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Investigation of tick-borne viruses as pathogens of humans in South Africa and evidence of Dugbe virus infection in a patient with prolonged thrombocytopenia

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SUMMARY

In the course of investigating suspected cases of viral haemorrhagic fever in South Africa patients were encountered who had been bitten by ticks, but who lacked evidence of infection with Crimean–Congo haemorrhagic fever (CCHF) virus or non-viral tick-borne agents. Cattle sera were tested by enzyme-linked immunoassay to determine whether tick-borne viruses other than CCHF occur in the country. The prevalence of antibody in cattle sera was 905/2116 (42·8%) for CCHF virus, 70/1358 (5·2%) for Dugbe, 21/1358 (1·5%) for louping ill, 6/450 (1·3%) for West Nile, 7/1358 (0·5%) for Nairobi sheep disease, 3/625 (0·5%) for Kadam and 2/450 (0·4%) for Chenuda. No reactions were recorded with Hazara, Bahig, Bhanja, Thogoto and Dhori viruses. The CCHF findings confirmed previous observations that the virus is widely prevalent within the distribution range of ticks of the genus *Hyalomma*, while antibody activity to Dugbe antigen was detected only within the distribution range of the tick *Amblyomma hebraeum*. Cross-reactivity for the nairoviruses, Hazara, Nairobi sheep disease and Dugbe, was detected in serum samples from 3/72 human patients with confirmed CCHF infection, and serum from 1/162 other patients reacted monospecifically with Dugbe antigen. The latter patient suffered from febrile illness with prolonged thrombocytopenia.

INTRODUCTION

From January 1980–December 1993 inclusive we examined 3219 specimens from 1717 patients in southern Africa suspected to be suffering from viral haemorrhagic fever, and diagnosed 109 cases of Crimean–Congo haemorrhagic fever (CCHF) [1–5]. Among patients for whom no cause of illness could be found there were some who had been bitten by ticks. Preliminary screening had eliminated tick-borne typhus (*Rickettsia conorii* infection), Q fever (*Coxiella burnetti* infection), ehrlichiosis (*Ehrlichia* spp. infection) and Lyme disease (*Borrellia burgdorferi* infection), and we were prompted to investigate the possibility that tick-borne viruses other than CCHF were involved. Twelve viruses, including CCHF, were selected for investigation for reasons discussed below.

Most ticks which bite humans parasitize farm animals by preference, and hence it was appropriate to test cattle sera by enzyme-linked immunoassay (ELISA) to determine whether there was evidence that the selected viruses occur in South Africa. Sera taken during convalescence from patients with confirmed CCHF infection were tested to determine whether the virus induces antibody which is cross-reactive with antigenically related viruses, and to check whether dual infections had occurred. Finally, the antibody tests were applied to convalescent sera from patients

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who lacked evidence of CCHF infection, but either had a history of tick bite, or lived in or visited a rural location where exposure to ticks was possible. Antibody activity to one or more members of the genus *Nairovirus* of the family *Bunyaviridae* (viruses antigenically related to CCHF) was detected in sera from 4 patients. Three of the patients were no longer accessible for investigation, but rising titres of antibody to Dugbe virus were detected in the fourth who suffered febrile illness with prolonged thrombocytopenia.

MATERIALS AND METHODS

Virus strains

Viruses used in the study included strains of CCHF, Chenuda and West Nile isolated in South Africa [1, 6, 7], and Hazara, Nairobi sheep disease (NSD), Dugbe, Bahig, Bhanja, louping ill, Kadam, Thogoto and Dhori obtained from Yale Arbovirus Research Unit, New Haven, CT, USA.

Antigens and antisera

Sucrose-acetone extracted antigens were prepared from infected mouse brain, and hyperimmune mouse ascitic fluids (HMAF) were prepared in adult mice for all viruses as described previously [8, 9].

Positive control sera were prepared by inoculating two guinea-pigs per virus intraperitoneally with 0·2 ml of a 1/10 dilution of infected mouse brain suspension. Boosters were administered 1 and 3 weeks later, and the guinea-pigs were bled 2 months after receiving the initial dose of virus. Sera from uninfected guinea-pigs were used as negative controls.

IgG sandwich ELISA for antibody to tick-associated viruses

A sandwich ELISA for the detection of IgG antibodies to the test viruses in cattle and human sera was performed as described previously for CCHF virus [4, 9], using the appropriate anti-species immunoglobulin horseradish peroxidase (HRPO) conjugate (Zymed Laboratories Inc., San Francisco, CA, USA), and 2, 2′ azino-di-(3-ethyl-benzthiazole-6-sulphonate) (ABTS) substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD, USA). Optimal working dilutions of HMAF coating antibodies (1/1000–1/5000) and antigens (1/50–1/200) for the tick-associated viruses were determined by chessboard titration with control guinea-pig sera and anti-guinea-pig IgG HRPO conjugate. Optimal dilutions of anti-cattle and anti-human IgG HRPO conjugates (1/500–1/2000) were determined in chessboard titrations with known CCHF positive cattle and human sera [4, 9]. Specimens were tested in doubling dilutions from 1/50 upwards, and recorded as IgG antibody positive if the absorbance at 402 nm was at least twice that of negative control serum. Titres were recorded as the reciprocal of the highest serum dilution producing a positive result.

IgM capture ELISA for antibody to tick-associated viruses

An ELISA for the detection of IgM antibodies to the test viruses in human serum was performed as described previously for CCHF virus [4], using μ-chain specific anti-human IgM (Cappel, Organon Teknika nv, Turnhout, Belgium) as capture antibody and ABTS substrate. Antigens immobilized by captured IgM were detected with reference HMAFs and anti-mouse IgG HRPO conjugate (Zymed). Optimal dilutions of the coating antibody (1/5000) and anti-mouse IgG HRPO conjugate (1/2000) were determined by chessboard titration with known CCHF positive serum [4]. Antigens and HMAFs of the tick-associated viruses were used at the optimal dilutions determined above. Human sera were tested in doubling dilutions from 1/200 upwards, and results recorded as described above.

Test sera

A total of 2116 cattle sera from 46 herds distributed throughout South Africa, which had been tested by reversed passive haemagglutination inhibition (RPHI) for antibody to CCHF virus in a survey in 1983–4 [2] and stored at −70 °C, were screened at initial dilution for antibody to CCHF virus by ELISA in order to evaluate the latter technique by comparing the two sets of results. Lesser numbers of sera were tested for antibodies to the remaining viruses, as indicated in the results. Fifty sera were tested per herd where possible, and in smaller herds all cattle were tested. For each virus, herds were selected to include areas where known vector species or at least ticks of the same genus are present, as well as areas where the ticks are absent [10, 11], as illustrated for Dugbe virus in the results.

Sera from human patients had been submitted to the laboratory from 1984–93 for the diagnosis of

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suspected viral haemorrhagic fever, and had been stored at −70 °C after the initial investigations had been performed. Specimens which had been collected during convalescence 1–2 months after the onset of illness were tested where possible, although a few sera taken earlier were also examined. The sera came from 72 patients in whom CCHF had been confirmed by isolation of virus and/or demonstration by indirect immunofluorescence (IF) of seroconversion or rising titres of IgG and IgM antibody, and a further 162 patients with a history of tick bite or possible exposure to ticks, for whom no evidence of CCHF infection was found. Serological evidence of *R. conorii* infection had been found in 28 of the latter patients, and other diagnoses had been established in a further 19 instances, but no definitive diagnosis had been obtained in the remaining 115 cases.

Investigation of a patient with suspected Dugbe virus infection

Observations were made over a period of 34 months following the onset of illness on a patient in whom rising titres of antibody to Dugbe virus were detected. Tests included attempts to isolate virus from serum samples and cerebrospinal fluid (CSF) by inoculation of suckling mice and Vero cell cultures [1, 2, 4], and efforts to demonstrate Dugbe virus S segment RNA in sera by reverse transcription and the polymerase chain reaction (RT-PCR) [12]. Tests for neutralizing antibody to Dugbe virus (strain AR1792) were based on fluorescent focus reduction in Vero cell cultures as described for CCHF [13, 14]. To eliminate the possibility that thrombocytopenia in the patient was caused by parvovirus B19 infection, sera were tested for viral DNA by PCR [15] and for IgG and IgM antibodies to the virus by means of commercially available ELISA kits (Genoclin GmBH Laboratories, Hamburg, Germany). Further tests for which results are reported, including clinical pathology assays, were performed in other laboratories. The farm where the patient had been bitten was visited 4 months after the event to collect ticks which were tested for virus content as described previously [1].

