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Comparative pathogenicity and antigenic cross-reactivity of Rift Valley fever and other African phleboviruses in sheep

By R. SWANEPOEL, J. K. STRUTHERS, M. J. ERASMUS, S. P. SHEPHERD, G. M. McGILLIVRAY, A. J. SHEPHERD, D. E. HUMMITZSCH

Department of Virology, University of the Witwatersrand and Special Pathogens Unit, National Institute for Virology, Sandringham 2131, Republic of South Africa

B. J. ERASMUS and B. J. H. BARNARD

Veterinary Research Institute, Onderstepoort 0110, Republic of South Africa

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SUMMARY

Homologous and heterologous haemagglutination-inhibition (HAI), complement-fixation (CF), immunodiffusion (ID) and mouse neutralization tests were performed with the Lunyo (LUN) and a Zimbabwean strain of Rift Valley fever (RVF) virus, the prototype and a South African strain of Arumowot (AMT) virus and prototype strains of Gordil (GOR), Saint-Floris (SAF) and Gabek Forest (GF) viruses, using immune mouse ascitic fluids prepared against these viruses. Reactions of identity occurred in all tests between LUN and the Zimbabwean strains of RVF and between the two strains of AMT virus. Otherwise, cross-reactions occurred between all the phleboviruses in HAI tests, while reactions in CF, ID and neutralization tests were monospecific for virus serotypes, except that weak cross-reaction occurred between GOR and SAF viruses in CF and ID tests.

Four sheep infected subcutaneously with the Zimbabwean strain of RVF virus developed transient fever, viraemia, leucopaenia, relative thrombocytopaenia, haemoconcentration and raised serum enzyme levels, which indicated that the sheep had developed necrotic hepatitis. Disseminated focal necrotic hepatitis was confirmed in a sheep killed for examination on day 4 post-infection. The other three sheep recovered uneventfully after only mild depression and anorexia. Groups of three sheep infected with SAF, GOR, AMT and GF viruses had no demonstrable viraemia or other sign of infection or illness, except that the sheep infected with AMT developed mild fever lasting less than 24 h.

Antibody responses were monitored at intervals over a period of 24 weeks in all sheep by homologous and heterologous HAI, CF and cell culture neutralization (CPENT) tests. Homologous antibody responses were marked in the RVF-infected sheep and their sera cross-reacted strongly in HAI tests with antigens of the other viruses. The sera of the RVF-infected sheep cross-reacted less markedly in CF and CPENT tests. Homologous antibody responses were poor in all the sheep infected

with phleboviruses other than RVF, and the cross-reactivity of their sera for RVF antigen or virus was negligible. All sheep were challenged with RVF virus 48 weeks after their initial infection. The sheep which had originally been infected with RVF virus were immune and developed neither fever nor viraemia. All other sheep developed fever, viraemia and antibodies to RVF virus.

It was concluded that the African phleboviruses, other than RVF, are unlikely to cause disease in livestock or to induce antibodies which could cause confusion in the diagnosis of RVF.

INTRODUCTION

Rift Valley fever (RVF) is a viral disease of veterinary and medical importance in Africa. Until recently the virus was unclassified and thought to be antigenically unique (Shope, 1978). Antibodies demonstrated in diagnostic tests were considered to be specific. The discovery that the virus is a member of the *Phlebovirus* genus of the *Bunyaviridae* (Shope, Peters & Walker, 1980; Shope *et al.* 1981; Tesh, Peters & Meegan, 1982) raises the possibility that antibodies induced by other phleboviruses may cross-react in diagnostic tests with RVF antigen. Moreover, the other phleboviruses may themselves be pathogenic and induce disease which could be confused with RVF.

We recently examined techniques for demonstrating antibodies to RVF virus (Swanepoel *et al.* 1986), and in extension we have performed comparative studies with RVF and the other known phleboviruses of sub-Saharan Africa, namely Arumowot (AMT), Gordil (GOR), Gabek Forest (GF) and Saint-Floris (SAF) viruses (Shope *et al.* 1981). As a preliminary, antigenic cross-reaction between the viruses was studied by performing homologous and heterologous antibody tests with reagents prepared in mice. Lunyo (LUN) virus, the only strain to have been described as a variant of RVF virus (Weinbren, Williams & Haddow, 1957), was included in the experiments with mouse reagents to check whether RVF virus itself exhibits antigenic variation of sufficient magnitude to cause problems in the diagnosis of the disease. A South African strain of AMT virus was also included, to confirm its antigenic identity with the prototype strain of AMT.

Next, groups of sheep were infected with RVF and the other pleboviruses and monitored for (*a*) signs of disease and (*b*) the induction of cross-reactive antibodies. Finally, the sheep were challenged to determine whether or not the other phleboviruses induced immunity to infection with RVF virus, irrespective of antibody status.

