The prevalence of bovine herpesvirus-1 in traditional cattle in Southern Province, Zambia

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Summary

A study was conducted to determine the prevalence of bovine herpesvirus-1 (BHV-1), which causes infectious bovine rhinotracheitis, in cattle destined for market in Southern Province, Zambia. A total of 116 nasal secretion samples were tested using the direct fluorescent antibody test, while blood samples from the same cattle were examined by a commercial enzyme-linked immunosorbent assay kit. The prevalence of the BHV-1 antigens in cattle was 23.28% (27/116), while the mean prevalence of the BHV-1 antibodies was 48.28% (56/116). This study showed that cattle in transit to markets could easily spread the virus, which was reactivated by the stress of trekking for long distances under unfavourable conditions, to the other cattle with which they came into contact. Thus, these transit cattle posed a serious threat to other bovines. Systems of cattle trading where cattle must be transported a long way to market should be reviewed by the authorities to minimise the conditions that may exacerbate the spread of infection.

Keywords

Antibody prevalence – Antigen detection – Bovine herpesvirus-1 – Cattle – Direct fluorescent antibody test – Enzyme-linked immunosorbent assay – Infectious bovine rhinotracheitis – Zambia.

Introduction

Bovine herpesvirus-1 (BHV-1) is a member of the subfamily *Alphaherpesvirinae*, genus *Varicellovirus*. This virus causes the respiratory infection infectious bovine rhinotracheitis; a genital infection known as infectious pustular vulvovaginitis (or balanoposthitis in bulls); conjunctivitis; and systemic infections leading to abortions and foetal death (2, 12, 13). On the other hand, infection can occur subclinically (3, 10), in which case the infection may spread through a herd without being noticed.

Following an acute infection, the latent virus is established mainly in the sensory ganglia (8), probably for the rest of the life of the animal. This latent virus can be reactivated and consequently excreted, with the risk of infecting herd mates which are free from BHV-1. This reactivation may occur as a result of stressful conditions. Although clinical disease induced by BHV-1 can be controlled by vaccination, latent infection often cannot be prevented (4). Therefore, once cattle have been infected, these animals must be regarded as lifelong potential shedders of BHV-1. Bulls infected with BHV-1 are lifelong carriers and may potentially shed virus intermittently in their semen (9). The use of sensitive serological tests to detect specific antibodies is of crucial importance in identifying latently infected cattle. Natural BHV-1 infections normally induce a considerable and long-lasting titre of antibodies (5). However, there are instances where cattle may have low serum antibody titres (2).

Zambia is a landlocked country lying 10 to 18 degrees south of the equator in south central Africa and has an area of approximately 725,600 km^2. The country has nine provinces, as depicted in Figure 1. In 1998, in Lusaka Province, a prevalence range of BHV-1 antibodies of 0% to 80% in bovine

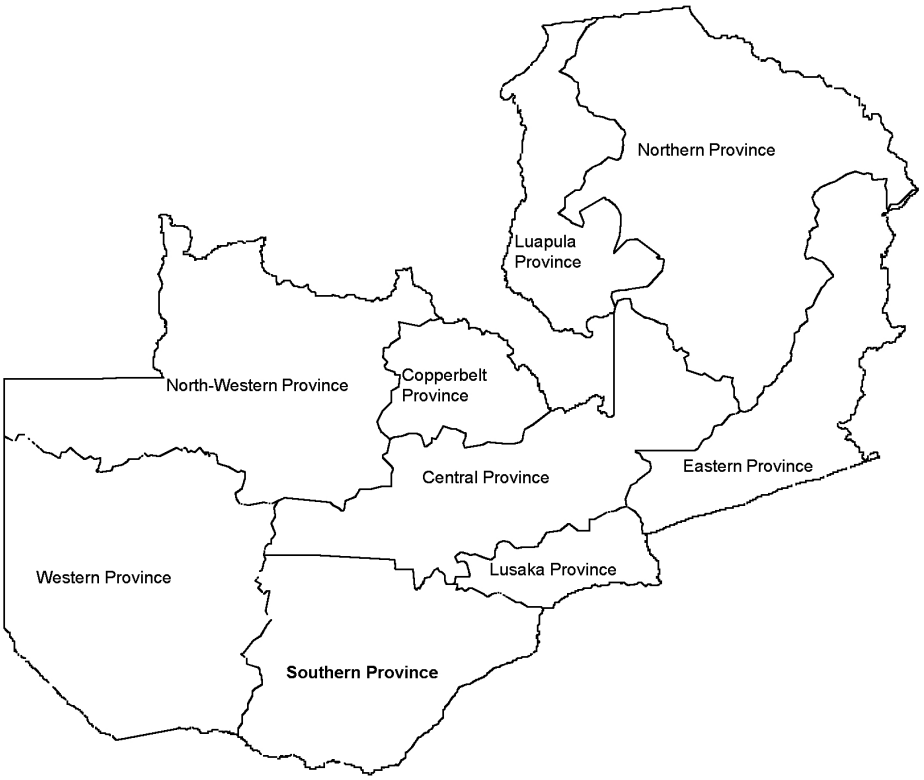

Fig. 1
Map of Zambia showing the nine provinces
The cattle tested in this study were from Southern Province

