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Source: Journal of Wildlife Diseases, 17(4) : 605-608

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-17.4.605
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Abstract: Sera from various wild ruminants co-existing in the same habitats with cattle had haemagglutination-inhibition (HI) antibodies as high as those in the cattle. Sera from wildebeest (Connochaetes taurinus), kongoni (Alcelaphus cokesi), Thomson's gazelle (Gazella thomsonii), eland (Taurotragus oryx) and the African buffalo (Syncerus caffer) had titers ranging from 64 to 128. Zebra (Equus burchelli), bushbuck (Tragelaphus scriptus), wart hog (Phacochoerus aethiopicus), and oryx (Oryx spp.) sera were negative to the HI test. It was suggested that some of the wild ruminants with high titers could be a possible reservoir of parainfluenza-3 virus.

INTRODUCTION

Bovine parainfluenza-3 (PI-3) virus affects the respiratory tract of cattle and together with Pasteurella has been associated with the shipping fever complex. In East Africa, PI-3 virus or a related agent was first isolated from lymph nodes of cattle. A serological survey has shown that haemagglutination-inhibition antibodies to PI-3 virus are widespread in cattle in East Africa, as well as wildlife species in other parts of Africa. Kamijnjlo et al. confirmed the results of Plowright by isolating PI-3 virus from an acute respiratory disease of cattle in Kenya. In an extensive survey of wildlife sera from several African countries, Hamblin and Hedger demonstrated neutralising antibodies to PI-3 virus in 20 of the 44 species examined and suggested the African buffalo (Syncerus caffer) might be a particularly important reservoir of infection in the wild. Natural infection of the African buffalo was later confirmed by isolations of PI-3 virus from 3 of 51 clinically normal buffaloes in Zimbabwe, whereas Thorsen et al. isolated the virus from one fallow and one mule deer and four of the free-ranging pronghorns in Canada. In both Kenya and Tanzania, Masai cattle graze together with numerous species of wild game, some of which are resident while others are on a seasonal migratory route. Furthermore, on many ranches within East Africa, most of the cattle co-exist with many species of wildlife in the same habitat. In view of this a survey for antibodies to PI-3 in wildlife species co-habiting the same areas with cattle was deemed necessary.

MATERIALS AND METHODS

Serum samples. Bovine sera were collected from herds located along migratory routes of wildlife and in game reserves where cattle and various wildlife species intermingled. The wildlife sera were collected in the same geographical areas from immobilized game animals or during game cropping programmes from ranches in the same areas. All wildlife were immobilized with a combination of xylazine, etorphine hydrochloride and acepromazine maleate. Serum samples were stored at -20 C or -70 C until tested.

Virus antigen. A strain of PI-3 virus isolated from Kenya was used. The virus was propagated in fetal bovine kidney
cell cultures grown in Eagle's minimum essential medium (MEM)\(^5\) containing 5% bovine fetal calf serum, 100 I.U. penicillin and 25 mg mycostatin per ml. The maintenance medium contained the same ingredient with 2% horse serum replacing the fetal calf serum. The virus was harvested after the full cytopathic effects had been observed and stored at -70 C until used. The virus was used at 4 haemagglutinating units (HU) per ml.

Erythrocytes. Heparinised blood was obtained from bovine and guinea pig donors and centrifuged at 400 x g for 10 min. The erythrocytes (RBCs) were washed three times in phosphate buffered saline (PBS) pH 7.2 and bovine RBCs resuspended to a concentration of 10% for serum adsorption. Guinea pig RBCs were resuspended to 0.5% and 1.0% concentration for the haemagglutination-inhibition test after Kaminjolo and Paulsen.\(^6\)

Treatment of sera. Both bovine and wildlife sera were adsorbed: 0.2 ml of serum were added to 0.6 ml of PBS pH 7.2 and 0.8 ml of 10% bovine RBCs. The mixture was incubated at 37 C for 15 min and then centrifuged to recover the serum which was inactivated in a waterbath at 56 C for 30 min.

**Hemagglutination-inhibition test.** World Health Organization (WHO) hemagglutination plates were used for HI test using the method of Rentscher et al.\(^7\) modified by Kaminjolo and Paulsen\(^6\) in which the inactivated sera were diluted in two-fold series. The HI titer was taken as the reciprocal of the highest serum dilution which completely inhibited 4 HU.

### RESULTS

Antibody titers of up to 128 were detected in the sera from wildebeest (*Connochaetes taurinus*), kongoni (*Alcelaphus cokesi*), Thomson's gazelle (*Gazella thomsonii*) and the African buffalo (*Syncerus caffer*), whereas the sera of eland (*Taurotragus oryx*) had a titer of 512 (Table 1). Zebra (*Equus burchelli*), bushbuck (*Tragelaphus scriptus*), warthog (*Phacochoerus aethiopicus*) and oryx (*Oryx spp.*) sera were negative to the HI test. Most bovine sera obtained from two areas where wildlife lived or migrated through had high HI titers.

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Reciprocal serum antibody titers</th>
<th>&lt;16</th>
<th>16</th>
<th>32</th>
<th>64</th>
<th>128</th>
<th>256</th>
<th>512</th>
<th>1024</th>
<th>Total</th>
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<td>2</td>
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<td>-</td>
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<td>-</td>
<td>6</td>
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<td>-</td>
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<td>1</td>
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<td>1</td>
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<tr>
<td><strong>Totals</strong></td>
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<td>10</td>
<td>15</td>
<td>14</td>
<td>9</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>121</td>
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</tbody>
</table>

\(^5\) Wellcome (Kenya) Ltd.

\(^6\) Dr. Rentscher et al., Bakteriolongisches Institut, 6908, Laupheim, West Germany.
corresponding to those observed for the wildlife species (Table 2). One wildebeest 
(Connochaetes taurinus) serum and one bovine serum (both obtained from the 
Masai Mara region which is on the yearly migratory route of the wildebeests) had a titer of 1024. On 
examining all bovine and wildlife serum samples it was apparent that HI antibody titers were of the same order for 
both types of animals.

DISCUSSION
Cottrial believes that in bovine HI titers of 10 and 40 show evidence of 
previous infection by PI-3 virus, whereas bovine with clinical disease would show 
titers of 320 or higher. Kaminjolo et al. reported that cattle which were 
seronegative to PI-3 on the HI test or had low titers (e.g., 4) later showed titers of 
512 and 1024, respectively, after an interval of 5 weeks following the outbreak of 
disease in the herds. Thus it would appear that the titers reported herein indicate previous exposure to PI-3 virus 
for both the bovine and wildlife. The presence of HI antibodies to PI-3 virus in 
both the domestic and wild species indicates that the virus is ubiquitous 
throughout the geographic areas shared by these ruminants.

Table 2. Prevalence of haemagglutination-inhibition titers to parainfluenza-3 (PI,)

<table>
<thead>
<tr>
<th>Reciprocal serum antibody titers</th>
<th>&lt;16</th>
<th>16</th>
<th>32</th>
<th>64</th>
<th>128</th>
<th>256</th>
<th>512</th>
<th>1024</th>
<th>Totals</th>
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<tr>
<td>Totals</td>
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<td>22</td>
<td>9</td>
<td>1</td>
<td>19</td>
<td>1</td>
<td>120</td>
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</tbody>
</table>

Acknowledgements
The authors are grateful to Dr. L. Karstad of the Wildlife Section, Veterinary 
Research Laboratory, Kabete, Kenya for the supply of some of the wildlife sera; and to 
the staff of the Wildlife Conservation Department, Kenya, for all relevant help 
rendered in obtaining sera from live wildlife species.

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Received for publication 29 January 1981