Maintenance of foot and mouth disease viruses in buffalo
(Syncerus caffer Sparrman, 1779) in Southern Africa

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Summary: Using age-related infection rates derived from serological data in available deterministic and specially developed stochastic simulation models, it has been possible to establish that the basic reproductive rates for South African Territory (SAT) type foot and mouth disease virus in buffalo (Syncerus caffer) are high. The models predict that there is a periodicity of infection within herds and possibly the population as a whole. Thus, buffalo herds are likely to be more infectious at some times than at others. However, because most infections in buffalo are inapparent, such episodes are difficult to identify.

There is wide intratypic variation within the SAT type virus populations circulating in buffalo. This was determined by sequencing part of the 1D gene of buffalo isolates and establishing antigenic profiles with neutralising monoclonal antibodies and conventional antisera.


INTRODUCTION

Circumstantial evidence indicates that buffalo (Syncerus caffer Sparrman, 1779) populations in Southern Africa are the major source of foot and mouth disease (FMD) viruses which periodically infect domestic livestock (principally cattle) (7). Such outbreaks and the presence of a free-living maintenance host for the South African Territory (SAT) types of FMD virus place a major constraint on the export of agricultural products of animal origin from the subregion. However, precisely how transmission from buffalo to cattle usually occurs is a matter of contention which is unlikely to be resolved until a clearer understanding is obtained of how FMD viruses are maintained in buffalo populations.

Buffalo acutely-infected with the SAT types of FMD virus excrete the virus by the same routes and in approximately the same quantities as acutely-infected cattle (7) but, unlike cattle, most naturally-infected buffalo do not develop obvious signs of FMD.

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(9; Bengis, unpublished findings). For this reason, and because buffalo are herd animals and usually experience infection early in life, it has been postulated that SAT type viruses are maintained in buffalo populations as “childhood” infections (2). However, during such “epidemics”, breeding herds of buffalo would be likely to provide a potent source of infectivity for other wild or domestic species in the immediate vicinity.

Following the acute phase of infection (< 2 weeks), a high proportion of buffalo maintain the virus in the oesophago-pharynx for periods of five years and probably longer, becoming so-called “carriers” (4, 9). However, carriers are inefficient transmitters of the infection to cattle in close proximity (2), although such events have been recorded (10).

This paper summarises the progress which has been made towards understanding the maintenance of SAT type infections in buffalo in the Kruger National Park (KNP), based on two different types of mathematical model and the measurement of intratypic variation within SAT 1 and SAT 2 viruses isolated from this population.

**MATERIALS AND METHODS**

**Study area**

The KNP covers an area of slightly less than two million hectares in the Eastern Transvaal and has a buffalo population of approximately 27,000.

**Animal material**

Sera and oesophago-pharyngeal scrapings (OPS) (“probang specimens”) were obtained from young buffalo (≤ 2.5 years of age) culled during routine population-control programmes in the KNP. The ages of culled animals were estimated as described by Sinclair (13).

**Serology**

Antibody levels to SAT 1, 2 and 3 viruses in buffalo sera collected in 1987 were determined using a blocking enzyme-linked immunosorbent assay (ELISA) as described previously (8).

**Virus isolation from oesophago-pharyngeal scrapings**

Primary pig kidney cells were used for initial virus isolation, with further passage on IB-RS-2 cells, using standard techniques.

**RNA sequencing of viral isolates**

The dideoxy sequencing procedure for ribonucleic acid (RNA) templates described by Zimmern and Kaesberg (16) and modified by Palmenberg and colleagues (12) and Xie and colleagues (15) was used with minor modifications. The dendrograms were drawn using PHYLIP version 3.3 (6).

**Stochastic simulation model for the behaviour of a SAT type virus in a herd of buffalo**

A stochastic model was constructed to simulate the interaction of factors which are believed to be important in the maintenance of a single SAT type infection in a breeding herd of buffalo. The model employed has the following characteristics:

- the number of animals in the herd can be varied at the outset of the simulation period, but the age and sex structure is predetermined
"new" infectious (contagious) individuals can be introduced into the herd at fortnightly intervals
- the sex, age and health of each individual are recorded
- the health status of each individual is updated every fortnight
- a seasonal peak in calving is allowed for (most buffalo in Southern Africa calve in mid summer)
- the herd is divided into groups of eight animals which associate more closely with each other than with other members of the herd
- the health of each buffalo with respect to FMD is classified as "susceptible", "contagious", "immune" or "carrier"
- the proportion of infected individuals which remain carriers is predetermined at the start of the simulation run
- the virus is transmitted by close contact between contagious and susceptible individuals.

The model recognises four different modes of transmission:

a) from carrier to susceptible individuals within a group
b) from acutely-infected to susceptible individuals within a group
c) from carrier to susceptible individuals within the herd at large
d) from acutely-infected to susceptible individuals within the herd at large.