sera was reported in *Bos indicus* (6). Ghirotti *et al.* (1) reported a prevalence of BHV-1 antibodies of 42.1% in cattle in the Kafue Flats, which are the largest and most important wetlands in the country, situated just west of Lusaka, the capital of Zambia.

A study was undertaken to determine the prevalence of BHV-1 in *Bos indicus* in Southern Province. It was hoped that the results would provide information on the reactivation of the virus in these herds, due to the stressful conditions that they are subjected to during grazing and when being taken long distances to markets. The cattle in the study, especially those near national parks, frequently came into contact with wildlife, sometimes due to husbandry practices which included seasonal grazing.

Materials and methods

Sample collection

Nasal swab samples were collected by inserting a sterile cotton swab into one nostril for a period of approximately five minutes. Then the same swab was transferred to the other nostril and held there for the same period of time. An impression smear from the swab was made immediately on a clean microscope slide. After air drying, the slide was put in a slide box and transported to the laboratory for examination.

Direct fluorescent antibody test

A nasal swab smear from a cow known to be free from BHV-1 infection was put next to the sample smear on each slide. This second smear was used as a negative control. The slides were examined as previously described by Hage *et al.* (3). Briefly, the slides were fixed in acetone for 15 minutes at –20°C. The slides were then dried on filter paper and fluorescein isothiocyanate-conjugated immunoglobulin (infectious bovine rhinotracheitis-fluorescent antibody), diluted in phosphate buffered saline, was added. The slide was then incubated at 37°C for 30 minutes. After washing with phosphate buffered saline three times, for three minutes per wash, the slides were covered with 50% phosphate buffered saline-glycerol and a cover slip was applied. The slides were then examined by fluorescent microscope.

Serology

Blood samples were taken from the coccygeal vein. The presence of BHV-1 antibodies was determined using the Trachitest kit, which consisted of microplates (coated with an inactivated BHV-1 antigen in even-numbered wells and control antigen in odd-numbered wells), control sera (positive and negative), and concentrated washing buffers, conjugates and chromogens. The assay procedures were done using the Trachitest, following the instructions of the manufacturer.

Results

The fluorescent antibody test for antigen detection and the enzyme-linked immunosorbent assay (ELISA) kit for the detection of the presence of antibodies to BHV-1 infection were used as previously described by Hage *et al.* (3) and Kadohira *et al.* (6), respectively. The direct fluorescent antibody test and the ELISA kit for BHV-1 were straightforward to use and gave rapid, reproducible results.

The prevalence of the BHV-1 antigen in cattle in Southern Province was 23.28% (27/116), while the mean prevalence of the BHV-1 antibodies was 48.28% (56/116).

Discussion

It should be noted that the cattle sampled were those taken for slaughter from their respective areas of origin. In some instances, these cattle would have travelled for two to three days before reaching the slaughter areas. The stress that this travelling caused the animals was thought likely to have reactivated BHV-1 in latently infected cattle. The distances that these cattle travelled varied from 20 km to 180 km. They were always driven on foot for the entire distance, sometimes through mountains where there was very little grass and water was scarce. These cattle could spend between one and three days travelling to their destination. There are no established markets for buying or selling cattle within these areas. Buying and selling cattle in these regions depends on individual arrangements between cattle owners. The data obtained in this study indicated that the practice of moving cattle for long distances to slaughter areas could contribute to the spread of BHV-1 infection through the shedding of the virus by carrier cattle. The results on the prevalence of BHV-1 antibodies obtained in this study are in agreement with those reported by Kadohira *et al.* (6), in which a prevalence range of 0% to 80% was reported in traditional cattle in Lusaka Province. The results of this study are also similar to the prevalence of 42.1% reported by Ghirotti *et al.* (1), during studies on the sero-surveillance of selected cattle diseases in the Kafue Flats.

