus PCR were sequenced (Eurofins MWG Operon, Ebersberg, Germany). The sequences were analysed and compared using bioinformatics software with sequences retrieved from GenBank (13).

Samples for histopathological examination

Gross pathological lesions were recorded and appropriate tissue samples were collected for histopathological examination. These samples were obtained from the lungs, heart muscle with carotid artery, brain (cerebrum, cerebellum, midbrain), lymph nodes, tonsil, liver, kidneys, adrenal glands, ureter, tongue, bile duct, bronchial wall, blood vessels and eye (cornea, lens, choroid). The tissues were fixed in 10% buffered formalin and processed using paraffin wax; 4 µm sections were cut and stained with haematoxylin and eosin (14).

Results

Gross pathological findings

The major pathological lesions encountered were enlarged liver and kidneys, oedematous, haemorrhagic and/or hyperaemic lymph nodes, haemorrhagic renal cortex on incision, especially along the corticomedullary junction, pinpoint haemorrhages on the mucosa of the bile duct and ureters and haemorrhagic adrenal glands. Bilateral corneal opacity was observed, together with sloughing of mucous membranes on the hard palate, dorsum of the tongue and gums, with a foul-smelling oral cavity. There was a yellow hue on the walls of the nasal and frontal sinuses, congestion of the turbinates, frothy blood-tinged fluid in the trachea and bronchi, and slight greyish discoloration of the cranioventral portion of the lungs. On opening of the cranium, congested blood vessels in the brain and black discoloration on the upper part of the cerebrum were noted.

Histopathological findings

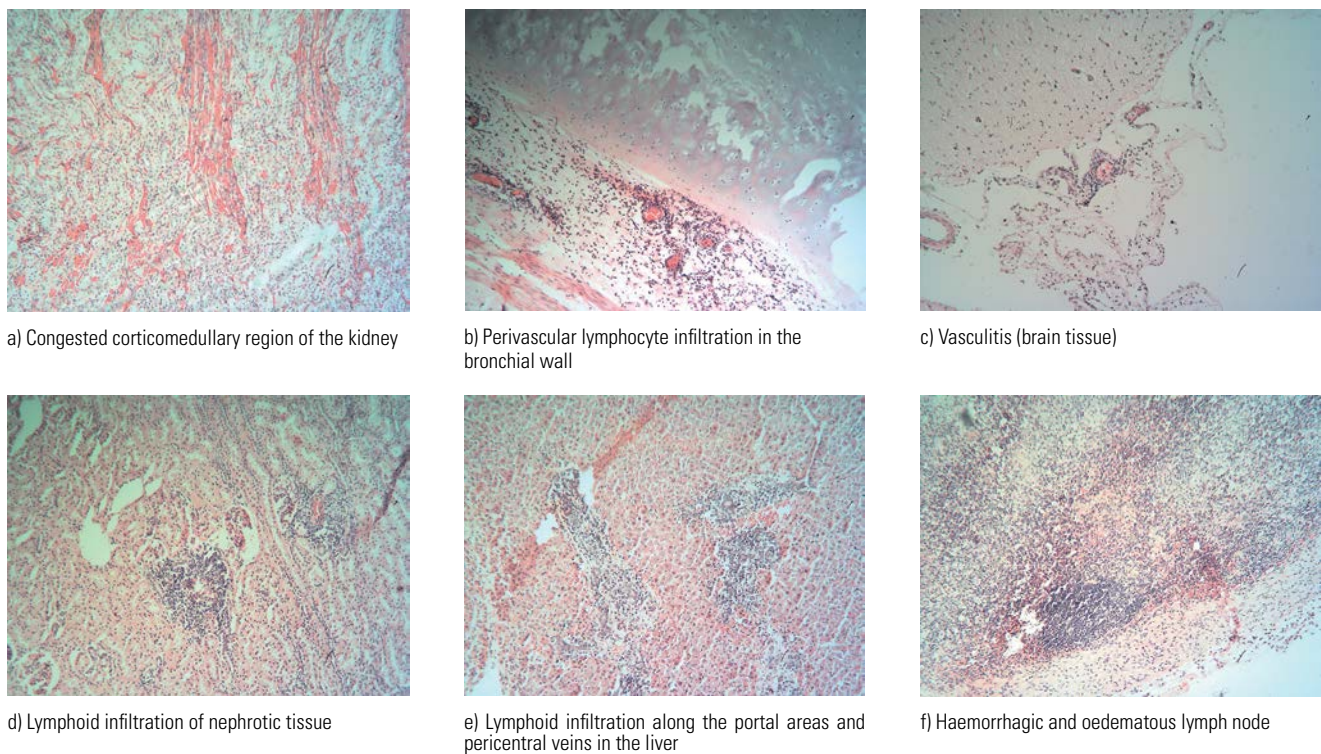
Histopathological examination demonstrated fibrinous oedema and necrosis in the lymph nodes; vasculitis in blood vessels of the brain and kidney; inflammation and necrosis of the mucosal epithelium of the tongue, bronchi and ureters; necrosis of the renal cortex, glomerular atrophy, congestion of blood vessels in the corticomedullary region of the kidney; mononuclear infiltration in the adrenal glands; necrotic liver and chronic cholangitis with remarkable proliferation of bile ducts replacing the hepatic tissue and infiltration of the lymphocytes in the portal areas (Fig. 2).

Molecular findings

Ovine herpesvirus type 2 DNA was detected by real-time PCR in all 16 pathological tissue samples tested. Six positive PCR products were generated by the degenerate nested PCR from the pathological samples tested and were sequenced; all six sequences were identical to each other and to OvHV-2 DNA polymerase gene sequences from GenBank (NCBI, BLAST v2.2.25). Sequence information has been deposited in GenBank with accession number HQ450395.