Each mode is represented by a different transfer coefficient which is determined at the outset of the simulation run.

Determination of antigenic variation

Neutralising monoclonal antibodies (MAbs) as well as antisera obtained from cattle immunised with conventional vaccines were examined using cross-neutralisation tests as previously described (5).

RESULTS

Age-related infection rates

The proportion of sera in various age groups which tested positive against SAT 1, 2 and 3 are shown in Figure 1. The proportion positive to SAT 1 approached 100% in all age groups, while the rates of increase with age were slower for SAT 2 and 3. From this data, estimates of approximate basic reproductive rates of SAT 1, 2 and 3 viruses were made using methods previously described (11). This is elaborated in the section entitled "Discussion".

Rate of infection with a SAT type virus in a buffalo herd

Using the stochastic simulation model for a herd set initially at 500 animals and variable parameters set at values which are reasonable estimates on the basis of present understanding, it was predicted that the rate at which new infections occur waxes and wanes cyclically (Fig. 2). An arbitrary number of acutely-infected individuals (ten) were
Age-related infection rates of buffalo in the Kruger National Park, 1987, with SAT type foot and mouth disease virus

Parameters:
1) Proportion of carriers in the herd 0.90
2) Transmission coefficients:
   a) carriers: i) within herd 0.01
      ii) within group 0.10
   b) acutely-infected animals:
      i) within herd 0.20
      ii) within group 0.95

Incidence of new infections with a single SAT type foot and mouth disease virus in a herd of 500 buffalo, predicted by a stochastic simulation model
used to introduce the infection into the herd; no other "introductions" were made during the simulated period. The duration of each cycle is a little more than a year, and peaks and troughs do not appear to be seasonal. There is a period between each cycle when no acutely-infected animals are present in the herd.

Genetic variation in virus isolates

Dendrograms depicting the genetic relationships between isolates of SAT 1 and 2 based on percentage nucleotide differences are shown in Figure 3. Maximum variation was in the region of 15% for SAT 1 and 19% for SAT 2. The relationship between SAT 3 isolates is not shown since only a few isolates have been sequenced. However, at least 10% variation exists (results not shown). For both SAT 1 and SAT 2 isolates, the degree of variation is such that it is difficult to make definite conclusions concerning relationships over time and place. Isolates made from one herd on a single day are not necessarily closely related, although this is often the case (unpublished findings).

**FIG. 3**

Dendrograms depicting the intratypic variation of SAT type foot and mouth disease isolates taken from buffalo in the Kruger National Park between 1986 and 1990.
Antigenic relationships between virus isolates

Six SAT 2 isolates tested against a panel of seven neutralising monoclonal antibodies gave widely differing profiles with the exception of KNP 10/88/2 and KNP 13/89/2, where the patterns were almost identical, and KNP 7/86/2 and KNP 9/88/2, where the similarities were not as clear (Fig. 4).

**Fig. 4**

Reactivity of seven neutralising monoclonal antibodies with six SAT foot and mouth disease isolates taken from buffalo in the Kruger National Park

To establish the ability of standard SAT 2 vaccine strains to protect against the buffalo isolates, antisera derived from cattle immunised with a bivalent vaccine containing ZIM 7/83 and BOT 3/77 (both SAT 2 viruses) were assessed for neutralising capacity both following a single inoculation of vaccine and after two inoculations two weeks apart. Figure 5 shows that even after two inoculations, in three instances (KNP 9/88/2, KNP 14/88/2 and KNP 19/89/2) the r-value did not reach 0.4, which is considered the minimal acceptable value for adequate protection.
DISCUSSION

As is the case for most viral infections of ungulates, young buffalo acquire maternal immunity to the SAT types of FMD virus soon after birth, through the ingestion of colostrum. It was previously found that maternally-derived antibodies do not persist effectively beyond six months (2). In the Hwange National Park in Zimbabwe, buffalo calves were only solidly protected from infection during the first three months of life (3).

The serological data presented in Figure 1 show that, particularly for SAT 1, infection in buffalo in the KNP in 1987 occurred soon after the waning of maternal immunity, and that the interval between the disappearance of maternal immunity and active infection was shortest for SAT 1 and longest for SAT 3.

As shown by Nokes and Anderson (11), the basic reproductive rate \( R_0 \) of an infection is a useful epidemiological parameter. This rate can be estimated as follows:

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R_0 = \frac{B}{A-F}
\]
where $B$ is the reciprocal of the birth rate per head of population, $A$ is the average age at which an individual typically experiences infection and $F$ is the average duration of protection by maternal immunity.