MATERIALS AND METHODS

Viruses

RVF 1678/78 was isolated from bovine organs during the 1978 epizootic in Zimbabwe (Swanepoel, 1981). The LUN strain of RVF virus was isolated from mosquitoes in Uganda (Weinbren, Williams & Haddow, 1957) and brought to the National Institute for Virology (NIV) by Dr M. P. Weinbren. Phleboviruses obtained from Dr R. E. Shope of the Yale Arbovirus Research Unit, New Haven, Conn., USA, included the Dak An BR496d strain of GOR virus and the Dak An

BR512d strain of SAF virus, both isolated from the same individual wild rodent in the Central African Republic, the AR1284–64 strain of AMT virus (AMT 1), isolated from mosquitoes in the Sudan and the Sud An 754–61 strain of GF virus, isolated from a rodent in the Sudan. The AR13532 strain of AMT virus (AMT 2) was isolated from mosquitoes in South Africa by Dr B. M. McIntosh by NIV.

Serological reagents and techniques

Sucrose–acetone extracted haemagglutinating (HA) antigens were prepared as described previously (Swanepoel *et al.* 1986). Infected mouse liver was used for preparation of RVF antigen, and mouse brain for the other viruses.

Hyperimmune mouse ascitic fluids were prepared as described by Sartorelli, Fischer & Downs (1966), except that the first two doses of RVF used for immunizing mice were inactivated with β-propriolactone (Shope & Sather, 1979). Complement-fixation (CF), haemagglutination-inhibition (HAI), immunodiffusion (ID) and micro-neutralization (CPENT) tests were performed as described previously (Swanepoel *et al.* 1986). Viruses other than RVF had to be adapted to produce cytopathic effect by passage in Vero cell cultures.

Constant serum-varying virus dilution neutralization tests using mice were performed by incubating tenfold dilutions of virus with equal volumes of undiluted test serum for 45 min at room temperature (22 °C). The control titration of virus was incubated with fetal calf serum. Sera were inactivated at 59 °C for 30 min prior to tests. Each serum–virus mixture was inoculated intracerebrally in a litter of day-old mice, and deaths occurring during 2 weeks of observation were recorded. End-points were calculated by the method of Kärber (1931) and $\log_{10}$ neutralizing indices calculated by subtracting test from control titres.

Cross-reactivity of mouse ascitic fluids

Homologous and heterologous HAI, CF, ID and neutralization tests in mice were carried out with all combinations of antigens, viruses and immune mouse ascitic fluids. In ID tests antibody, in the form of undiluted ascitic fluid, was placed in the central well and homologous and heterologous HA antigens in alternate peripheral wells.

Sheep experiment

Fourteen of the sheep used were cross-bred Dorpers and Merinos, while two were cross-bred Karakuls. All were reared in stables and lacked neutralizing antibodies to the five phleboviruses before they were included in the experiment at the age of approximately 18 months. They were housed in isolation stables and infected in groups with RVF 1678/78, AMT 2, GOR, SAF and GF viruses. Four sheep were infected with RVF virus and groups of three with the other viruses. All sheep were infected subcutaneously with doses calculated to contain 10^6 plaque-forming units (p.f.u.) of virus. Three of the sheep infected with RVF were, in fact, the same sheep used for comparing serological techniques as reported previously (Swanepoel *et al.* 1986). The fourth RVF-infected sheep was sacrificed during fever on day 4 following infection and a sample of liver was taken for histopathological examination. Temperature and viraemia were monitored for the first 14 days following infection in all sheep, as described previously (Swanepoel *et al.* 1986). The daily

monitoring included homologous HAI, CF and CPENT response, total and differential leucocyte counts, platelet counts, packed cell volumes (PCV) as determined by microhaematocrit, liver enzymes and fibrin degradation products (FDP).

Platelets were separated by centrifugation of heparinized blood at a dilution of 1 in 20 in a mixture of sodium metrizoate, ficoll and Isoton II (Coulter Electronics Ltd, Harpenden, Herts, U.K.) solutions as described by Archer, Allen & Baldwin (1978). The platelet-rich supernatant fractions were fixed by addition to glutaraldehyde in Isoton II to yield a final dilution of 1 in 50 of the platelets in 1 % final concentration of glutaraldehyde. The fixed suspension of platelets were stored at 4 °C to be counted electronically at the end of the 14 days of observation of the sheep.

Kits for glutamate dehydrogenase (GLDH), sorbitol dehydrogenase (SDH), gamma-glutamyltransferase (GGT) (Boehringer Mannheim GmbH, Mannheim, W. Germany), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Abbott Laboratories, South Pasadena, Calif., USA) enzyme tests were used in accordance with the manufacturer's instructions, as were kits for FDP tests (Wellcome Reagents Ltd, Beckenham, Kent, UK). The full range of enzyme tests was applied to the sera of the RVF-infected sheep, but the sheep infected with the other phleboviruses were monitored for GLDH values only.