This limited study was undertaken to investigate the situation of BHV-1 infections in Southern Province, especially among cattle destined for market, with the aim of formulating and implementing appropriate disease control measures. The antigenic and serological evidence indicated widespread BHV-1 infection. Identifying which cattle are latent carriers of BHV-1 is important for control programmes and international trade activities, so that the risk of introducing the virus into a BHV-1-free herd or into artificial insemination centres is minimised. Detection of latent carriers is crucially dependent on the use of highly sensitive serological tests which are able to detect low levels of BHV-1-specific antibodies (7).

Vaccines play a very important role in the control of BHV-1 infections. There is a wide variety of live and killed vaccines that usually prevent the development of severe clinical signs after BHV-1 infection, and hence reduce economic losses. Although most of these conventional BHV-1 vaccines also reduce the amount of virus shed after infection, there has been no attempt to use them to restrict the spread of the infection in herds or regions. Since marker BHV-1 vaccines allow differentiation between infected and vaccinated cattle, their use offers a good opportunity to implement eradication programmes for BHV-1. In such control programmes, measures to improve hygiene and husbandry management must also be taken into consideration (11).

The problem of BHV-1 infection deserves urgent attention and field veterinarians need to be educated about the complexities of this disease. At present the disease is of particular interest since, due to its ability to modify the upper respiratory tract environment and cause immunomodulation, the virus is considered to be one of the major initiators of the bovine respiratory disease complex.

In this study, the prevalence of BHV-1 antigen in nasal secretions of cattle was 23.28% (27/116), while the mean prevalence of the BHV-1 antibodies in sera was 48.28% (56/116). It was observed that cattle in transit to slaughter areas posed a serious threat to other cattle with which they came into contact, as these transit cattle could easily spread the virus upon reactivation caused by the stress of trekking. These preliminary observations should be followed by a further large-scale survey to establish the extent of BHV-1 infection in Zambia. It is proposed that adequate diagnostic facilities for BHV-1 infections be provided in all disease investigation laboratories in Zambia.

The authorities could review systems of cattle-trading, where markets are very far away from where the cattle originate to minimise conditions that may exacerbate the spread of these infections. Proper grazing management systems should also be encouraged, especially in areas like Southern Province, where the pastures are sparse and there is little drinking water for the animals. These problems are especially pronounced during the dry season and dry-spell periods, which have now become a very common feature of animal husbandry in Zambia.

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Prévalence de l'herpèsvirus bovin de type 1 dans les élevages traditionnels de la province du Sud de la Zambie

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Résumé
Une étude a été réalisée en vue de déterminer la prévalence de l'herpèsvirus bovin de type 1 (BHV-1), responsable de la rhinotrachéite infectieuse bovine, parmi des bovins destinés aux marchés de la province du Sud de la Zambie. Un total de 116 prélèvements de sécrétions nasales ont été analysés par immunofluorescence directe. Par ailleurs, des échantillons sanguins issus des mêmes animaux ont fait l'objet d'un dosage immuno-enzymatique à l'aide d'une trousse commerciale. La prévalence des antigènes BHV-1 chez ces bovins était de 23,28 % (27/116), pour une prévalence moyenne d'anticorps anti-BHV-1 de 48,28 % (56/116). Cette étude a montré que les bovins pouvaient facilement propager le virus, par ailleurs réactivé par le stress causé par les déplacements sur de longues distances et dans de mauvaises conditions, et contaminer les bovins avec lesquels ils entraient en contact durant leur déplacement vers les marchés. Ces bovins en transit peuvent donc constituer une grave menace pour d'autres bovins. Il appartient aux autorités de se pencher sur les systèmes de commerce du bétail qui impliquent le transport des animaux vers des marchés éloignés, afin de limiter les conditions susceptibles de favoriser la propagation de l'infection.

Mots-clés
Bovins – Dépistage des antigènes – Dosage immuno-enzymatique – Épreuve d'immunofluorescence directe – Herpèsvirus bovin de type 1 – Prévalence d'anticorps – Rhinotrachéite infectieuse bovine – Zambie.

Prevalencia del herpesvirus bovino 1 en el ganado vacuno tradicional de la Provincia Meridional de Zambia

