The prevalence of bovine viral diarrhoea antibodies in selected South African dairy herds, and control of the disease

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ABSTRACT

The prevalence of bovine viral diarrhoea (BVD) serologically positive animals in 18 dairy herds with clinical and pathological lesions suggestive of BVD infection, the post-vaccinal seroconversion rates in negative animals vaccinated twice with an inactivated BVD vaccine, and the control measures taken, are described. The pathological and histopathological findings in 6 necropsies performed on animals that died in 5 separate herds closely resembled published descriptions. Positive immunohistochemistry results in 3 cases confirmed the diagnosis in those animals. In 1 herd the prevalence of prevaccinal BVD antibodies was only 36.8 %, while the prevalence varied from 79.85 to 100 % in the remainder. Control measures taken included immunoprophylaxis with an inactivated vaccine, culling animals that were serologically negative after vaccination that were regarded as probably persistently infected (PI) and the implementation of additional biosecurity measures. The prevalence of serologically negative PI animals in 10 herds varied from 0.38 to 4.04 %, with 8 herds less than 1 % and 2 herds at 2.79 % and 4.04 %, respectively. Methods based on vaccinating the herd, followed by serological testing and culling cattle that did not develop an antibody titre, are not reliable. The identification of PI animals should be confirmed by isolation of the virus or identification of the antigen.

Key words: bovine viral diarrhoea, control, dairy herds, persistently infected animals, post-vaccinal seroconversion rates, prevalence of BVD antibodies.

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INTRODUCTION

Bovine viral diarrhoea (BVD) is a sporadic disease with a worldwide distribution that causes a complex of disease syndromes in cattle. Major advances in the understanding of the clinical syndromes, epidemiology and pathogenesis of BVD have been made and have been extensively reviewed[4,6,10,27].

A wide variety of clinical manifestations of bovine virus diarrhoea virus (BVDV) infection occur in cattle. These vary from a benign infection resulting in minimal clinical signs, sometimes recognised as BVD, subclinical infections and reproductive syndromes. The virus is also associated with fatal acute mucosal disease (MD) and chronic mucosal disease in persistently infected immunotolerant animals[4,6,10,12,14,22,25,27].

BVDV isolates are classified as cytopathic or non-cytopathic biotypes. This is an important characteristic of the virus. It refers to the capacity of strains to produce cellular damage *in vitro* and not to the behaviour of the virus in an animal. In the case of non-cytopathic isolates, indirect methods for the detection of virus or viral antigens must be used. There are numerous strains of both cytopathic and non-cytopathic BVDV, and all are equally capable of causing disease in the field[2,6,27].

Susceptible cattle infected in early pregnancy before about 125 days of gestation with a non-cytopathic strain of BVDV may produce persistently infected serologically negative calves following transplacental infection of the foetus. These carriers are persistently infected (PI) and viraemic, specifically immunotolerant and continuously shed virus in a wide variety of secretions and excretions[2,5,6].

The diagnosis and control of BVDV follows the basic principles of a systematic and cost-effective protocol for the investigation of the disease, identification and elimination of persistently viraemic animals, which are reservoirs of infection, the vaccination of immunocompetent heifers and cows, biosecurity measures and the monitoring of these control measures[1,13,26,27].

This paper describes the prevalence of serologically positive animals in dairy herds with clinical and pathological lesions suggestive of BVDV infection, the post-vaccinal seroconversion rates in negative animals vaccinated twice with an inactivated BVD vaccine, and the control measures taken to eradicate infection and prevent new infections in these herds.

MATERIALS AND METHODS

History

From January 1992 to July 1994, cases of diarrhoea and reproductive failure in 18 commercial Friesland herds in the Potchefstroom and Carletonville districts of the North-West Province, South Africa, were investigated. These herds had not been previously vaccinated against BVDV. All the herds were well-managed and had above-average milk yields, good general health standards and were tuberculosis and brucellosis free. The range and severity of clinical signs seen varied between and within herds from mild diarrhoea to acute haemorrhagic diarrhoea, dehydration and death. The number of recorded cases of diarrhoea varied from 2 to 18 cases per herd. Other signs seen included pyrexia, decrease in milk production, anaemia, depression, salivation (even blood-stained), nasal discharge and varying degrees of erosion and ulceration of the buccal mucosa and tongue, varying degrees of bloat, reduced appetite and emaciation. Provisional diagnosis of BVD was made on the basis of the presence of characteristic clinical signs.

A significant number of cows that were confirmed pregnant by rectal palpation at approximately 42 days subsequently returned to oestrus and were determined non-pregnant during the next examination. Repeat breeders were also evident in

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many of the herds. This led to a diagnosis of early embryonic loss. No cases of congenital malformations were reported in calves born to cows pregnant during this time nor was there an increase in the incidence of abortion in any of the herds.

