Serological evidence in sheep suggesting phlebovirus circulation in a Rift Valley fever enzootic area in Burkina Faso

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Abstract
Within the Phlebovirus serogroup, Rift Valley fever (RVF) virus is endemo-enzootic in the African sahelian zone. Recently an RVF epizootic in West Africa prompted a serosurvey in the major sheep and cattle raising areas. Because of the close antigenic relationship between the phleboviruses it appeared of interest to evaluate the prevalence of the other phleboviruses also. In 1987, 482 sheep serum samples were collected in 2 different ecological zones of Burkina Faso and tested for the presence of phlebovirus antibodies. A sensitive but non-specific immunofluorescent antibody test and a specific enzyme-linked immunosorbent assay (ELISA) were used, with the following African phlebovirus antigens: Rift Valley fever (RVF), Arumowot, Gabek Forest, Gorgi, Saint Floris and Odenariou. A total of 13.8% of the sera sampled had anti-RVF antibody in the ELISA. RVF virus appeared to be more active in drier areas such as the sahelian region, known to be an enzootic area for the disease. Antibodies to other phleboviruses were found in 11.8% of the samples, independent of RVF virus activity. It is assumed that sheep can be infected by different phleboviruses.

Introduction
The Phlebovirus genus consists of 38 virus serotypes. Six of them are known from sub-Saharan Africa, including the Rift Valley fever (RVF), Arumowot (AMT), Gor-dil (GOR), Gabek Forest (GF), Saint Floris (SAF) and Odenariou (ODR) viruses. RVF virus is distributed over the entire area and has been associated with dramatic epidemics, severe sporadic human cases, and large-scale epizootic abortion in domestic ruminants (SMILUZZO et al., 1987; JOUAN et al., 1988; KHAZER et al., 1989). However, the ecology and epidemiology of the other phleboviruses are poorly documented and only limited serological studies in humans are available (TSH et al., 1976; PETTE & LEDUC, 1984).

A serosurvey for RVF virus infection in sheep conducted in 1987 in Burkina Faso gave us the opportunity to study phlebovirus circulation (AKAKPO et al., 1989). A high prevalence of fluorescent antibody was found against RVF antigen and in various environments. Because the immunofluorescent antibody (IFA) test cross-reacts with RVF virus and others phleboviruses (TESH et al., 1982), we decided to investigate the circulation of the other phleboviruses known from sub-Saharan Africa.

Materials and Methods
Study area
Three phyto-geographical regions can be identified from the north to the south of Burkina Faso: sahelian, Sudano-sahelian and medio-Sudanian. We investigated the sahelian and medio-Sudanian regions, the former is known to be a focus of enzootic RVF in West Africa (AKAKPO et al., 1989) and the latter is not.

Study population and serum sampling
Sheep sera had been previously collected in Burkina Faso for an RVF IFA serosurvey. Methods of sampling have been described in detail elsewhere (SOME, 1988; AKAKPO et al., 1989). Herds were selected at random from farms in rural areas which had not experienced domestic ruminant importation. A representative sample of more than 85% of each herd was examined. Blood samples were taken by jugular venipuncture, decanted and sera refrigerated at 4°C for less than 24 h before storage at -20°C.

Serological tests
The following virus strains were provided from the World Health Organization Collaborating Centre of Reference and Research on Arboviruses in Dakar (Institut Pasteur): RVF (Ar1976), AMT (Ar1284-64), GOR (AnB496), GF (EgAn754-61), SAF (AnB512R) and ODR (ArAI131). Sera were screened and titrated using both the IFA test and enzyme-linked immunosorbent assay (ELISA).

A classical IFA test was performed on infected Vero cells (WULF & LANGE, 1975). Sera were tested at two-fold dilutions starting at 1:8 and samples were considered positive when they showed fluorescent antibodies at a dilution greater than 1:16. RVF reacting sera were considered as specific for RVF virus if they did not react with other phleboviruses antigens or when they had an RVF titre at least twice that against the other phleboviruses antigens.

A double (sandwich) ELISA for immunoglobulin (Ig) G detection was used (NIKLASSON et al., 1984). Tests were performed in 96-well microplates (Immulon 1®, Dynatech Laboratories, Alexandria, Virginia, USA), slightly modified by using phosphate-buffered saline containing 0.05%-0.2% Tween 20® and 1% non-fat bovine milk (GUILAUD et al., 1988). The plates were coated with a diluted specific phlebovirus hyperimmune mouse ascitic fluid (HD49199 strain). Phlebovirus antigen in crude suckling mouse brain, heat inactivated at 60°C for 1 h, was then captured. Test sera diluted 1:400 were added and specific IgG detected by anti-sheep IgG sera conjugated with horse-radiolase peroxidase (Biosys, Compiègne, France). A chromogenic substrate (o-toluidine, Sigma, La Verpillière, France) was added. All plates included a control of crude suckling mouse brain without virus antigen. Optical density (OD) values were measured at 450 nm using a Multiskan® reader. Sera were considered positive for antibody if the OD of the test was greater than the mean background value of negative controls plus 2 standard deviations.