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Resumen
Los autores describen un estudio destinado a determinar la prevalencia del herpesvirus bovino 1 (HVB-1), causante de la rinotraqueítis infecciosa bovina, en ganado vacuno destinado a la venta en mercados de la Provincia Meridional de Zambia. Se aplicó la prueba de inmunofluorescencia directa a un total de 116 muestras de secreciones nasales, a la vez que se analizaban muestras sanguíneas de los mismos animales mediante un ensayo inmunoenzimático (ELISA) de venta en el mercado. La prevalencia de antígenos del HVB-1 en los bovinos resultó ser de un 23,28% (27/116), mientras que la prevalencia media de anticuerpos contra el virus fue de un 48,28% (56/116). El estudio vino a demostrar

que los bovinos en tránsito hacia los mercados podían fácilmente transmitir el virus a otros animales con los que entraran en contacto, tanto más cuanto que el microorganismo se reactiva ante el estrés que genera en el animal una larga caminata en condiciones desfavorables. Estos bovinos en tránsito suponen pues una grave amenaza para otros bovinos. Las autoridades deberían replantearse estos sistemas de comercialización de bovinos que entrañan largos desplazamientos hasta los mercados y tratar de encontrar fórmulas que reduzcan al mínimo los factores susceptibles de exacerbar la propagación de la infección.

Palabras clave
Bovinos – Detección de antígenos – Ensayo inmunoenzimático – Herpesvirus bovino 1 – Prevalencia de anticuerpos – Prueba de inmunofluorescencia directa – Rinotraqueítis infecciosa bovina – Zambia.

References

1. Ghirotti M., Semproni G., De Meneghi D., Mungaba F.N., Nannini D., Calzetta G. & Paganico G. (1991). – Sero-prevalences of selected cattle diseases in the Kafue Flats of Zambia. *Vet. Res. Commun.*, **15**, 25-36.

2. Gibbs E.P.J. & Rweyemamu M.M. (1977). – Bovine herpesviruses. I. Bovine herpesvirus 1. *Vet. Bull.*, **47** (5), 317-343.

3. Hage J.J., Schukken Y.H., Barkema H.W., Benedictus G., Rijsewijk F.A.M. & Wentink G.H. (1996). – Population dynamics of bovine herpesvirus 1 infection in a dairy herd. *Vet. Microbiol.*, **53** (1-2), 169-180.

4. Kaashoek M.J., Moerman A., Madic J., Rijsewijk F.A.M., Quak J., Gielkens A.L.J. & van Oirschot J.T. (1994). – A conventionally attenuated glycoprotein E-negative strain of bovine herpesvirus type 1 is an efficacious and safe vaccine. *Vaccine*, **12** (5), 439-444.

5. Kaashoek M.J., Rijsewijk F.A.M. & van Oirschot J.T. (1996). – Persistence of antibodies against bovine herpesvirus 1 and virus reactivation two to three years after infection. *Vet. Microbiol.*, **53** (1-2), 103-110.

6. Kadohira M., Mweene A.S. & Samui K.L. (1998). – Prevalence and risk factors of bovine viral diarrhoea and infectious bovine rhinotracheitis in traditional cattle in Lusaka province, Zambia. *Zamb. J. vet. Sci.*, December (2), 1-9.

7. Kramps J.A., Perrin B., Edwards S. & van Oirschot J.T. (1996). – A European inter-laboratory trial to evaluate the reliability of serological diagnosis of bovine herpesvirus 1 infections. *Vet. Microbiol.*, **53** (1-2), 153-161.

8. Pastoret P.-P., Thiry E., Brocher B., Derboven G. & Vindevogel H. (1984). – The role of latency in the epizootiology of infectious bovine rhinotracheitis. *In* Latent herpesvirus infection in veterinary medicine (G. Wittmann, R.M. Gaskell & H.J. Rziha, eds). Martinus Nijhoff Publishers, Dordrecht, 211-227.

9. Snowdon W.A. (1965). – The IBR/IPV virus: reaction to infection and intermittent recovery of virus from experimentally infected cattle. *Aust. vet. J.*, **41**, 135-142.

10. Van Oirschot J.T., Straver P.J., van Lieshout J.A.H., Quak J., Westenbrink F. & van Exsel A.C.A. (1993). – A subclinical infection of bulls with bovine herpesvirus type 1 at an artificial insemination centre. *Vet. Rec.*, **132** (2), 32-35.

11. Van Oirschot J.T., Kaashoek M.J. & Rijsewijk F.A.M. (1996). – Advances in the development and evaluation of bovine herpesvirus 1 vaccines. *Vet. Microbiol.*, **53** (1-2), 43-54.

12. Wyler R., Engels M. & Schwayzer M. (1989). – Infectious bovine rhinotracheitis/vulvovaginitis. *In* Herpesvirus diseases of cattle, horses and pigs (G. Wittmann, ed.). Kluwer Academic Publishers, Boston, Dordrecht, London, 1-72.

13. Yates W.D.G. (1982). – A review of infectious bovine rhinotracheitis, shipping fever pneumonia and viral-bacterial synergism in respiratory disease of cattle. *Can. J. comp. Med.*, **46** (3), 225-263.