After the first 14 days, the sheep were bled at weekly and later at fortnightly intervals over a period of 24 weeks as indicated in the results. The sera were tested by HAI, CF and CPENT against homologous and heterologous antigens and viruses. HAI, CF and neutralization tests were chosen as representing the most widely used techniques for serodiagnosis of arthropod-borne virus diseases (Shope & Sather, 1979).

All sheep were bled again at week 48 and challenged with 10^6 p.f.u. of RVF 1678/78 administered subcutaneously. Temperature and viraemia were again monitored for 14 days. Sera collected immediately prior to challenge and on day 14 after challenge were tested by HAI and mouse neutralization tests against RVF virus.

RESULTS

In HAI, CF, ID and neutralization tests performed with mouse ascitic fluids, reactions of identity occurred between LUN and RVF in all instances, confirming that they are strains of a single virus. Reactions of identity also occurred between the prototype (AMT 1) and the South African (AMT 2) strain of AMT virus. Otherwise, marked cross-reactions between phleboviruses occurred only in HAI tests (Table 1). SAF HAI antigen produced the greatest cross-reaction and thus tended to behave as a universal antigen for the group. Homologous HAI titres nevertheless equalled or exceeded heterologous titres in all instances. Reactions in CF tests were monospecific for individual virus serotypes, except that a weak bi-directional cross-reaction occurred between GOR and SAF viruses. Antigen titres in homologous CF systems were all 128 and antibody titres ranged from 256 to 1024. Heterologous antigen titres were 16 and 4 in the cross-CF tests with GOR and SAF viruses, while the antibody titres were 8 and 4. Three lines of precipitation, corresponding to antibody reactions with different viral proteins (Swanepoel *et al.*

Table 1. *Results of cross-HAI tests with African phlebovirus antigens and immune mouse ascitic fluids*

	Immune mouse ascitic fluid						
Antigen	RVF	LUN	AMT1	AMT2	GOR	SAF	GF
RVF	**2560***	2560	160	160	80	80	80
LUN	2560	**5120**	320	80	320	320	320
AMT1	40	40	**1280**	1280	40	20	40
AMT2	40	40	1280	**2560**	20	10	20
GOR	160	160	40	20	**2560**	640	10
SAF	1280	1280	640	640	1280	**5120**	640
GF	20	20	40	20	40	160	**640**

* Antibody titres are expressed as reciprocals of serum dilution.

1986) were evident in RVF and LUN ID tests. Only one or two lines of precipitation occurred in the other homologous systems, indicating that the reagents were less potent. The reactions in the ID tests were monospecific, except for a one-way cross-reaction between GOR and SAF viruses: GOR ascitic fluid produced a line of precipitation with SAF antigen which did not correspond to the line of precipitation seen in the homologous GOR system. The homologous $\log_{10}$ neutralizing indices of the ascitic fluids were low, ranging from 1·4 to 2·6, as can be expected in tests utilizing intracerebral inoculation of mice (Shope & Sather, 1979), but the reactions confirmed the separate identity of each phlebovirus.

Sheep infected with RVF virus in pathogenicity tests exhibited transient fever and viraemia with mild hyperpnoea, depression and anorexia during fever (Fig. 1). Marked leucopaenia, involving both lymphocytes and neutrophils, occurred during the fever and viraemia. There was a gradual drop in platelet counts and rise in PCV values following infection, and the changes were most marked on day four, when fever and viraemia subsided. Thereafter, PCV values did not vary markedly from pre-infection values, but platelet counts increased steadily over a few days and then fluctuated at overcompensatory levels. There were no marked changes in FDP values (data not shown).

A range of liver enzyme tests was applied to the sera of the RVF-infected sheep. For sake of clarity, only the curve for GLDH values is included in Fig. 1 and the results for the full range of enzyme tests are presented separately in Fig. 2. GLDH and AST values increased markedly from the second day post-infection, but high AST values were already evident in sera collected prior to infection. Values for both enzymes declined to pre-infection levels 1 week after infection. SDH enzyme was detected at low concentrations, but significant increases in values were discernible from day 4 following infection onwards. GGT values did not vary significantly during the 14 days of monitoring following infection, while ALT values underwent irregular but non-significant fluctuations.

Data from the sheep which was killed on day 4 post-infection were omitted in compiling Figs. 1 and 2, but fever, viraemia, enzyme and all other changes in this sheep conformed to the patterns shown by the remaining three RVF-infected sheep. The liver from the sheep which was killed had extensive, disseminated focal necrotic lesions.