Necropsies

Six necropsies were performed on animals between 6 and 15 months of age that had died in 5 separate herds. Histopathological examination was performed on kidney, small intestine (Peyer's patches), cerebellum, mesenteric lymph nodes, spleen, liver and heart muscle. Owing to cost considerations, virus isolation was not attempted, but the immunoperoxidase staining for BVD antigen was performed on formalin-fixed tissue samples from 3 of the cases.

Serological screening

In each herd all the animals above 6 months of age (to obviate the influence of maternal immunity) were bled and screened serologically for the presence of antibodies. The indirect fluorescent antibody (IFA) screening test at a serum dilution of 1:20 was carried out. The prevalence of positive animals was calculated as the number of animals serologically positive to the IFA test, related to the total number of animals in the herd older than 6 months that were tested, irrespective of the time that had elapsed between contact with the virus and the serum tested. Serologically negative animals were regarded as either susceptible animals that had not been exposed to BVD virus, as animals that had been exposed recently, or possibly as persistently infected animals.

Immunisation

Following the initial screening tests, all animals older than 6 months were vaccinated twice at an interval of approximately 28 days. A vaccine containing a freeze-dried preparation of chemically altered strains of IBR and PI3 viruses and modified live BRSV plus a liquid adjuvant preparation of inactivated cytopathic and non-cytopathic strains of BVD virus (Cattlemaster 4, Pfizer) was used.

Post-vaccination serology

Subsequent to vaccination, blood was collected from all the prevaccinal serologically negative animals 14–21 days after the 2nd vaccination, and antibody detection was carried out using the IFA method. Seroconversion in these negative animals, expressed as a percentage, was calculated as the number of animals serologically positive post-vaccination divided by those initially negative. Post-vaccination serologically negative animals were regarded as immunotolerant to the BVD virus, *i.e.* probably persistently infected carriers, and were therefore culled. The percentage of PI animals for each herd was calculated as the number of animals that tested negative after the second vaccination, divided by the total number of animals initially tested.

Control

Measures taken included immunoprophylaxis with an inactivated vaccine, culling of post-vaccinal serologically negative animals that were regarded as PI animals, and the implementation of general biosecurity measures.

All the calves born to cows known to be pregnant during the time the PI animals were identified were tested when they reached the age of 6 months. Routine vaccination with Cattlemaster 4 vaccine was continued in all animals at approximately 6 months of age when passive immunity had declined. To avoid possible maternal antibody interference with active immunisation in calves and to achieve better titres, 2 doses approximately 28 days apart were given. All replacement heifers and cows were vaccinated at least 3 weeks before breeding. Breeding animals were revaccinated annually with a single dose.

Farmers were advised on general principles of biosecurity such as maintaining a closed herd, testing of purchased animals for BVDV, use of approved semen, minimisation of stress factors, separating age groups, optimal nutrition and management.

RESULTS

Post mortem examinations on animals in herds II, IV, V, XII and XIV revealed erosions and shallow ulcerations on the buccal mucosa, external nares and abomasum. Congestion and petechial haemorrhages were also seen in the abomasum. There was evidence of catarrhal enteritis with enlargement of mesenteric lymph nodes as well as Peyer's patches.

Histopathological examination revealed acute enteritis with necrosis and sloughing of the epithelial cells, dilated crypts of Lieberkuhn filled with mucous, inflammatory cells and cellular debris. Multifocal ulceration with hydropic and vascular degeneration and necrosis was present in the mucous membranes with lymphocytic infiltration in the submucosa. Lymphoid tissue showed acute lymphoid atrophy and necrosis, especially in the germinal centre. Bronchopneumonia, pneumonia, atelectasis and necrosis with accumulation of exudate and neutrophils in the debris was seen in the terminal bronchi and bronchioli. Secondary bacterial infection was thought to be involved. BVD antigen using the immunoperoxidase staining technique was detected in the lamina propria of the oesophagus, the macrophages of the lung as well as in the smooth muscle of the small intestine in 3 animals from herds II, IV and XIV.

In herd XII the antibody prevalence was only 36.8 %, while the antibody presence varied from 79.8 % to 100 % in the rest of the herds (Table 1). To simplify the results, the 4 herds with a prevalence of 100 % were excluded from the table.

In 4 of the remaining 14 herds (29 %) the post-vaccinal seroconversion rate was 100 %. In herd XIV the seroconversion rate was only 40 % (15/38 animals) while rates varied from 80 % to 96 % in the remainder. The prevalence of post-vaccinal serologically negative animals in 10 of the herds varied from 0.38 % to 4.04 %, with 8 herds less than 1 % and 2 herds at 2.79 % and 4.04 %, respectively (Table 1).

