A serological survey for foot-and-mouth disease in wildlife in East Africa

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Abstract:

Serosurveillance for FMD in Africa is complicated by the need to screen for up to six of the seven serotypes of FMD using VNT which is time consuming, requires virus containment and is expensive. The availability of the non-structural 3ABC ELISA kits has the potential to improve this situation. This study used the Ceditest ® to screen 731 sera from East African wildlife, predominantly buffalo, for FMD NSP antibodies. The results suggest that there are high levels of exposure in buffalo populations and only very low levels of exposure in other wild ungulates. We also describe preliminary attempts at parameter estimation analysis of the data using a Bayesian formulation of the Hui-Walter model for parameter estimation in the absence of a gold standard.

Introduction:

Foot and Mouth Disease (FMD) is a highly contagious viral disease of even-toed ungulates (Artiodactyla) caused by the single stranded +ve sense RNA foot-and-mouth disease virus (Aphthovirus, Picornaviridae). There are seven distinct serotypes recognised globally known as O, A, C, SAT1, SAT2, SAT3 and Asia1, of which only Asia one has not been seen in Africa. These provide little cross-protection although it has been suggested that there may be problems differentiating exposure in multiple infected or exposed buffalo using virus neutralisation tests (VNT) (Hedger et al. 1982). The Cape buffalo (Syncerus caffer) has been identified as natural hosts for SAT serotypes of FMD, although they may be infected by all serotypes (Hedger et al. 1973; Hedger 1976) and frequently become infected, subsequently circulating virus(es) amongst the herd(s) and acting as a reservoir. This discovery along with clear spatial associations with outbreaks in cattle has lead to the belief that any control strategy for FMD in cattle must therefore address control in buffalo (Thomson et al. 2003). However, the role of 'carrier' animals and particular 'carrier' buffalo still remains a point of debate as it is not clear whether or how transmission occurs (Bengis et al. 1986; Dawe et al. 1994; Dawe et al. 1994), although it has been suggested that sexual transmission may be an important route from buffalo to cattle (Bastos et al. 1999).

This paper describes a serological study of FMD using a non-structural protein (NSP) assay and a preliminary estimation of the test parameters using a combined VNT for all the SAT serotypes as a comparison in a Bayesian formulation of the Hui-Walter latent class model (Hui and Walter 1980).

Materials and Methods:

Sampling: Serum samples collected between 1994 and 2005 as part of wildlife health surveillance project by the Kenya Wildlife Service, the Pan African Rinderpest Eradication Campaign (PARC) and the Programme for the Control of Epizootics (PACE), under the auspices of the African Union Inter African Bureau for Animal Resources (AU-IBAR) were screened for antibodies to non-structural proteins. A total of 731 sera from 27 species of wildlife from Kenya, Tanzania, Ethiopia, Sudan, and Chad were initially screened for FMD virus antibody using a Ceditest® FMDV-NS test kit (Cedi Diagnostics B.V.). (Table 1)
Table 1- Number of each species sampled.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number Sampled</th>
<th>Number Sampled</th>
</tr>
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<tbody>
<tr>
<td>Buffalo <em>Syncerus caffer</em></td>
<td>483</td>
<td>Beisa and fringe eared oryx</td>
</tr>
<tr>
<td>African and desert Warthog <em>Phaeocherus</em></td>
<td>52</td>
<td>Bovine</td>
</tr>
<tr>
<td><em>Africans and P. aethiopicus</em></td>
<td></td>
<td>Roan antelope</td>
</tr>
<tr>
<td>White eared kob <em>Kobus leucotis</em></td>
<td>50</td>
<td><em>Hippotragus</em></td>
</tr>
<tr>
<td>Giraffe <em>Giraffa camelopardalis</em></td>
<td>34</td>
<td>Wildebeest</td>
</tr>
<tr>
<td>Eland <em>Taurotragus oryx</em></td>
<td>19</td>
<td><em>Connochaetes</em></td>
</tr>
<tr>
<td>Thomson’s and Grant’s Thomson Gazelle</td>
<td>17</td>
<td><em>Beatragus</em></td>
</tr>
<tr>
<td>Grantii <em>Gazella thomsoni and G. grantii</em></td>
<td></td>
<td><em>scriptus</em></td>
</tr>
<tr>
<td>Impala <em>Aepyceros melampus</em></td>
<td>13</td>
<td>Gerenuk <em>Litocranius walleri</em></td>
</tr>
<tr>
<td>Lelwel, Swayne’s and Coke’s hartbeest</td>
<td>14</td>
<td>Mountain nyala</td>
</tr>
<tr>
<td><em>Alcelaphus busefalaphus</em></td>
<td></td>
<td><em>Tragelaphus</em></td>
</tr>
<tr>
<td><em>lelwel, A.B. swaynei, A.B. busefalaphus</em></td>
<td></td>
<td><em>buxtoni</em></td>
</tr>
<tr>
<td>Lesser and greater kudu</td>
<td>9</td>
<td>Sable <em>Hippotragus niger</em></td>
</tr>
<tr>
<td>Topi and tiang</td>
<td>9</td>
<td>Water buck</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Kobus ellipsyprimenus</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>defassa</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>731</strong></td>
<td></td>
</tr>
</tbody>
</table>