A value of 5.0 (1 divided by 0.2) was obtained for $B$ based on pregnancy rates found in culled buffalo in breeding herds between 1988 and 1991 ($n = 7,812$) (Whyte, personal communication). From the data available it is impossible to determine $A$–$F$ accurately. Nevertheless, it is clear that the interval is short, particularly for SAT 1. In the case of SAT 3, where less than 50% of animals had antibody in the age interval 0-0.5 year but more than 50% had antibody by the ages of 0.6-1.0 year, the average age of infection can assumed to be approximately 6 months. If protection by maternal antibody is assumed to be approximately 3 months this would result in an $R_0$ value of 20. For SAT 2, and SAT 1 in particular, $R_0$ would have higher values comparable to human infections, which characteristically produce epidemics, e.g. measles and rubella (11).

Infections which have high $R_0$ values and induce lasting immunity to re-infection tend to exhibit oscillatory fluctuations in incidence (11). Although the duration of immunity to SAT type viruses in buffalo is unknown, the fact that antibody levels show no evidence of decline over 7-8 months following active infection (14) indicates that the requirement of long-lasting immunity is probably fulfilled.

The predictions of the stochastic simulation model support the suggestion that there is oscillation in the rate at which new infections arise in breeding herds (Fig. 2). Interestingly, the peaks and troughs of the cycle do not appear to be seasonal in spite of the fact that buffalo are seasonal breeders, a feature which was built into the model. The rate at which new infections in the herd occur, predicted in Figure 2, obviously only reflects the situation with one virus type. The other two types would be superimposed, not necessarily identically, on the pattern shown. Antigenic variation within each type (Figs 4 and 5) further complicates the issue.

Viral infections with high reproductive rates also tend to display antigenic heterogeneity (1). In the context of FMD, antigenic heterogeneity is well recognised but virus populations which are associated exclusively with buffalo have not yet been examined in detail in this respect. Based on both intratypic genetic (Fig. 3) and antigenic (Fig. 4) variation, the viruses in this study are clearly heterogenic. Furthermore, this is undoubtedly a continuous process, with the result that it is difficult to produce effective vaccines against all antigenic variants for the immunisation of cattle farmed in areas adjacent to game reserves (e.g. the KNP). This is illustrated in Figure 5.

**CONCLUSION**

Evidence from a number of sources indicates that SAT type infections in buffalo have high basic reproductive rates. This implies oscillation in the incidence of infection within breeding herds over time and suggests that when peaks in incidence occur, breeding herds are a potent source of infection for other susceptible animals in the vicinity.

The marked intratypic antigenic heterogeneity in SAT 1 and 2 viruses circulating in the buffalo population of the KNP results in difficulty in ensuring that vaccines are always appropriate for all the variants circulating in buffalo breeding herds.
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Résumé : En se fondant sur l'étude sérologique des taux d'infection en fonction de l'âge, à l'aide de modèles de simulation déterministes existants et de modèles stochastiques spécialement conçus, il a été possible de montrer que le taux de renouvellement des infections par le virus de la fièvre aphteuse de type SAT (South African Territory) chez le buffle d'Afrique (Syncerus caffer) est élevé. Ces modèles permettent de prédire une périodicité de l'infection au sein du troupeau, voire dans l'ensemble de la population. Ainsi, les troupeaux de buffles sont-ils susceptibles d'être plus contagieux à certaines périodes qu'à d'autres. Cependant, étant donné que la plupart des infections sont inapparentes, ces épisodes sont difficiles à identifier.

Il existe une forte variation intratypique au sein des populations virales de type SAT qui circulent chez les buffles. Ce fait a été révélé par le séquençage d'une partie du gène 1D des isolats provenant de buffles, et par la détermination des profils antigéniques à l'aide d'anticorps monoclonaux ou polyclonaux neutralisants.

MOTS-CLÉS : Anticorps monoclonaux - Buffle d'Afrique - Fièvre aphteuse - Modèles - Séquençage de l'ARN - Taux d'infection.

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Resumen: La utilización de las tasas de infección en función de la edad, que se obtienen a partir de datos serológicos durante simulaciones realizadas siguiendo los modelos determinísticos existentes o modelos probabilísticos desarrollados ad hoc, ha permitido determinar que la tasa de reproducción básica de las infecciones por virus aftoso del tipo SAT (South African Territory) en el búfalo africano (Syncerus caffer) es alta. Los modelos predicen una infección periódica en las manadas y probablemente también en el conjunto de la población. Por lo tanto, existen posiblemente periodos durante
los cuales las manadas de búfalos son más infecciosos. Sin embargo, teniendo en cuenta que la mayoría de las infecciones no son aparentes, estos periodos difícilmente se pueden determinar.

Existe una fuerte variación dentro de las poblaciones virales del tipo SAT que circulan en los búfalos. Este hecho fue revelado gracias a la secuenciación de una parte del gen 1D presente en los aislados procedentes de búfalo y a la determinación de los perfiles antígenicos, utilizando anticuerpos neutralizantes monoclonales o policlonales.


REFERENCES