DISCUSSION

There are many similarities between the clinical and reproductive syndromes encountered in this study and those described in the literature for BVD[1,6,7,14,22,25]. However, there is a whole array of differential diagnoses of diseases in cattle where there are either buccal lesions and/or diarrhoea in the same animal[10], and the diagnosis of BVD on clinical grounds is therefore seen as subjective.

Reproductive syndromes and economic losses within a herd due to BVDV have been well described[1,14,24,27]. Intra-uterine infection with both the non-cytopathic and cytopathic biotypes can lead to problems such as lowered conception rates, early embryonic losses, repeat breeders, abortions, mummification and various congenital malformations.

It is believed that the mortalities that occurred in 5 of the herds where *post mortem* examinations were performed were probably due to mucosal disease (MD) in immunotolerant carrier animals. The pathological and histopathological findings in these cases were similar to published descriptions and supported such a diagnosis[1,4,6,12,27,28]. The positive results of the immunoperoxidase test in 3 cases from herds II, IV and XIV, respectively, confirmed the diagnosis.

The recorded prevalence of BVD antibodies correlates with published figures from other parts of the world[10,26,27] and suggests that infections with BVD virus are widespread in South African dairy herds. It has been estimated that 70–90 %

Table 1: **Prevalence of BVDV antibodies, post-vaccinal seroconversion rate and percentage of permanently infected animals.**

Parameter	Herd I	Herd II	Herd III	Herd IV	Herd V	Herd VI	Herd VII	Herd VIII	Herd IX	Herd X	Herd XI	Herd XII	Herd XIII	Herd XIV
Total number animals tested[a]	86	174	134	262	206	300	359	383	262	120	122	179	125	569
Number sero positive[b]	83	149	107	249	199	290	346	367	257	118	116	66	117	531
Prevalence of BVD antibodies[c]	96.59	85.63	79.85	95.03	96.60	96.66	96.37	95.37	98.09	98.33	95.08	36.87	93.60	93.32
Number sero negative[d]	3	25	27	13	7	10	13	16	5	2	6	113	8	38
Number of animals negative post-vaccination[e]	—	1	—	2	1	2	2	2	1	—	1	5	—	23
Seroconversion rate (%)[f]	100	96	100	84.6	86	80	85	87.5	80	100	83	95.6	100	40
Percentage of PI animals[g]	—	0.6	—	0.76	0.48	0.66	0.56	0.52	0.38	—	0.81	2.79	—	4.04
Post mortem[h]		+		+	+							+		++
Immunoperoxidase[i]		+		+										+

[a]Number of animals >6 months tested for BVDV antibodies in each herd.
[b]Number of animals positive for BVDV antibodies with 1:20 serum dilution IFA screening tests.
[c]Represents the percentage of pre-vaccinated animals that tested sero positive for BVD virus antibodies.
[d]Number of pre-vaccinated animals negative for BVDV antibodies.
[e]Number of animals BVDV negative post-vaccination.
[f]Number of animals tested seropostive for BVDV antibodies post-vaccination divided by the number of animals tested seronegative for BVDV antibodies pre-vaccination in the IFA test.
[g]Percentage of animals that tested negative for BVDV antibodies post-second vaccination in relation to all the animals that were tested in the herd before vaccination.
[h]Herds and number of *post mortems* that were performed.
[i]Herds where there was a positive immunoperoxidase test.

of infections in susceptible immunocompetent cattle are subclinical[10].

Various serological tests can be used to detect BVD antibody[5,8,13,16,20,27]. The indirect fluorescent antibody (IFA) screening test for antibodies at a serum dilution of 1:20 was used, because it is rapid, sensitive and relatively cost-effective.

When BVDV infection is suspected it should be confirmed by isolation of the virus in cell culture or antigen detection and by serological testing of acute and convalescent samples at approximately 2–4 week intervals[2,8,12,13,16,26,27,30]. However, this was not performed in the present study.

In this documented approach, animals that had not seroconverted after vaccination, in the absence of any other explanation for their negative status, were regarded as likely carriers and culled. The detection of such serologically negative cattle has been suggested as a method of identifying persistently infected cattle[1,3,27], and it has been shown that PI animals given the inactivated vaccine do not have detectable amounts of neutralising antibodies in their blood 6 weeks post-vaccination[3].

However, methods based on vaccination, serological testing, and culling of cattle that do not develop an antibody titre, are not reliable. These serologically negative animals could either be persistent carriers unable to mount an antibody response or normal animals that did not react to the vaccine. Furthermore, carriers exposed to heterologous BVD strains in vaccines or in the field can show seroconversion [2,3,5,8,27,28,30]. These animals would then continue to be a source of infection despite their circulating antibodies. Antibody responses during field outbreaks or those resulting from any vaccination are clearly inadequate if used as the sole assay in identifying persistently infected animals and should be interpreted with caution.