Test plates were read by measuring Optical Density (OD) at a wavelength of 450nm. The OD of all values including the controls were calculated and expressed as a Percentage Inhibition (PI) relative to the mean OD of the negative control (OD max):

\[
P I = 100 - \left( \frac{OD_{\text{test sample}}}{OD_{\text{max}}} \right) \times 100
\]

A PI of < 50% was considered negative and it was interpreted that the animal tested had not been exposed to FMD for 40 days. A PI of ≥ 50% was considered positive and recent exposure <40> days to FMD was assumed. More specifically, a PI value of ≥ 50% but < 70% was considered a weak positive result and a PI value of ≥70% was considered a strong positive result (Soresen et al., 1998).

**Analysis**: Hui & Walter (1980) introduced a latent class approach to the evaluation of diagnostic tests in absence of a “gold-standard”. The Hui-Walter paradigm for test evaluation in the absence of a “gold-standard” requires the presence of two (or more) tests evaluated in two (or more) populations and furthermore that: the prevalence of the disease is different within each population; the tests have the same properties across populations; and the tests must be conditionally independent given the disease status. Conditional independence given disease status between two tests implies that if the true status of the test subject is known, then knowing the outcome (e.g. positive) of one of the tests will not change our belief in a specific test result (e.g. positive) of the other test.

The Bayesian version of the Hui-Walter model assumes that for the ith subpopulation the counts \((O_i)\) of the different combinations of test results, e.g. +/+ , +/-, -/+ , and -/- for two tests, follow a multinomial distribution:

\[O_i \mid Se_j, Sp_j, p_i \sim \text{Multinomial}(Pr_i, n_i) \text{ for } i = 1, 2, ..., S \text{ and } j = 1, 2, ..., T\]

where S is the number of subpopulations and T is the number of tests and Pr_i is a vector of probabilities of observing the individual combinations of test results. Conditioning on the (latent) disease status, these probabilities can be specified using Se and Sp of the tests and the prevalence (p) of the subpopulations. As well as conditional independence the model assumes that the test Se and Sp are the same in each population. As an example, for two tests the probability of observing both tests positive in the ith subpopulation is given as:
= Se1 Se2 p1 + (1-Sp1) (1-Sp2) (1-p1)
The open access software “Winbugs” was used to run the model.

Results:

Ceditest-descriptive: Results among the vertical duplicates were consistent and with the exception of a PI of -77%, all values fit into a reasonable range compared to the Ceditest controls.

The results of the screening with the Ceditest are given in Figure 1 below. Of the 731 sera tested 339 (46.4%) were positive based on the Ceditest PI result of ≥ 50%. Buffalo comprised a significant majority of the positive results, 327 out of the 339, leaving only 12 positives from all other wildlife species. Sampling was carried out from 1994-2004 and positive Ceditest results occurred for all 10 years in buffalo and in the years 1996, 1999, and 2000 for the other wildlife species. Of the total of 483 buffalo sampled 327 (67.7%) of samples were NSP FMD positive.

(a) (b)

Fig. 1. (a) The map gives the distribution of the animals sampled both positive and negative (b) Boxplot of Ceditest results for all species sampled. A PI value of ≥50% indicates a positive test result. * = outlier.