Antibodies became demonstrable by CPENT on day 4 following the infection

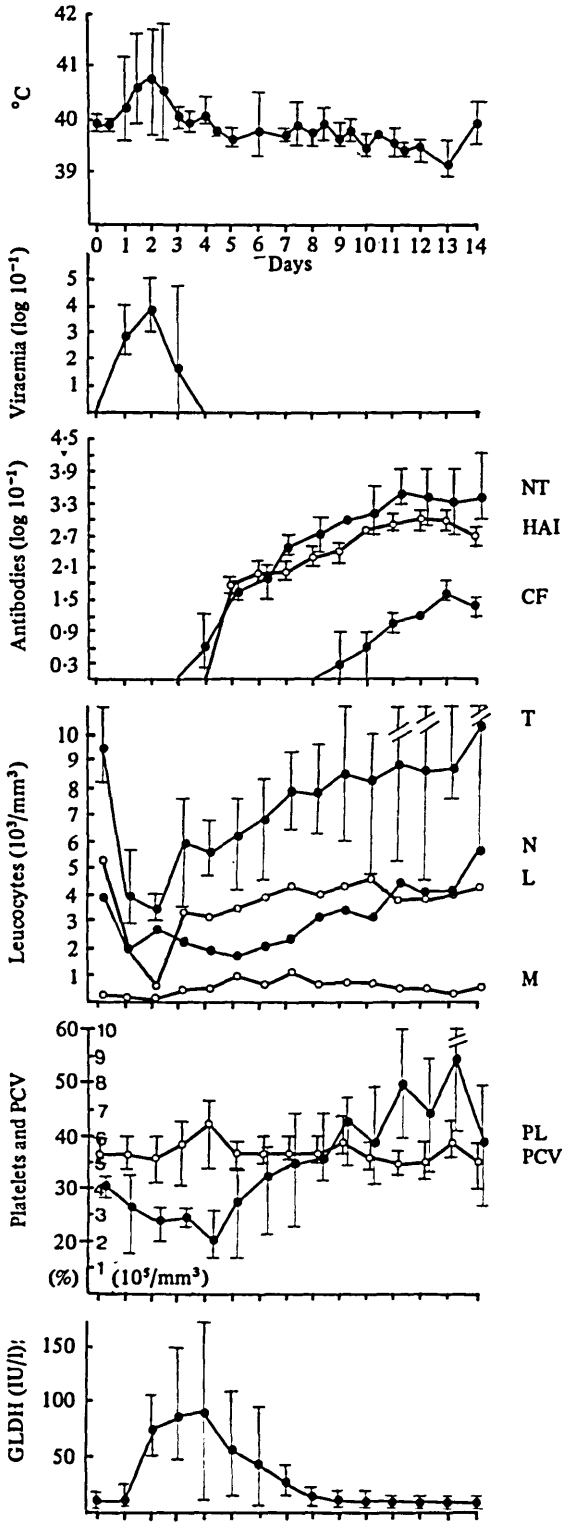

Fig. 1. Response of three sheep to infection with RVF virus. Antibody curves show geometric mean values and range. All other curves show mean values and range. T, total leucocytes; N, neutrophil leucocytes; L, lymphocytes; M, monocytes; PL, platelets; PCV, packed cell volume.

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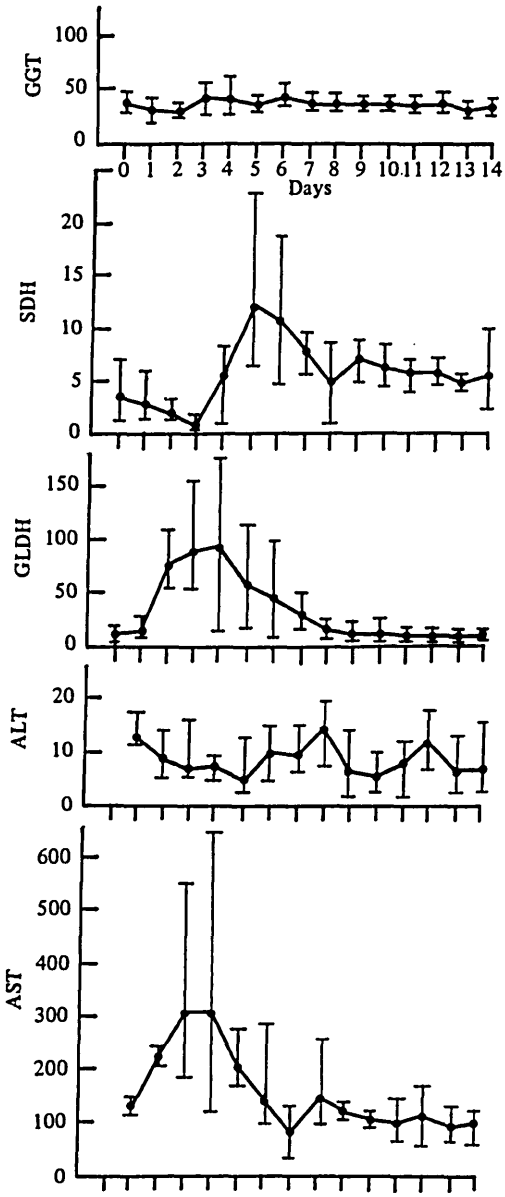

Fig. 2. Serum enzyme levels recorded in three sheep following infection with RVF virus. All values are International Units per litre. Curves show mean values and range.

with RVF virus (Fig. 1). Antibodies were demonstrable on day 5 by HAI test and on day 9 by CF test. The titres increased steadily over the next few days of observation.

By contrast with the RVF-infected sheep, the sheep infected with the other phleboviruses exhibited minimal or no signs of disease. The features of infection were all similar to those shown in Fig. 3 for the group of sheep infected with SAF virus. Only the three sheep which received AMT virus had significantly elevated

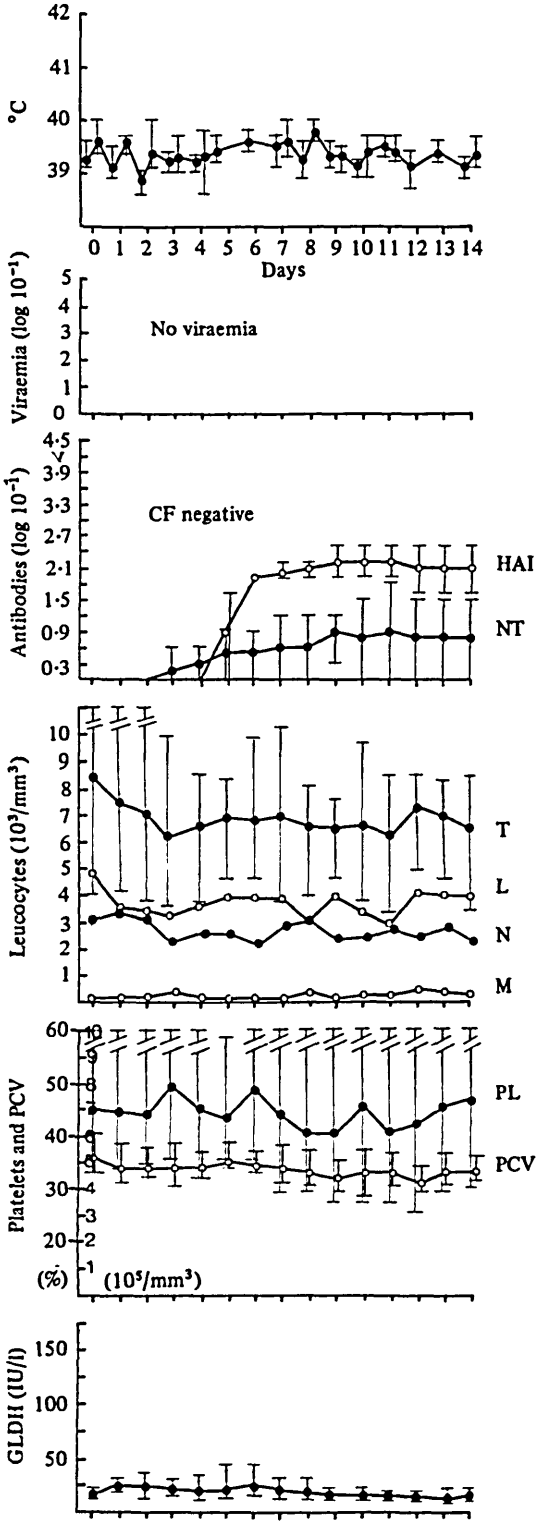

Fig. 3. Response of three sheep to infection with SAF virus. Legend as in Fig. 1.

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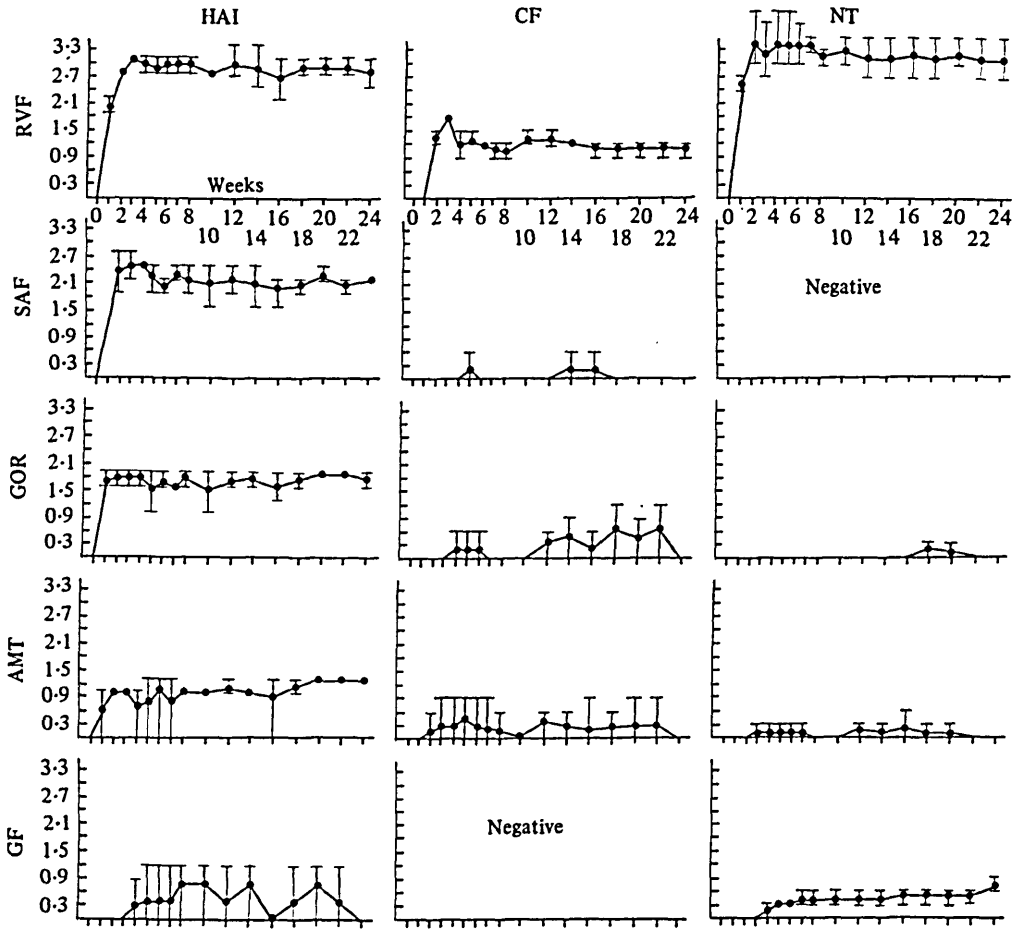

Fig. 4. Cross-reactivity for African phleboviruses of antibodies induced in three sheep by infection with RVF virus. Antibody titres are expressed as $\log_{10}^{-1}$ of serum dilution. Curves show geometric mean titres and range.

temperatures, and even these mild fevers lasted less than 24 h in individual sheep. None of the viruses induced demonstrable viraemia, leucopaenia or significantly raised GLDH values. A few sheep, particularly the two cross-bred Karakuls, had inherently high leucocyte counts, but this could not be associated with specific disease or internal parasite burdens. PCV values did not vary markedly and, while there was a tendency for mean platelet counts to fluctuate at high levels in all groups of sheep, there was also a tendency for individual sheep to have consistently high or consistently low platelet counts.

Homologous antibody responses in all other groups were weaker than in the RVF-infected sheep. CF titres were not demonstrable in the groups of sheep infected with SAF, GOR and AMT viruses while HAI titres were not demonstrable in the GF-infected group during the first 2 weeks of observation.

In all the antibody cross-reactions between phleboviruses recorded with sera from the various groups of sheep over the full 24 weeks of observation, homologous

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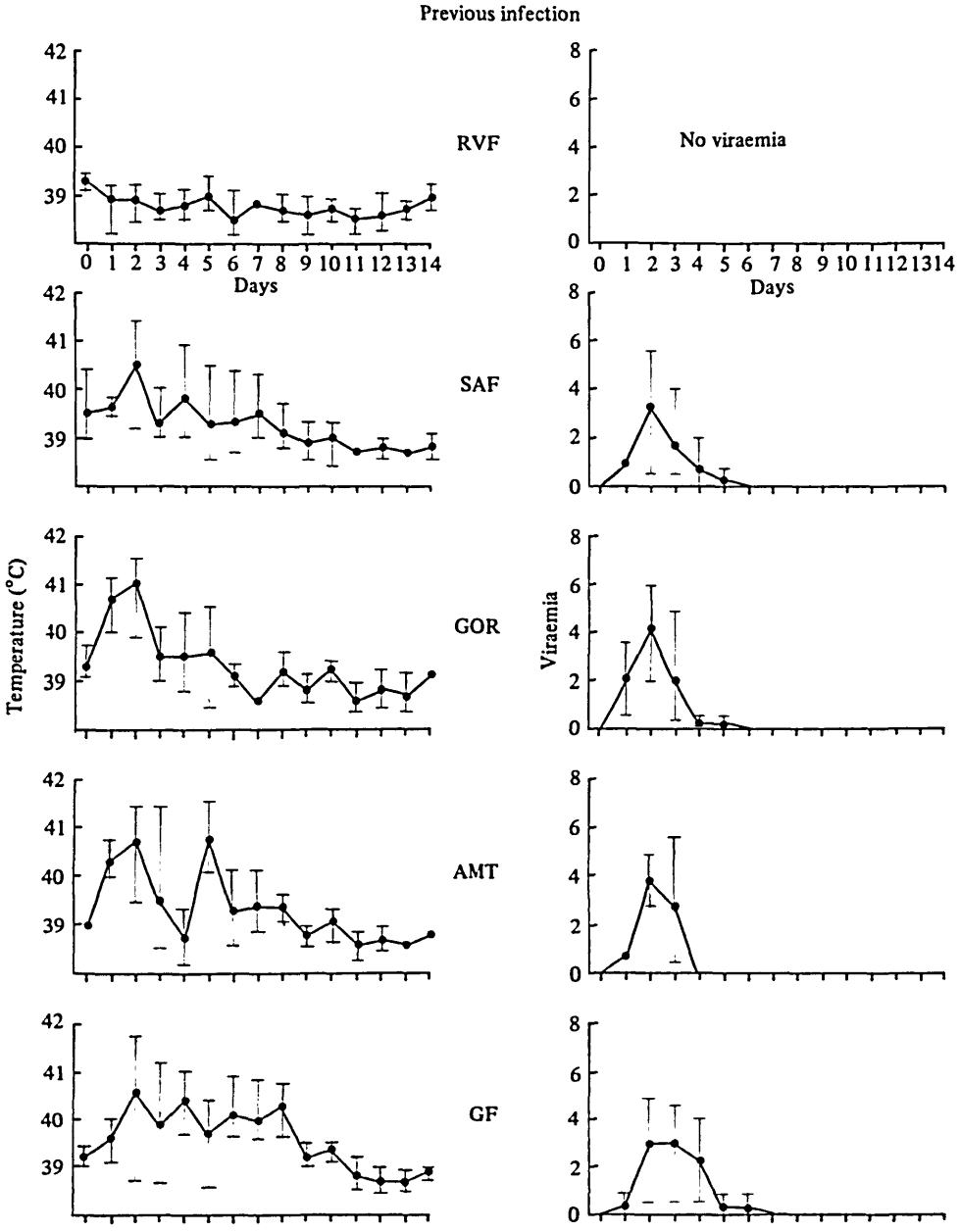

Fig. 5. Temperature and viraemia curves recorded in groups of three sheep following challenge with RVF virus. The groups had been infected 48 weeks previously with African phleboviruses as indicated. Curves show mean values and range.

antibody titres greatly exceeded heterologous titres. Nevertheless, infection with RVF virus induced moderate to high HAI antibody responses to the other phleboviruses (Fig. 4). Antibodies from the RVF-infected sheep were less cross-reactive in the CF test, and heterologous titres were very low in the CPENT test. Infection of sheep with phleboviruses other than RVF induced poor to moderate

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Table 2. *RVF log_{10} neutralizing indices (NI) and HAI titres of sheep before and 14 days after challenge with RVF virus. The sheep had previously been infected with phleboviruses as indicated.*

Sheep number	Initial infection	Pre-RVF virus challenge		Post-RVF virus challenge	
		RVF NI	RVF HAI titre	RVF NI	RVF HAI titre
1	RVF	1·9	320	2·3	640
2	RVF	2·1	540	3·5	1280
3	RVF	1·8	640	1·6	1280
4	SAF	0·0	—	2·0	5120
5	SAF	0·8	—	2·0	2560
6	SAF	0·0	—	2·2	1280
7	GOR	0·0	—	1·3	5120
8	GOR	0·0	—	1·3	5120
9	GOR	0·0	—	0·9	5120
10	AMT	0·9	—	1·8	640
11	AMT	0·0	—	1·3	320
12	AMT	0·0	—	1·7	2560
13	GF	0·2	—	1·5	10240
14	GF	0·2	—	2·3	5120
15	GF	0·0	—	1·0	2560

homologous antibody responses and little cross-reactivity. Heterologous antibody reactivity for RVF virus or antigen was particularly weak and transient in all groups. No cross-reaction was evident in HAI tests with RVF antigen. A GF-infected sheep had a CF titre of 8 with RVF antigen on a single occasion and two serum samples, from sheep infected with SAF and AMT viruses, had CPENT titres of 16 and 8 with RVF virus. Otherwise the few sera which cross-reacted in CF and CPENT tests with RVF virus or antigen all had titres of less than 8, which could be disregarded as non-significant in routine diagnostic tests.