The focus of eradication programmes should shift to the use of more costly viral isolation or antigen detection tests[8,13,16,27,28]. If infection is confirmed, the herd must be screened for persistently infected carriers by repeated virus isolation or antigen detection techniques. Various diagnostic methods (virus isolation, fluorescent antibody tests, immunoperoxidase staining, enzyme-linked immunosorbent assay, DNA probes and polymerase chain reaction) are available for antigen detection in PI animals[13,15,16,19,20,28].

To identify all persistently infected animals, individual blood samples of all cattle older than 6 months must be tested. They may be clinically normal or unthrifty before overt disease develops. The persistently infected carrier is an animal of any age from which BVDV can be isolated from any body fluid on 2 occasions at least 4–6 weeks apart, regardless of its serological status. PI animals are important in providing reservoirs within a herd for the initiation of new cases and the maintenance of PI carriers[2,23,24,27,28,30].

The use of antigen detection methods to find the small percentage of persistently infected carriers would require large sample sizes. The financial resources were not available for such an undertaking in this study. The high cost per sample currently places virus isolation out of the economic reach of many farmers. In addition, isolation of the virus may require several weeks and is insensitive because it is dependent on the presence of infectious virus[18].

A vaccination programme implemented at strategic times is currently probably the most cost-effective method of controlling the disease, but deciding which type of vaccine to use is difficult. The key to solid BVDV protection is a vaccine's ability to cross-protect against as many strains as possible[10].

Both modified live and inactivated vaccines are available. Inactivated vaccines are safe and can be used in pregnant animals. However, they take longer to stimulate an antibody response and depend on a high antigenic content, multiple vaccinations and the use of effective adjuvants to enhance the immune response. The same level of safety cannot be reported for modified live BVD vaccines, as breaks in immunity, immunosuppression, mucosal disease and symptoms similar to BVDV infection with field strains have been reported in the past. Modified live vaccines provide better efficacy and immunogenicity at the expense of safety. Their use is contra-indicated in pregnant animals, as spread of virus to the foetus causing foetopathic effects can occur[1,3,5,7–9,11,27].

Herd immunity to BVD is likely to decline over time, and long-term control of the disease should include a recommendation to vaccinate replacement heifers and cows annually 3 weeks before breeding. This is the key to the successful control of foetal BVDV infection and its consequences. In adult cattle, the main aim is to protect unborn calves from transplacental infection. Yearly vaccination additionally enhances the level of passive immunity passed on to offspring[1,3,5,7–9,11,27]. The inactivated vaccine can be used in pregnant cows and lactating animals, as no negative side-effects

have been described. It will not result in vaccine breaks and does not cause immunosuppression. It is also possible to achieve foetal protection between days 25 and 80 of gestation following vaccination of pregnant animals with an inactivated vaccine[9].

The reintroduction of BVDV must be prevented by maintaining a closed herd together with sound management practices[1,2,10,17,21,24,27,29]. These should aim at testing cattle for persistent infection before entry into a herd, using semen for artificial insemination and embryos in embryo transfer programmes from BVDV-negative bulls and donor cows, avoiding as far as possible overcrowding, stressing and mixing of cattle and optimising nutrition. BVDV can be introduced into herds with contaminated equipment such as needles and gloves, so hygienic measures must be strictly applied by practitioners. Young stock must be housed separately from mature animals to ensure that carriers amongst them cannot transmit virus to pregnant animals. It is important to take cognisance of the role of other animals such as sheep, pigs and perhaps other species that can act as reservoirs for the virus. Thus, contact between cattle and other species should be minimised[11].

No further clinical and or reproductive syndromes were observed in any of the herds after the initial outbreaks of BVD and after control measures had been implemented. It would appear that in dairy herds managed under intensive conditions, although initial losses associated with infections may be considerable, infections may be self-limiting due to rapid increase in herd immunity, protecting cows from further foetal infections. As the endemicity of the virus depends almost entirely on the presence of one or more carriers, and these animals are at a survival disadvantage compared to normal animals, infection will be naturally eliminated from some herds[10,27].

The involvement of BVDV in South African dairy herds should be investigated further, as large numbers of unprotected animals may be at risk. The introduction of infected cattle into susceptible herds is a risk factor, as few South African dairy farmers maintain closed herds, and the economic implications for individual farmers can be severe.

The results of this study illustrate the practical difficulties involved in studying the epidemiology and confirmation of infection in commercial dairy farms within economic constraints. Nevertheless, eradication of the pathogen must focus on identification and subsequent culling of persistently infected carriers, which, together with immunoprophylaxis and sound management, could be useful in controlling the adverse effects of the infection in dairy herds.

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