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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, Gordil, Saint Floris and Odrenisrou. A total of 15·8% 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 asumed 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), Gordil (GOR), Gabek Forest (GF), Saint Floris (SAF) and Odrenisrou (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 (SALUZZO *et al.*, 1987; JOUAN *et al.*, 1988; KSIAZEK *et al.*, 1989). However, the ecology and epidemiology of the other phleboviruses are poorly documented and only limited serological studies in humans are available (TESH *et al.*, 1976; PETERS & 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 RFV 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 (Institut Pasteur): RVF (Ar1976), AMT (Ar1284-64), GOR (AnB496), GF (EgAn754-61), SAF (AnB512R) and ODR (ArA1131). 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 plebovirus antigens or when they had an RVF titre at least twice that against the other phlebovirus antigens.

A double (sandwich) ELISA for immunoglobulin (Ig) G detection was used (NIKLASSON *et al.*, 1984). Tests were performed in 96-well microplates (Immulon II®, Dynatech Laboratories, Alexandria, Virginia, USA), slightly modified by using phosphate-buffered saline containing 0·05% Tween 20® and 1% non-fat bovine milk (GUILLAUD *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-radish peroxidase (Biosys, Compiegne, France). A chromogenic substrate (*o*-tolidine, 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 χ^2 test was used at the 0·05 level of significance. Results were plotted and analysed by means of 2×2 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%) (χ^2=9·2, $P<0·01$ for ELISA and χ^2=38·1

Table 1. Phlebovirus antibody prevalence determined by ELISA in a randomized sample of sheep sera from Burkina Faso, 1987

Region	No. in sample	No. of sera positive[a]					
		RVF	AMT	GF	GOR	SAF	ODR
Sahelian	306	60 (19·6%)	8 (2·6%)	4 (1·3%)	2 (0·7%)	16 (5·2%)	8 (2·6%)
Medio-Sudanian	176	16 (9·1%)	3 (1·7%)	2 (1·1%)	7 (4·0%)	1 (0·6%)	6 (3·4%)
Total	482	76 (15·1%)	11 (2·3%)	6 (1·2%)	9 (1·9%)	17 (3·5%)	14 (2·9%)

[a]RVF = Rift Valley fever; AMT = Arumowot; GF = Gabek Forest; GOR = Gordil; SAF = Saint Floris; ODR = Odrenisrou virus antigens.

phlebovirus antigens, RVF, AMT and SAF virus antibodies had a significantly higher prevalence (χ^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 (χ^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

Serum no.	Antigen[a]					
	RVF	AMT	GF	GOR	SAF	ODR
1	+	−	−	−	−	+
2	+	−	−	−	+	−
3	+	−	−	−	−	+
4	+	−	−	−	−	+
5	+	+	−	−	−	−
6	+	+	−	−	−	−
7	+	+	−	−	−	−
8	−	−	−	+	+	−
9	+	+	−	−	−	+
10	−	+	+	−	−	−
11	+	−	−	+	−	−
12	−	−	−	−	+	+

[a]RVF = Rift Valley fever, AMT = Arumowot, GF = Gabek Forest, GOR = Gordil, SAF = Saint Floris, ODR = Odrenisrou virus antigens; + = positive reaction, − = negative reaction.

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

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 *Lemniscomys striatus*, the latter being less restricted to a dry area than *Tatera* sp. (GONZALEZ *et al.*, 1983). However, SAF antibody prevalence is significantly higher in drier areas, as previously observed in Somalia, Sudan and Egypt (TESH *et al.*, 1976). This distribution must be related to the distribution of the only known potential rodent host (*Tatera* sp.).

ODR virus is known only by its original isolate from a mosquito pool in Ivory Coast. While it is antigenically more closely related to AMT, its biology remains unknown (Hervé Zeller, personal communication).

Previous study showed that serum samples collected in field conditions often cross-react with several phlebovirus antigens in IFA and complement fixation tests (TESH *et al.*, 1982; MEUNIER *et al.*, 1988). EITREM *et al.* (1991) found cross-reactivity in human sera within the sandfly phlebovirus group. 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. However, we demonstrated the high specificity of the ELISA for detecting RVF human antibodies that did not react with other phlebovirus antigen, even when the sera had a titre >16000 against RVF antigen (GONZALEZ *et al.*, 1989). Moreover, GOR and SAF viruses were first isolated from the same rodent specimen (KARABATSOS, 1985). From our experience we can assume that the few sheep sera reacting against 2 or more antigens in the ELISA represent multiple infections by dif-

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