Discussion

The disease outbreak caused morbidity and mortality in 18 cattle out of a total of more than 2,000 kept by farmers in the village and surrounding area. This observation is in agreement with a previous report (1) that OvHV-2 occurs worldwide in cattle and other species, normally appearing sporadically but occasionally causing local epizootic outbreaks. Thus, the current cattle disease problem represented a local epizootic outbreak of MCF. It was not

**Fig. 2****Histopathology findings in tissues from cattle that died during the outbreak of disease, 100 × magnification**

clear why this particular disease problem occurred only in these cattle in one village. This might have been due to the nature of the virus, the host reaction to the pathogen, environmental factors, animal movement, or the probability of having had contact with wild ruminants. The clinical presentation, pathological findings and epidemiological data strongly suggested the occurrence of MCF. Corneal oedema is a frequent clinical finding in diseased cattle with MCF and this is consistent with previous reports (1, 3). The gross and histopathological findings also supported the existence of MCFV infection. Virus isolation was conducted to detect the presence of cytopathic virus in the pathological samples, but there was no evidence of virus growth after three blind passages. This finding is in line with the study of Li *et al.* (15), who reported that OvHV-2 does not grow in cell culture. Therefore, MCF-specific diagnosis was performed by PCR detection of the virus genome. A rinderpest-specific competitive enzyme-linked immunosorbent assay (C-ELISA) was also conducted on serum samples collected from cattle showing clinical signs and from apparently healthy animals to confirm the absence of specific antibody to rinderpest virus (data not shown), because rinderpest is a known differential diagnosis with similar clinical signs to MCF.

Real-time PCR analysis confirmed the existence of OvHV-2 viral DNA in the pathological tissue samples collected, supporting a diagnosis of sheep-associated MCF (1, 4, 15, 16). The sequences of the OvHV-2 DNA polymerase (UL30) gene obtained were aligned with each other and with the

OvHV-2 genome sequence (17) from the GenBank database and revealed 100% sequence identity. As far as the authors are aware, there is no previous report of the existence and confirmatory diagnosis of MCF in Ethiopia. Thus, the current report of molecular characterisation and sequencing of the OvHV-2 DNA polymerase gene from clinical cases in Ethiopian cattle has confirmed the existence of sheep-associated MCF in Ethiopia.

The authors recommend that veterinary laboratory diagnosticians and researchers should consider MCF as one of the potential differential diagnoses for disease in ruminants. Further studies are warranted to increase understanding of the circulating virus strains, and to estimate the impact of the disease on livestock production.

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Détection d'un virus d'origine ovine du coryza gangréneux chez des bovins présentant des signes cliniques en Éthiopie

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Résumé

Divers signes cliniques (écoulement nasal, perte de poids, diminution de la production de lait, diarrhée, salivation, dyspnée, fièvre, larmoiement, opacification bilatérale de la cornée et présence de sang dans les urines) ont été observés chez des bovins élevés dans le district d'Arbe Gona, au sud de l'Éthiopie. Le taux de létalité associé à cette maladie était élevé : les bovins atteints décédaient dans les quatre à cinq jours suivant l'apparition des signes cliniques. Au vu des manifestations cliniques, de l'examen macroscopique, des résultats de l'histopathologie et des données épidémiologiques disponibles, la maladie incriminée était manifestement le coryza gangréneux. Par la suite, une amplification en chaîne par polymérase (PCR) nichée ciblant spécifiquement l'ensemble des herpèsvirus et une PCR en temps réel ont permis de détecter la présence dans des échantillons de tissus pathologiques de l'ADN polymérase d'un gène (UL30) de l'herpèsvirus ovins de type 2 (OvHV-2). À la connaissance des auteurs, il s'agit de la première détection notifiée de l'OvHV-2 ovin chez des bovins et du premier rapport confirmant la présence du coryza gangréneux d'origine ovine en Éthiopie.

Mots-clés

Amplification en chaîne par polymérase en temps réel – Amplification en chaîne par polymérase nichée ciblant spécifiquement tous les herpèsvirus – Bovins – Coryza gangréneux – Éthiopie – Herpèsvirus ovin de type 2.



Detección de fiebre catarral maligna asociada a las ovejas a partir de casos clínicos en bovinos etíopes

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Resumen

Los autores refieren la aparición en bovinos del distrito etíope de Arbe Gona, al sur del país, de signos como rinorrea mucóide, pérdida de peso, reducción de la producción lechera, diarrea, salivación, disnea, fiebre, lagrimeo, opacidad corneal bilateral o hematuria. Esa patología se acompañaba de una elevada tasa de letalidad: los bovinos enfermos morían a los cuatro o cinco días de empezar a manifestar síntomas. Tanto el cuadro clínico como las observaciones patológicas macroscópicas, los resultados histopatológicos y los datos epidemiológicos apuntaban con gran probabilidad a la fiebre catarral maligna. Ulteriormente, aplicando técnicas de reacción en cadena de la polimerasa (PCR) en tiempo real y PCR anidada para pan-herpesvirus, se detectó el gen de la ADN polimerasa (UL30)

del herpesvirus ovino de tipo 2 (OvHV-2) en muestras de tejido patológico. Hasta donde saben los autores, es la primera vez que una investigación diagnóstica depara la detección del OvHV-2 en bovinos y confirma la existencia de fiebre catarral maligna asociada a las ovejas en Etiopía.

Palabras clave

Bovinos – Etiopía – Fiebre catarral maligna – Herpesvirus ovino de tipo 2 – Reacción en cadena de la polimerasa anidada para pan-herpesvirus – Reacción en cadena de la polimerasa en tiempo real.



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