In addition, the animal’s age and date of sampling were recorded for the majority of the buffalo. Buffalo that tested positive ranged from 1 month of age to over 15 years (Figure 2a). The data indicates that the majority of buffalo aged from approximately 18 months to 10 years are seropositive to one or more of the serotypes of FMD. The two buffalo aged 0-9 months had a very low median PI value of 14.5%. Percentages of animals with positive Ceditest results were above 50% for every age grouping except buffalo ranging from 0-9 months of age.
Buffalo samples were collected over a ten-year period from 1994-2004 (Figure 2b). The proportion that had a positive Ceditest result was well below 50% for each year from 1994-1997 and well above 50% for each year from 1999-2004. Results from a bar chart of the data show a gradual rise in the proportion of buffalo that tested positive from 1996-1999 and the overall trend of an increase in the proportion of buffalo that tested positive from 1994-2004.

![Boxplot of PI for Ceditest for buffalo stratified by age group (years) and by year of sampling.](image)

**Fig. 2.** Boxplot of PI for Ceditest for buffalo stratified by (a) age group (years) and (b) by year of sampling (thickness of the box is proportional to the number of observations).

Buffalo samples were collected over a ten-year period from 1994-2004 (Figure 2b). The proportion that had a positive Ceditest result was well below 50% for each year from 1994-1997 and well above 50% for each year from 1999-2004. Results from a bar chart of the data show a gradual rise in the proportion of buffalo that tested positive from 1996-1999 and the overall trend of an increase in the proportion of buffalo that tested positive from 1994-2004.

![Posterior distributions of Ceditest Se and Sp based on uniform and Beta priors.](image)

**Fig. 3.** Posterior distributions of Ceditest Se and Sp based on (a) uniform prior and (b) Beta priors based on estimates from systematic review of Ceditest performance where there is good agreement on Sp but wide ranging estimates of Se which is reflected in the priors.

**Ceditest-parameter estimation:** A preliminary analysis of the parameter estimates was made to demonstrate the model and its potential usefulness. The first model used uniform (0,1) prior distributions for the Ceditest Se and Sp reflecting the lack of knowledge about these were parameters when the test is used in buffalo. The results are given in figure 3a below. The combined VNT for the three SAT serotypes was used as the second test for comparison with a uniform (0,1) prior in both models.
Discussion:

The Ceditest suggests very high seroprevalence rates in buffalo compared to all other species of wildlife sampled in East Africa (though the numbers are very small for many species). However these are very consistent with the findings or others (Thomson et al. 2003). Interestingly there was no evidence of significant seroconversion in either the warthog or the desert warthog. There is little information about warthogs and FMD and if they were particularly good excreters of virus, similar to domestic pigs, this could be important. However, possibly because of behaviour aspects or resistance they do not appear to be important epidemiologically. Most data was available for buffalo and the stratification by age and year of sampling suggest that buffalo calves are seroconverting very early in life and are continuously reexposed since their NSP antibodies remain high. Also there is a clear increase in exposure over the period of sampling with much lower levels in the 1990’s and this may reflect the introduction of a new SAT2 into the region in the late 1990’s (Vosloo et al. 2002).

The parameter estimation is still not complete and will require validation and sensitivity analysis. However, it illustrates the use of the latent class approach to estimate Se and Sp in the absence of a gold standard test. The posterior for the Se reflects the very wide range of estimates from the literature while the posterior for the Sp is much narrower reflecting the strong prior evidence of test Sp.

The validation of the Ceditest or other NSP tests will provide a useful new tool for serosurveillance in African wildlife and particularly buffalo. Removing the need to carry out VNT for each serotype in an area where there are potentially 6/7 serotypes circulating will make screening much more economic in what is already a very expensive exercise sampling wildlife. This may become increasingly important as efforts are stepped up for eradication. Control in much of sub-Saharan Africa, East Africa in particular, is likely to depend on control in the buffalo reservoir. Fencing that has been used in Southern Africa is unlikely to be an option here. The results here are very consistent with previous studies in other areas and the Ceditest appears to give reliable and consistent results with useful parameters for Se and Sp. However the imperfect nature of the test means that as in cattle it probably will have more use as a herd level test.

Conclusion:

FMD is a disease that continues to have a significant influence on livestock industries in developing countries throughout Africa. Although the epidemiology of the disease has been studied for many years, many questions still remain about the role of wildlife in transmission and maintenance of the disease. Results of the serological survey support the assertion that the prevalence of the disease is high in African buffalo and showed that other wild ungulates such as antelope are also susceptible, confirming past findings. Further examination of the role of different wildlife species in maintenance and spread of FMD is important and could have a positive impact on control strategies implemented in developing countries throughout Africa to prevent outbreaks of the disease in the future.

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References:


