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Source: Journal of Wildlife Diseases, 36(1) : 165-168

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-36.1.165

Journal of Wildlife Diseases, 36(1), 2000, pp. 165–168

Typing of Pestiviruses from Eland in Zimbabwe

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ABSTRACT: Pestiviruses were isolated from three eland (*Taurotragus oryx*) in Zimbabwe. The viruses were characterised by typing with monoclonal antibodies and by partial genetic sequencing. All were similar to bovine viral diarrhea viruses commonly isolated from cattle. This suggests that bovine viral diarrhea virus can spread from cattle to eland.

Key words: Antigenic typing, bovine virus diarrhea virus, eland, Pestivirus, sequencing, *Taurotragus oryx,* transmission.

Pestiviruses are important infectious agents of domestic cattle, sheep, and pigs. The genus *Pestivirus* of the family Flaviviridae is comprised of at least four virus genotypes, namely bovine viral diarrhea virus types 1 and 2 (BVDV-1 and -2), ovine border disease virus (BDV) and classical swine fever virus (CSFV; Wengler et al., 1995). Although these viruses are named according to their principal animal reservoir, they are not strictly host specific (Paton et al., 1995). Thus, individual domestic species of artiodactyl can be infected by different pestiviruses. The BVD viruses mainly infect cattle and sheep. However, BVDV has been isolated from pigs, goats, and from wild animals, including buffalo (*Syncerus caffer*) and giraffe (*Giraffa camelopardalis*) (Plowright, 1969), various species of deer (Romvary, 1965; Nettleton et al., 1980; Neumann et al., 1980; Nettleton, 1990), and of captive exotic ruminants (Doyle and Heuschele, 1983). Antibodies against BVDV have been detected in over 40 ruminant species (Hamblin and Hedger, 1979; Elazhary et al., 1981; Doyle and Heuschele, 1983; Kocan et al., 1986). A question remains whether wildlife species have their own indigenous pestiviruses or are infected from domestic animals.

The genetic organization of all pestiviruses is similar. The positive sense RNA is approximately 12.3 kb long and has untranslated regions at each end (5′UTR and 3′UTR). A variety of methods are available for typing pestiviruses, including use of monoclonal antibodies (Paton et al., 1995) and sequencing of parts of the viral RNA, such as the 5′UTR (Hofmann et al., 1994; Vilcek et al., 1997). The aim of our work was to use antigenic and genetic typing to characterize pestiviruses obtained from eland (*Taurotragus oryx*) and to determine whether or not the isolates appeared to be of a unique type.

In 1994, blood samples were collected from 1539 eland in southeastern Zimbabwe (general map coordinate 11°25′S, 30°43′E) where they range widely throughout this cattle ranching area. Thirty-two per cent of these samples contained antibodies to BVDV when tested by an indirect ELISA method (Anderson and Rowe, 1998). Three hundred and three seronegative samples that came from areas where seropositives also were found were tested for the presence of virus using an antigen capture ELISA. Antigens were prepared from Triton X100 (Sigma Chemical Company, St. Louis, Missouri, USA) lysed white blood cells (Drew et al., 1993), with confirmation of positive findings by cell culture isolation (Anderson and Rowe, 1998). Three animals were found to contain non-cytopathic BVD virus. All three animals were clinically normal at the time of sampling. A subadult female (eland 29) was persistently infected as shown by repeated isolation of virus from samples collected on separate occasions. This animal subsequently lost condition, developed a febrile illness and died. Whether or not this was related to persistent infection with BVDV is unknown. Another two eland

(eland 44 and 353) were virus positive on the first sampling, but not on subsequent occasions. In the case of 353, seroconversion was demonstrated. Samples from the viremic animals were stored at −70 C, as cell culture supernatants (eland 29 cc, eland 44 cc and eland 353 cc), and also in the case of animals 29 and 44 as Triton X-100 extracts of white blood cells (eland 29 wbc and eland 44 wbc).

For antigenic analysis, virus from the persistently infected eland (eland 29 cc) was grown on bovine turbinate cells which were fixed and immunostained with a panel of monoclonal antibodies (mAbs). Results were compared with those obtained previously using the same and additional monoclonal antibodies for examination of a wide range of pestiviruses (Paton et al., 1995). Genetic typing was carried out on all three viruses, including both cell culture supernatants and detergent treated white blood cells from animal 29. The method used the reverse transcription-polymerase chain reaction (RT-PCR) to amplify and then sequence a 288 nucleotide fragment of the 5′UTR of the virus genome using the 324 and 326 primers (Vilcek et al., 1997). The new sequences were compared with those of representative pestivirus strains deposited in GenBank (NCBI, Bethesda, Maryland, USA). Sequence alignment and preparation of a phylogenetic tree used the Clustal and Megalign programs of DNASTAR (DNASTAR Inc., Madison, Wisconsin, USA).

Typing with mAbs showed a reaction pattern typical of BVDV-1. Positive staining was seen with the BVDV-1 specific mAbs WB163 (anti-E2), WB162 (anti-E2) and WB210 (anti-E^{rns}), whereas negative results were obtained with a panel of seven other mAbs specific for BDV, BVDV-II or CSFV (WS363, WS368, WV433, WV437, WA538, WA548, WH303; Paton et al., 1995). All samples analysed were positive by RT-PCR providing an expected size of DNA fragment around 290 bp. An additional non-specific band of approximately

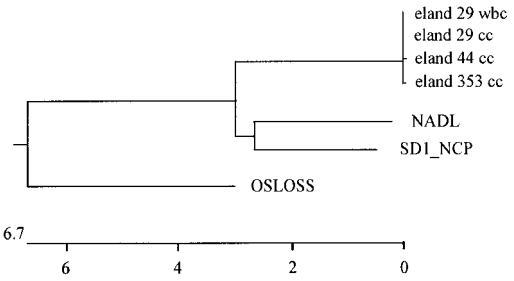

FIGURE 1. Dendrogram of eland and reference pestivirus strains prepared using program MegAlign of DNASTAR. Sequences for pestivirus reference strains NADL (BVDV 1a), SD-1 (BVDV 1a), Osloss (BVDV 1b), 890 (BVDV 2), Moredun (BDV), Alfort (CSFV) and Brescia (CSFV) were taken from Genbank. The scale indicates relative substitution events. Eland sequences came from white blood cell extracted RNA of the persistently infected animal (eland 29 wbc) and from RNA derived from cell cultures infected with viruses from the persistently infected (eland 29 cc) and acutely infected (eland 44 cc and eland 353 cc) animals.

100 bp in size was observed with sample eland 44 wbc. Four positive PCR products were sequenced including that amplified directly from the white blood cells of the persistently infected eland 29. All of the sequences were identical (Fig. 1). This implies that the culture derived isolates were genuinely from eland and do not represent laboratory contaminants introduced from cell cultures supplemented with bovine calf serum.

Genetic comparison with laboratory reference pestivirus strains revealed that the sequences obtained from the eland samples are very similar to BVDV NADL strain representative of the BVDV la subgenotype (Fig. 1). The genetic similarity of the eland isolates was 93% to NADL (BVDV 1a), 92% to SD 1 (BVDV 1a), 87% to Osloss (BVDV 1b), but only 65% to the 890 strain (BVDV 2), 66.5% to Alfort (CSFV), 64% to Brescia (CSFV) and 62% to Moredun (BDV).

Some pestiviruses isolated from deer and buffalo are also very similar to classical BVDV at the genetic level (Becher et al., 1997). A BVDV isolated from a roe deer (*Capreolus capreolus*) in Germany was found to be genetically intermediate be-

tween BVDV type 1a and 1b (Fisher et al., 1998). Another BVDV isolated from a fallow deer (*Dama dama*) in England was more distinctive, possibility representing a new genotype of pestivirus (van Rijn et al., 1997). A pestivirus isolated from a giraffe (*Giraffa camelopardalis*) in Kenya 30 yr ago is even more distinctive and probably forms a separate genotype (Becher et al., 1997; van Rijn et al., 1997). However, contemporary cattle isolates from the same part of the world are not available for comparison. Pestivirus isolates from cattle in South Africa have been shown to include a variety of different subgenotypes, including BVDV 1a, 1b, 1c, and 1d (Baule et al., 1997).

A number of pestiviruses have been isolated from captive exotic ruminants, but their genetic characterisation has not been reported (Doyle and Heuschele, 1983). Our results provide the first characterisation of pestiviruses from eland, revealing isolates that are very closely related to commonly encountered BVD viruses from domestic cattle. Transmission of BVDV to other animals is usually explained by close contact with cattle and spread from cattle seems the most likely explanation for the introduction of this BVDV 1a virus into the eland population. It is not certain that the virus was being sustained by eland to eland spread, but the presence of a persistently infected carrier animal and of two other eland infected with indistinguishable isolates suggests that this may have been the case.

The expert technical assistance of G. Ibata is gratefully acknowledged. S. Vilcek was supported by an International Fellowship from The Wellcome Trust (grant no. 049929/Z/96/Z).

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Received for publication 17 December 1998.