RESULTS

Antibody survey on cattle sera

The results of ELISA for antibodies to the tick-associated viruses in cattle sera are summarized in Table 1. Findings for CCHF virus were similar to those reported previously using RPHI [2]. Antibody to the virus was found to be widely distributed in South Africa in accordance with the known distributions of the 3 *Hyalomma* tick species which occur in the country, with a tendency for herds to be seronegative or have a low prevalence of antibody along the southern coast where *H. truncatum* is the sole representative of the genus. Results were concordant (either positive or negative) for 1681/2010 (83·6%) sera tested by both RPHI and ELISA. However, ELISA appeared to be the more sensitive technique and produced positive results for 231/2010 (11·5%) sera for which RPHI was negative, while the

Table 1. *Results of ELISA for antibodies to tick-associated viruses in cattle sera from South Africa*

Virus	Sera positive/tested (%)	Herds positive/tested
Family *Bunyaviridae*, Genus *Nairovirus*, Serogroup CCHF		
CCHF	905/2116 (42·8)	41/46
Hazara	0/785 (0)	0/16
Serogroup Nairobi sheep disease		
Nairobi sheep disease	7/1358 (0·5)	3/28
Dugbe	70/1358 (5·2)	7/28
Genus *Bunyavirus*, Serogroup Tete		
Bahig	0/400 (0)	0/9
Genus unassigned, Serogroup Bhanja		
Bhanja	0/435 (0)	0/9
Family *Flaviviridae*, Genus *Flavivirus*, Antigenic complex Tick-borne encephalitis		
Louping ill	21/1358 (1·5)	11/28
Antigenic complex St Louis encephalitis		
West Nile	6/450 (1·3)	4/9
Antigenic complex unassigned		
Kadam	3/625 (0·5)	3/13
Family *Reoviridae*, Genus *Orbivirus*, Serogroup Kemerovo		
Chenuda	2/450 (0·4)	2/9
Family *Orthomyxoviridae*, Genus unassigned		
Thogoto	0/1358 (0)	0/28
Dhori	0/785 (0)	0/16

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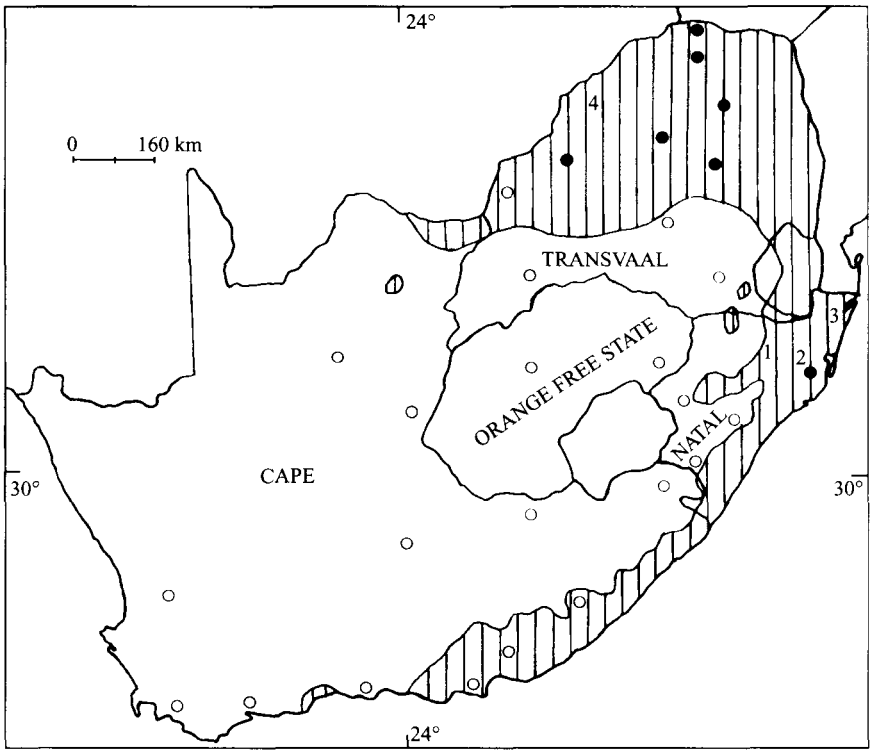

Fig. 1. Map showing locations where antibody to Dugbe virus antigen was detected by ELISA in cattle and human sera, in relation to the known distribution of the tick *Amblyomma hebraeum* (vertical hatching): closed circles (●), herds in which antibody was detected; open circles (○), herds in which antibody was not detected; arabic numerals, locations where the corresponding patients in Table 3 were exposed to infection.

Table 2. *Cross-reactivity for nairovirus antigens in 1385 cattle sera detected by ELISA*

	Numbers of sera reacting with indicated antigens							
Total sera tested (herds)	CCHF	CCHF Dugbe	CCHF Dugbe NSD	CCHF NSD	Dugbe	Dugbe NSD	NSD	Hazara
785 (16)*	365	24	3	1	24	1	2	0
600 (12)†	167	11	0	0	7	0	0	NT‡

* Tested with CCHF, Dugbe, NSD and Hazara antigens.
† Tested with CCHF, Dugbe and NSD antigens.
‡ NT, not tested.

reverse occurred in 98/2010 (4·9%) instances. There was a tendency for discrepancies between the two techniques to manifest as differences in prevalence of antibody within seropositive herds, and 5 herds lacked antibody by both methods.

Dugbe was the only other virus for which moderately high prevalences of antibody were detected in cattle sera (Table 1); this occurred only in 7 herds which fell within the known distribution range of the tick *Amblyomma hebraeum* in South Africa [10] (Fig. 1). Antibody was present in 70/350 (20%) sera from the 7 seropositive herds, and the prevalence in the individual herds ranged from 2–32% (⩾ 20% in 5 instances). The 7 reactions recorded with NSD virus, the closest known antigenic relative of Dugbe, fell within the distribution range of the tick *Rhipicephalus appendiculatus* (data not shown) [10], which is the principal vector of NSD virus in East Africa [16]. Six of the NSD reactions occurred in herds which contained Dugbe reactors, while the seventh occurred in the Orange Free State. No reactions were recorded with the remaining nairovirus, Hazara.

The cross-reactivity of individual cattle sera for nairoviruses is analysed in Table 2, in which dis-

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Table 3. *Antibody reactions to nairovirus antigens detected by ELISA in serum samples of four suspected haemorrhagic fever patients*

		ELISA titres							
		Serogroup CCHF				Serogroup Nairobi sheep disease			
		CCHF		Hazara		Nairobi sheep disease		Dugbe	
Patient	Day of illness	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
1*	12	3200	3200	200	—	100	—	3200	—
2*	14	12800	3200	400	—	800	1600	12800	1600
3*	13	800	—	100	—	—	—	3200	—
4	29	—	—	—	—	—	—	12800	51200

* Patients diagnosed as cases of CCHF infection on the basis of virus isolation and/or IF antibody response.

tinction is made between 785 sera tested with all 4 nairovirus antigens included in the study and 600 sera which were not tested with Hazara antigen. Altogether, 532/571 (93·2%) sera which reacted with CCHF antigen were monospecific, while 31/70 (44·3%) Dugbe reactors and 2/7 NSD reactors were monospecific.

Among the flaviviruses, very few reactions were recorded with West Nile and Kadam antigens, but antibody activity to louping ill virus was detected in 11 widely scattered locations which appear to coincide with the patchy areas of distribution of the 2 ticks of the genus *Ixodes* which parasitize livestock in South Africa, *I. pilosus* and *I. rubicundus* (data not shown) [10]. Maximum prevalence of louping ill antibody, 7/37 (18·9%), occurred in a herd in the Cape Province. Only 2 cattle sera reacted with Chenuda antigen, and no antibodies were detected to the remaining 4 viruses in the study, Bahig, Bhanja, Thogoto and Dhori (Table 1).

Antibody tests on sera of suspected haemorrhagic fever patients

Antibody activity to one or more nairoviruses, besides CCHF, was detected by ELISA in serum samples from 4 patients; 3/72 in whom a diagnosis of CCHF infection had been made, and 1/162 of the remaining patients in the study (Table 3). All 4 patients were ostensibly exposed to infection in locations which fell within the distribution range of the tick *A. hebraeum*, where antibody activity to Dugbe virus antigen was found in cattle sera (Fig. 1). The diagnosis of CCHF in the first patient (no. 1 in Table 3) was based on isolation of virus and demonstration of seroconversion, while rising titres of antibody were detected by IF in paired sera from patients 2 and 3. In addition, CCHF virus was isolated from and seroconversion demonstrated in the daughter of patient 2, who became ill 2 days after her father but had a monospecific antibody response. Patient 4 had monospecific antibody to Dugbe virus (Table 3). The 3 CCHF patients came from remote rural locations and could not be traced for further investigation, but extended observations were made on the suspected Dugbe patient as described below. None of the patients in the study had antibody to viruses tested other than nairoviruses.

Findings on a patient with suspected Dugbe virus infection

The patient (number 4 in Table 3), a previously healthy 51 year old male who lived in Johannesburg, visited a farm in the north-western Transvaal (Fig. 1) from 16–18 November 1990, and acquired about 300 larval tick bites on the first day. He consulted a doctor on 20 November when he felt 'light-headed' and had developed fever, headache and malaise, and was placed on oral tetracycline therapy. Over the next 2 weeks he experienced bouts of fever, headache, weakness, fatigue and severe myalgia (backache and thigh pains), which were exacerbated on days that he returned to work. On 26 November he became aware of 'flecks' (petechiae) on his arms and legs, and on 3 December developed swelling and tenderness of his right testicle, and noticed that he bruised easily. Blood taken on 7 December had a platelet count of 3×10^9/l, and he was admitted to hospital on the following morning.

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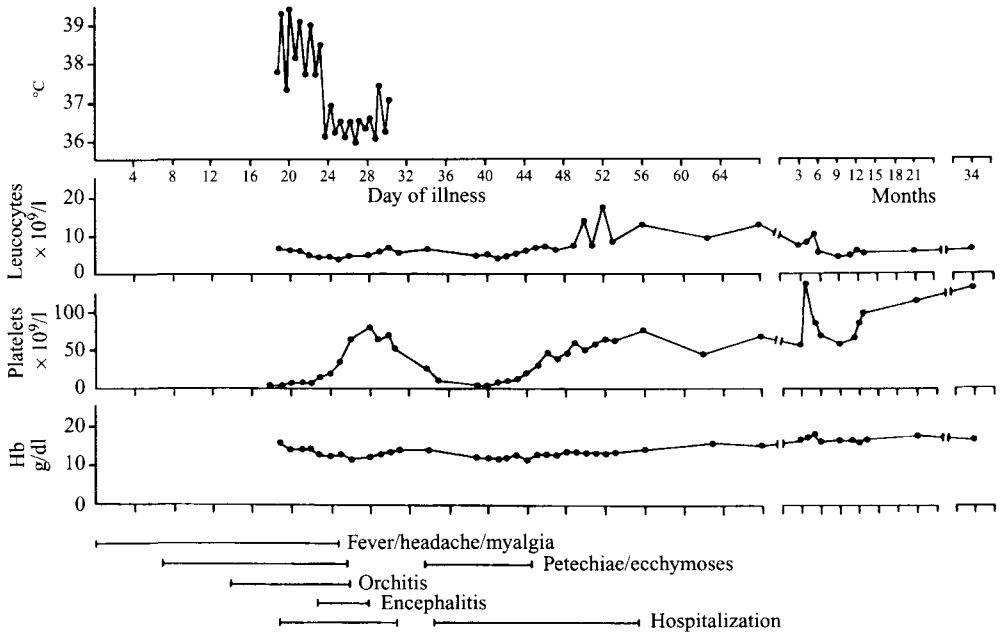

Fig. 2. Curves showing temperature, blood leucocyte, platelet and haemoglobin levels monitored over a period of 34 months in a patient with suspected Dugbe virus infection, in relation to the main signs and symptoms of illness.

On admission the patient was febrile, had numerous healing tick bites, bruises, petechiae and ecchymoses on the lower trunk and limbs, subconjunctival haemorrhages, palatal petechiae, generalized lymphadenopathy, myalgia, swollen right testicle and epididymis, tachycardia and hyperpnoea. On 12 December he developed visual hallucinations, nightmares and hyperaesthesia of the chest and trunk. Leucocyte, differential and erythrocyte counts fluctuated within reference value ranges, but haemoglobin values declined transiently from 15·9 g/dl on admission to 12·0 g/dl at the lowest (Fig. 2). Thrombocyte counts remained $< 20 \times 10^9$/l (Fig. 2) despite the administration of 750 ml of platelet concentrate over the first 5 days in hospital. Prolonged prothrombin times (INR 1·3–1·4) were recorded on 8 and 13 December, but at no stage were there abnormal findings in other coagulation studies to indicate the occurrence of intravascular coagulopathy. Bone marrow samples taken on 9 and 19 December were described as being of normal active appearance. Fever subsided on 13 December and other signs and symptoms abated over the next 3 days, while the platelet count rose steadily to 80×10^9/l by 17 December (Fig. 2). The patient was discharged on 20 December, but re-admitted on the 24th with renewed skin and palatal petechiae. Platelet counts were again $< 20 \times 10^9$/l and although tests for anti-platelet antibody were negative, immune thrombocytopenic purpura was considered as a possible diagnosis. Large doses of pooled immunoglobulin (28 g/day) were administered intravenously for 4 days, and the patient was placed on prednisolone therapy for 4 weeks. Platelet counts rose to 66×10^9/l by 10 January, while total leucocyte counts rose transiently to a maximum of $18{\cdot}0 \times 10^9$/l (Fig. 2). The patient was finally discharged from hospital on 14 January, and was still well when last seen 34 months after the onset of illness, despite the fact that his platelet counts had remained $< 100 \times 10^9$/l for most of the time. There was nevertheless an upward trend in platelet counts towards the end of the period of monitoring (Fig. 2), and a count of 158×10^9/l was recorded on the last occasion. No blood counts had been performed on the patient prior to the illness reported here for comparison of the results with the present findings.

Results of tests for liver and renal function were unremarkable throughout the periods of hospitalization. Blood cultures for bacteraemia and bloodsmears for malaria were negative. No virus was isolated from serum or CSF specimens. Antibody tests failed to produce evidence of infection with Coxsackie B1-6, mumps, Epstein-Barr, parvovirus B19, human immunodeficiency, chikungunya, Sindbis, West Nile, Wesselsbron, Rift Valley fever, CCHF, Marburg, Ebola, arena- and hantaviruses, or non-viral tick-borne agents mentioned in the introduction. The PCR for parvovirus B19 and the RT-PCR for Dugbe virus were negative. The IgM ELISA titre to Dugbe virus was 12800 in serum taken on day

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20 of illness, reached a maximum of 51200 by day 28, and the antibody was no longer demonstrable after day 65, while IgG titres rose from 1600 on day 20 to a maximum of 25600 by day 35, and remained demonstrable 34 months after onset of illness at a titre of 3200. Dugbe neutralizing antibody was detected on a single occasion only 65 days after the onset of illness, at the lowest dilution of serum tested, 1/10.

No questing ticks were found on the farm in the north-western Transvaal 4 months after the patient had been there, but 79 partially-engorged ticks of four species (*A. hebraeum*, *Rhipicephalus evertsi evertsi*, *H. truncatum* and *H. marginatum rufipes*) were collected from cattle, and tested for virus without positive result.

DISCUSSION

At least 24 viruses which occur in Africa are known or suspected to be tick-borne, while a few additional viruses which are transmitted by other vectors have been isolated from ticks on occasion [17, 18]. Four viruses, CCHF, NSD, Dugbe and Thogoto, were included in the study because they were known to have been associated with naturally occurring disease of humans, while a fifth, Bhanja, was known to have caused febrile illness following laboratory infection. Hazara virus, isolated from ixodid ticks in Pakistan, is the closest known antigenic relative of CCHF virus and it was included along with NSD and Dugbe viruses to assess the cross-reactivity for nairoviruses of antibody induced by CCHF infection. Similarly, Dhori, a tick-borne orthomyxovirus of Asia, Europe and North Africa, was included to assess the specificity of any antibody reactions recorded with the antigenically related Thogoto virus.

Louping ill virus was included to determine whether the tick-borne encephalitis complex of flaviviruses is represented in southern Africa. These viruses of the northern hemisphere are difficult to distinguish from each other in serological tests and two of them, Kyasanur Forest disease and Omsk haemorrhagic fever, are known to be associated with haemorrhagic syndromes in humans. Kadam, a tick-borne flavivirus from Uganda, was included as a check on the specificity of any reactions recorded with louping ill antigen. West Nile virus served as a further check for flavivirus cross-reactions. However, this well known mosquito-borne flavivirus of Africa, Europe and Asia, causes sporadic human infections each year in South Africa, and has been associated with a few cases of fulminant hepatitis resembling haemorrhagic fever in West Africa [19]. Moreover, the virus has been isolated from ticks on occasion, also in South Africa [20]. Bahig virus, associated with birds in Egypt and southern Europe, was included merely because it had been isolated from *Hyalomma* ticks of the same species as are involved in the transmission of CCHF in South Africa. Chenuda virus was originally isolated from argasid ticks of birds in Egypt, but was included because antibody had been found in livestock in Egypt, and because there had been multiple isolations of the virus in South Africa [6].

The fact that the findings on CCHF antibody in cattle sera were essentially in agreement with results obtained previously by RPHI, suggests that the ELISA constituted a valid and sensitive technique, and confirms that the virus is widely prevalent in South Africa. In contrast, antibodies to nairovirus antigens other than CCHF were detected only in 7 herds and 4 human patients from locations within the known distribution range of the tick *A. hebraeum*, with the exception of a single reaction to NSD antigen recorded in a herd in the Orange Free State. Because 532/571 (93·2%) cattle with antibody to CCHF virus and 69/72 (95·8%) patients with confirmed CCHF infection lacked antibody activity to the other nairoviruses tested, it can be surmised that primary or uncomplicated infection with CCHF virus in cattle and humans does not appear to induce the production of antibody which is cross-reactive with other nairoviruses. It follows that the suggested use of conventional or recombinant antigens prepared from heterologous, antigenically related viruses [21] is unlikely to be satisfactory for the serodiagnosis of CCHF infection.

It nevertheless remains clear that one or more nairoviruses other than CCHF are active in parts of South Africa, and the indications are that this includes Dugbe virus: 31/70 (44·3%) cattle which had antibody activity to Dugbe virus antigen, and the human patient monitored over a period of 34 months, reacted monospecifically with the virus (Tables 2, 3). The diagnosis of Dugbe infection in the patient is supported by the failure to detect antibody activity to NSD virus, the closest known antigenic relative of Dugbe virus (Ganjam virus of India is regarded as synonymous with NSD) [18, 22]. Although the weak and transient virus neutralizing activity demonstrated in the serum of the patient would seem to militate against the diagnosis, it is well known that nairo-

viruses induce poor neutralizing antibody responses [13, 23, 24], and it is notable that investigators in West Africa were unable to detect the response in patients from whom they had isolated Dugbe virus [25, 26].

