Detection of bovine viral diarrhoea virus in specimens from cattle in South Africa and possible association with clinical disease

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ABSTRACT

Studies covering all aspects of bovine viral diarrhoea virus (BVDV) have been conducted in several countries in Europe, Asia and America. In southern Africa, more information is required about the nature of BVDV infection, the prevalence of different strains and the economic importance of the disease. The presence of BVDV in southern Africa has been known since the early 1970s through serological surveys but few reports confirming its presence by virus isolation and correlation with clinical disease are available. Specimens (n = 312) collected in 1998/99, from live and dead cattle from different farming systems, were obtained from private practitioners, feedlot consultants and abattoirs throughout the country. Specimens (n = 37) from African buffaloes (*Syncerus caffer*) in the Kruger National Park were also included. All specimens were processed for virus isolation in cell culture with confirmation by means of immunofluorescent antibody tests and some also by means of an antigen capture ELISA. BVDV was isolated from 15 (4.7 %) cattle and were all noncytopathic biotypes. BVDV was not detected in 37 lymph nodes obtained from buffaloes in the Kruger National Park. Of the clinical signs in cattle from which virus were isolated, respiratory signs was the most frequent (10/15), followed by diarrhoea (5/15). Abortion, congenital malformations, haemorrhagic diarrhoea and poor growth were also included as criteria for selection of animals for specimen collection, but no BVD viruses were isolated from cattle manifesting these clinical signs.

Key words: antigen ELISA, buffaloes, BVDV, cattle, clinical disease, immunofluorescence, South Africa, *Syncerus caffer,* virus isolation.

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INTRODUCTION

Bovine viral diarrhoea virus (BVDV) is an economically important viral pathogen of cattle worldwide, causing a wide range of clinical syndromes. It is a single-stranded RNA virus and together with hog cholera virus (HCV) and border disease virus (BDV), are members of the genus *Pestivirus* in the family *Flaviviridae.* Its economic importance has been reported from several countries including Great Britain, USA, Sweden, Denmark, Canada, Poland, Australia and Belgium[2,6,14,17,25,26,31]. Losses are mostly due to reproductive failure as infection during pregnancy can result in embryonic resorption, abortion, stillborn calves, teratogenesis or the birth of persistently infected calves[3,4,6].

The presence of BVDV in southern Africa has been known since the early 1970s[33,34,35] and was found in association with diarrhoea, mucosal disease (MD), abortion, teratogenic defects, stillbirths and respiratory disease. During the last decade, several strains have been isolated in Mozambique and South Africa[7,8]. Several serological surveys have indicated that infection with BVDV is widespread in cattle, sheep, goats and wild ruminants[5,7,12,16,19,27,34–36]. During a study conducted in Namibia[12], the prevalence of antibodies to pestiviruses was found to be 58 % in cattle sera, 14 % in sheep, 4,6 % in goats and more than 40 % in game that included giraffe (*Giraffa camelopardalis*), eland (*Taurotragus oryx*) and kudu (*Tragelaphus strepsiceros*). A limited number of studies have been conducted in African buffaloes (*Syncerus caffer*) and water buffaloes (*Bubalus bubalis)* and were based on serological surveys[1,20,32,35,38]. The presence of the infection in water buffaloes was confirmed in Egypt[20] and in India[32] by the presence of neutralising antibodies but in Zimbabwe, negative results were obtained in African buffaloes[35]. In South Africa, there is no report available on BVDV infection in African buffaloes.

Some veterinary practitioners have suspected the presence of BVDV genotype II based on clinical signs compatible with the haemorrhagic syndrome described in the northern hemisphere[28,30]. However, in two previous studies,[8,23] the presence of genotype II could not be confirmed in southern Africa. Detection of BVDV in cattle was undertaken to broaden information on BVDV biotypes and genotypes circulating in South Africa and their possible association with clinical disease. Results of the molecular study have been published[23]. This report describes the clinical findings from cattle from which BVDV were isolated and emphasises the varied clinical manifestations that can possibly be associated with acute BVD.

MATERIALS AND METHODS

Specimen collection

Specimens (n = 312) were obtained from private practitioners and feedlot consultants and were received between January 1998 and October 1999. Specimens obtained from live cattle (n = 283) were limited to sick animals, and those from dead animals (n = 29) to cattle that had clinical signs prior to death. Mesenteric lymph nodes collected from 37 African buffaloes culled in the Kruger National Park were included in the study.

The following clinical criteria were used to define 'sick animals': congenital malformations, pyrexia, mild to severe diarrhoea, haemorrhagic diarrhoea, infertility, abortion, erosions on the feet, nasal discharge and oral ulcers. The specimens from live animals consisted only of blood in heparin-containing tubes. Necropsy specimens were collected from cattle that died in feedlots and in commercial beef and dairy herds and included either spleen, lymph nodes or lung. All specimens were kept cool during transport to the laboratory. The specimens were all subjected to virus isolation

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procedures in tissue culture, but only 154 specimens were additionally subjected to antigen detection by means of ELISA.

Reference viruses and cell cultures

Both cytopathic (cp) and noncytopathic (ncp) BVDV strains were used as controls in cell culture work. The cp reference strain C24V (Oregon) was obtained from the National Veterinary Services Laboratories, Ames, Iowa, USA. The ncp strain was a local strain (ALT3) obtained from Allerton Laboratories, Pietermaritzburg, South Africa. The passage number of C24V was unknown when received. The ALT3 strain was obtained at the 8th passage. After receipt, it was passaged more than 12 times in MDBK cells, stored in 2 ml freezing tubes (Nunc) and kept at –70 °C as stock virus. For attempted virus isolation, primary and secondary cells prepared from calf foetal kidney and Madin Darby bovine kidney (MDBK) line cells were used. The primary and secondary cells were used between passages 2 and 10. Cells were routinely tested for BVDV by means of fluorescent antibody to rule out any adventitious contamination that could have occurred in the laboratory during cultivation of the cells. The serum had been filtered and gamma irradiated at 28–30 KGY under conditions that preserve its biological integrity. Before use, it was inactivated at 56 °C for 30 minutes. Horse serum when available was also used to eliminate BVDV contamination of cell cultures.

Cells were grown in 75 cm^2 flasks in modified Eagle's medium with gentamycin (1 ml per 1000 ml of MEM) supplemented with 5 % foetal calf serum (FCS) (Highveld Biological Products). The maintenance medium for inoculated cell cultures contained a concentration of 2 % serum.

Virus isolation

Blood in heparin-containing tubes was centrifuged at 1500 *g* for 10 minutes, and the buffy coat was collected and stored in 2 ml freezing tubes (Nunc) at –20 °C. Blood without anticoagulant was centrifuged at 1000 *g* for 10 minutes and the sera collected and stored in 2 ml volumes in freezing tubes at –20 °C until used. Two grams of pooled organs were ground with sterile sand in a mortar and re-suspended in 10 ml phosphate-buffered saline with calcium (Ca^{++}) and magnesium (Mg^{++}) (PBS plus). The suspension was centrifuged at 1500 *g* for 10 minutes and the supernatant passed through a 0.22 μm filter. The filtrate was then poured into 2 ml freezing tubes and stored at –20 °C until inoculation into cell cultures The cells were checked daily for cytopathic effects (cytoplasmic vacuolation and detachment of cells). Blind passages were done after 4–8 days.

An 8-chambered glass slide system (Lab-Tek, Nalge Nunc International) was used to assay specimens. Each chamber was seeded with 20 μl of a suspension containing 4×10^5/ml MDBK cells in 400 μl of Eagle's minimum essential medium and 5 % heated FCS and then inoculated with 10 μl of inoculum 24 hours later. A known BVD virus was added to 1 well of the 8-chambered slide to act as a positive control. Chambered slides were incubated for 24 hours at 37 °C in a humidified atmosphere with 5 % carbon dioxide (CO_2) in air before staining with conjugated antibodies.

Immunofluorescence

The presence of noncytopathic BVD virus in cell cultures was detected either by direct or indirect immunofluorescent staining of cells in chamber slides. For the direct method, slides were stained for the presence of BVDV antigen using 20 μl of fluorescein-labelled specific antibody. The conjugate was produced from antibodies obtained from pigs immunised with several type 1 and type 2 bovine strains of BVDV and was obtained from the National Veterinary Services Laboratory, Ames, Iowa, USA. It was tested for efficacy and titrated to determine the optimal working dilution using cells infected with the cytopathic strain C24V. It was used at a working dilution of 1:30 to 1:40.

For the indirect fluorescent antibody (IFA) test, chamber slides containing cell monolayers were reacted with known BVDV-reactive antiserum and probed with 20 μl rabbit anti-bovine immunoglobulin G conjugated to fluorescein isothiocyanate (SA Scientific Products) diluted with 0.05 % Evans blue stain to a working dilution of 1:40.

Antigen ELISA test

Blood in heparin-containing tubes were the only specimens tested by means of an ELISA. The IDEXX commercial ELISA kit (HerdChek™, Idexx) was designed to detect a non-structural BVDV protein (NS3) with a molecular mass of 80 000 Daltons (p80) in peripheral blood leukocytes, blood clots, or whole heparinised blood.