Statistical analysis
The χ² test was used at the 0.05 level of significance. Results were plotted and analysed by means of 2 x 2 contingency table.

Results
By IFA 21% of 292 sera tested had RVF antibodies. By ELISA only 15.8% of the 482 sera tested were positive. ELISA and fluorescent antibody prevalences were significantly higher in the sahelian region (ELISA: 35.8%; IFA: 19.6%); than in the medio-Sudanian (ELISA: 6.3%; IFA: 9.1%) (χ²=9.3, P<0.01 for ELISA and χ²=4.5, P<0.05 for the IFA test). IFA prevalence was significantly higher than the ELISA prevalence in the sahelian domain (χ²=14.5, P<0.001).

When we compared these 2 areas by ELISA using the 6
phlebovirus antigens, RVF, AMT and SAF virus antibodies had a significantly higher prevalence ($\chi^2=4.9, 6.1, 7.1$ respectively, $P<0.05$) in the sahelian region (Table 1) than in the medio-Sudanian region. On the contrary, GOR virus appeared to be commoner in the medio-Sudanian region ($\chi^2=6.7, P<0.01$).

Of the 292 sera examined by the IFA test, 44 (14.7%) reacted with several antigens. However, of 482 sera tested by ELISA, only 12 were found to have antibodies reacting against 2 antigens (11 sera) or 3 antigens (one serum, no. 9 in Table 2). Ten of these samples were from the sahelian zone (Table 2, sera nos 1–10) and 2 came from the medio-Sudanian zone (Table 2, sera nos 11 and 12).

Table 2. Sheep sera reacting against more than one phlebovirus antigen by ELISA, Burkina Faso, 1987

<table>
<thead>
<tr>
<th>Serum no.</th>
<th>RVF</th>
<th>AMT</th>
<th>GF</th>
<th>GOR</th>
<th>SAF</th>
<th>ODR</th>
</tr>
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<tr>
<td>1</td>
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</tr>
</tbody>
</table>

GF, GOR and SAF viruses have been isolated only from rodents. They seem to have a low circulation rate when a potential rodent reservoir is present. Humans are rarely infected (Tesh et al., 1976; Gonzalez et al., 1983). GF virus is widely distributed in Africa with a low rate of circulation (Saluzzo et al., 1986). The higher seroprevalence rate of GOR virus in the medio-Sudanian region must be due to the circulation of a closely related virus extending the apparent distribution of this rare central African virus. GOR virus has been isolated from different rodents, Tatera sp. and Lemiscosmys striatus, the latter being less restricted to a dry area than Tatera sp. (Gonzalez et al., 1983). Nevertheless, it is difficult to conclude that sera collected in the field are cross-reacting rather than reflecting double infections, in the absence of a specific test using purified specific peptide antigens.

Discussion

RVF virus activity in the sahelian region of Burkina Faso, determined by the IFA test on the same serum samples, has been reported by Akakpo et al. (1989). Moreover, using an RVF IgM ELISA capture test, we found an IgM antibody prevalence of 4.9% (15 positive of 306 tested) within the sahelian region and 0.6% (1 positive of 176 tested) within the medio-Sudanian region (J. P. Gonzalez & B. Le Guenno, unpublished data). These observations suggested active circulation of the RVF virus in Burkina Faso during 1987.

About 5% of the sera which were positive by IFA remained undetected by ELISA. We and others (J. L. Sarthou, personal communication) previously observed such a discrepancy, probably associated with surface antigens detected by IFA but not by ELISA, depending on the degree of protein denaturation and antibody affinity.

Phleboviruses other than RVF virus had a low prevalence among domestic ruminants. AMT virus is a mosquito-borne virus with a cycle including rodents and domestic ruminants (Tesh, 1988; Peters & Leduc, 1984). Its presence has been previously suspected in domestic ruminants in Niger (Niamey), in a transitional Sudano-sahelian region (Saluzzo et al., 1987). AMT virus is widely distributed in Africa. Humans can be infected and develop neutralizing antibody, but its disease potential is unknown (Tesh et al., 1976).

In conclusion, phleboviruses other than RVF virus occur at a low level of endemicity but are not restricted to a specific phytogeographical region. For the first time GF, GOR, SAF and ODR virus antibodies have been detected in domestic ruminants. Their veterinary significance and their pathogenic potential remain unknown. Serological evidence of human infection has been reported without defining the disease potential of the virus (Tesh et al., 1976; Tesh, 1988). Further studies are needed to clarify this and other points.

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References


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