Dugbe virus has been isolated in Nigeria, Central African Republic and Ethiopia, and antibody has been found in Senegal and Uganda [17]. Although there have been no reports of the presence of the virus further south in Africa, there do not appear to have been specific investigations. The virus has been isolated on at least 598 occasions from ixodid ticks in West Africa, but it appears to have a particular relationship with *A. variegatum* from which the majority of the isolations were made, and in which transovarial transmission has been demonstrated following artificial infection [17, 27]. This tick is the most widely distributed *Amblyomma* in Africa and it overlaps in distribution with *A. hebraeum* in Zimbabwe [28], so it is theoretically possible that the dissemination of the virus historically extended to South Africa.

Despite the large number of isolations of Dugbe virus made from ticks in West Africa, serosurveys did not reveal widespread human infection, but seven isolations were made from the blood of persons, mainly children, with benign febrile illness in Nigeria and the Central African Republic, including a laboratory infection [25, 26]. One patient had transient meningitis and virus was isolated from cerebrospinal fluid. The mechanisms involved in the pathogenesis of the thrombocytopenia observed in our patient remain undetermined, but the failure of the administration of large doses of platelets to lead to immediate elevation of thrombocyte counts suggests that at least during the acute illness there was consumption of platelets. The performance of virological and RT-PCR tests on biopsy specimens may have revealed whether there was infection of bone marrow. It was not established whether platelet counts were inherently low in the patient for other reasons, but depression of thrombopoiesis due to parvovirus infection was excluded, and mumps was eliminated as a cause of the orchitis seen in the patient.

The inference is that although Dugbe virus infection of humans may occur infrequently, the condition should be borne in mind in the differential diagnosis of suspected cases of viral haemorrhagic fever in Africa, particularly when there is a history of tick bite. In South Africa, the potential exists for the infection to occur within the distribution range of the tick *A. hebraeum*, at least as far south as Natal-KwaZulu (Fig. 1).

Since antibody cross-reactivity broadens following sequential infection with two or more antigenically related bunyaviruses [18, 24], it is possible that the reactivity for Hazara and NSD viruses detected in the sera of 3 confirmed CCHF patients (Table 3) may have stemmed from the occurrence of CCHF infection in subjects who had previously experienced Dugbe infection. At least no other evidence was obtained to indicate that Hazara virus occurs in southern Africa, while the detection of antibody activity to NSD virus in a few cattle sera, including 2 monospecific reactions, at best constitutes inconclusive evidence of the presence of the latter virus. It is well documented that NSD virus causes livestock disease in East Africa [16, 29, 30]. Evidence has been cited to the effect that antibody to the virus has been detected as far south as Botswana, Mozambique and Natal-KwaZulu province of South Africa [17, 31]. However, examination of the original experimental records [5] reveals that undiluted human sera collected in 1957–9 were screened for ability to neutralize NSD virus without treatment for the removal of non-specific inhibitors of nairovirus infectivity [13], and that results regarded as positive (survival of 5–6/6 inoculated mice) were obtained for 1/131 specimens from South Africa, 1/450 from Mozambique, and 0/439 from Botswana and Namibia.

Since West Nile virus is transmitted mainly by mosquitoes, the lack of antibody to this virus in patients' sera, and the paucity of reactions recorded in cattle sera, probably relate to the drought conditions which have prevailed over large parts of the subcontinent in recent years [6]. The 3 reactions recorded with Kadam virus in cattle sera occurred in isolation from each other, and could well have been non-specific. In contrast, antibody to louping ill virus coincided in distribution with *Ixodes* ticks, a genus which includes most of the vectors of the tick-borne encephalitis complex viruses in the northern hemisphere [17].

Chenuda virus has been isolated from argasid tick parasites of swallows in South Africa on 38 occasions [6], but the present findings suggest that livestock and humans rarely encounter the infection, and no evidence was obtained to indicate that the remaining viruses in the study, Bahig, Bhanja, Thogoto and Dhori, occur in the country.

It can be concluded that serological evidence was obtained to indicate that certain tick-borne viruses, including Dugbe, may be present in South Africa, but that definitive evidence can probably best be obtained

through systematic attempts to isolate viruses from ticks collected in locations where antibodies were detected in cattle sera.

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