Table 1: **BVD viruses detected by means of virus isolation in cell cultures.**

	Serum	Whole blood	Spleen	Lung	Lymph nodes	Total
Total number of specimens tested	129	154	10	9	47[a]	349
Number positive	4	3	3	2	3	15
Number doubtful	2	3	2	1	0	8[b]
Number negative	123	148	5	6	44	326

[a]Including 37 lymph nodes from African buffaloes.
[b]Doubtful results represent those that yielded a weak (inconclusive) reading with the FA test.

RESULTS

Results of attempted BVDV isolation from specimens from calves and adult cattle with clinical signs suggestive of BVDV infection and from African buffaloes are summarised in Table 1. Four of 129 serum specimens, 3 of 154 buffy coats, 3 of 10 spleen specimens, 2 of 9 lung specimens and 3 of 47 lymph node specimens (including 10 lymph nodes from cattle and 37 from buffaloes) were positive with virus isolation in cell culture ($n = 15$).

Three of 154 blood specimens tested by means of an antigen-capture ELISA yielded positive results, and these 3 specimens were also positive when examined by means of cell cultures.

The disease condition associated with cattle from which each isolate was obtained, is presented in Table 2. Respiratory signs were the most frequent clinical signs (10/15), followed by cattle with diarrhoea (5/15).

Of the 15 isolates, 10 were from feedlot cattle, 4 from commercial beef cattle and one from a dairy cow. Ten of the cattle that yielded BVDV were between 7 and 12 months of age, and 5 were older than 12 months.

Table 2: **Frequency of clinical syndromes associated with cattle from which BVDV was isolated during 1998–1999** ($n = 15$).

Disease conditions	Number of positive specimens	Proportion
Respiratory signs with fever	7	7/15
Respiratory signs	3	3/15
Diarrhoea with fever	3	3/15
Diarrhoea	2	2/15

DISCUSSION

For BVDV isolation, a variety of specimens may be used, including blood, urine, nasal mucous, vaginal mucous, uterine fluids and internal organs. Faeces have been found to be an unsuitable specimen[13]. In this study, private practitioners and feedlot consultants submitted bovine clinical samples (sera, blood in heparin-containing tubes and specimens of spleen, lymph nodes or lung) as requested. Blood samples in heparin-containing tubes are the specimens of choice from live animals for virus isolation since BVDV has an affinity for lymphoid tissue and thus for peripheral blood leukocytes. Sera have been shown to be good diagnostic specimens in persistently infected animals as a result of the high concentration of virus in the serum, e.g. 10^3–10^5 $CCID_{50}$/ml. In addition, BVDV is very stable in serum, surviving at room temperature for at least 7 days[10].

No significance could be attributed to the absence of BVDV in lymph nodes obtained from 37 African buffaloes during investigations into tuberculosis in African buffaloes and other species in the Kruger National Park [9,24] as the number of specimens was small in comparison to the number of buffaloes (±30 000) in the Kruger National Park.

The age predilection of cattle included in the study was influenced by the farming system from which the specimens were collected. Considering the mean age of 7 months at arrival at the feedlot and of 12 months at departure, it was to be expected that the predominant age group for cattle infected with BVDV and expressing clinical signs compatible with BVDV infection during this study was less than 12 months.

There are no pathognomonic clinical signs of infection with BVDV in cattle[6,11]. In the USA[31], various cases of BVDV infection were classified into syndromes based on the predominant clinical manifestation: reproductive disease comprising abortion, repeat breeding, stillbirth, weak calves; acute/peracute BVD in animals of all ages often resulting in death; classical BVD represented by gastro-enteritic disease, pyrexia and respiratory disease; haemorrhagic syndrome with bloody secretions and petechial haemorrhages; MD characterised by gastroenteritis, digital erosions and ulcers; respiratory disease including pyrexia, bronchopneumonia and weakness. Results obtained from different reports showed that the association with enteric and respiratory disease predominated[15,18]. Bovine viral diarrhoea virus has been identified in outbreaks of respiratory disease, usually in association with other pathogens[21,22,29,36,37].

Bovine viral diarrhoea should be considered as a differential diagnosis in outbreaks of diarrhoea, reduced milk yield and of general reproductive problems (infertility, embryonic loss, abortion) especially where there is evidence of congenital abnormalities in calves. Based on the main clinical signs recorded from case histories, the diagnosis of BVD on clinical grounds is difficult. Bovine viral diarrhoea virus has the capacity to change its clinical manifestations and often serves as a challenge to astute clinicians. Diagnostic investigations therefore rely on laboratory detection of the virus, viral antigens or virus-induced antibodies in submitted specimens. The fatal acute/peracute form of BVDV infection caused by genotype II strains is more common in the northern hemisphere[28] and has not yet been recorded in Africa[8,23].

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