The results obtained in challenging the five groups of sheep with RVF virus, 48 weeks after they were initially infected with one or other of the phleboviruses, are presented graphically in Fig. 5. The sheep which had initially been infected with RVF virus were immune, and failed to develop fever or demonstrable viraemia. The other groups were all susceptible to RVF and developed fever and viraemia. The RVF neutralizing indices and HAI titres of the sheep on the day of challenge and 14 days later are presented in Table 2. There was moderate boosting of titres in the RVF-immune group and seroconversion in the other groups.

DISCUSSION

Despite the occurrence of minor differences between strains of RVF, as demonstrated by sophisticated techniques such as oligoncleotide mapping (Cash *et al.* 1981), the antigenic structure of the virus appears to have remained remarkably stable over the years since it was first isolated. Even Zinga virus from West Africa, only recently discovered to be RVF (Meegan *et al.* 1983), does not

seem to differ antigenically from other strains. The only exception is LUN virus from Uganda. This virus initially had low pathogenicity for mice, failed to yield haemagglutinin and was difficult to neutralize with RVF antiserum, but after it had been subjected to further passaging in mice its virulence was increased and it could be neutralized (Weinbren, Williams & Haddow, 1957).

Tomori (1979*a*, *b*) concluded that LUN differed from an attenuated, neuro-adapted strain (Smithburn, 1949) and a Nigerian strain of RVF. Cross-neutralization occurred between all three strains when hyperimmune mouse ascitic fluids were used, but reciprocal cross-neutralization did not occur with the sera of sheep which recovered from infection with the three viruses. Tomori found that LUN failed to yield haemagglutinin and produced a single line of precipitation in ID tests, as opposed to two lines produced by the other two viruses. Peters & Anderson (1981) demonstrated that LUN and an Egyptian strain of RVF were identical in cross-plaque reduction neutralization (PRNT) tests and that each strain produced three precipitation lines of identity in cross-ID tests, but they did not specify the type of antisera used in their tests. We experienced no difficulty in preparing haemagglutinin from LUN virus and obtained reactions of identity between LUN and RVF 1678/78 in HAI, CF, ID and neutralization tests with mouse reagents. We demonstrated previously that the occurrence of multiple lines of precipitation in ID tests corresponds to the appearance in antiserum of antibodies directed against different viral proteins, and this varies with the stage of the immune response (Swanepoel *et al.* 1986). It can be concluded that the demonstration of differences between LUN and other strains of RVF is largely a function of the potency of the reagents used, but that valid quantitative differences have been demonstrated in neutralization tests on occasion.

The existing information on antigenic relationships between RVF and the other phleboviruses was obtained by performing homologous and heterologous HAI, IF, CF and PRNT tests with potent RVF convalescent sera and reference antisera prepared in laboratory animals (Shope, Peters & Walker, 1980; Shope *et al.* 1981; Tesh, Peters & Meegan, 1982; Travassos Da Rosa *et al.* 1983). These published relationships between RVF and the other African phleboviruses are listed in Tables 3 and 4. Although the RVF reagents were not tested by all the study methods against each African phlebovirus, it is clear that marked cross-reaction occurred in HAI and IF tests and that little cross-reaction occurred in CF and PRNT tests. Since most of the antisera used in these taxonomic studies were prepared by repeated immunization of laboratory animals, it is difficult to draw conclusions concerning the extent to which natural infection with the African phleboviruses would induce cross-reactive antibodies.

For the same reason, the tests performed with mouse reagents in the present study have greater relevance to virological than to serological diagnosis of RVF. Mouse inoculation is a standard method for isolating arthropod-borne viruses (Shope & Sather, 1979) and it is used extensively for RVF (Swanepoel, 1981). CF, ID and neutralization tests utilizing intracerebral inoculation of mice are commonly used for identifying arthropod-borne viruses isolated in mice (Shope & Sather, 1979), and the present findings confirm the usefulness of these techniques for separating RVF from the other African phleboviruses. The broad cross-reactivity encountered in the HAI test renders the technique useful for identifying RVF

Table 3. *Summary of existing information on reactions between RVF virus or antigen and antibodies to African phleboviruses*

	Antibody					
Test	RVF	SAF	GOR	AMT	GF	Extracted from
HAI	> 640	ND*	160	ND	ND	Shope, Peters & Walker, 1980
IF	2048	< 8	32	8	< 8	Tesh, Peters & Meegan, 1982
PRNT	10240	ND	80	ND	ND	Shope, Peters & Walker, 1980
PRNT	5120	< 10	< 10	< 10	< 10	Tesh, Peters & Meegan, 1982
CF	32	ND	< 4	ND	ND	Shope, Peters & Walker, 1980
CF	128	< 4	8	< 4	< 4	Travassos da Rosa *et al.* 1983

* ND, test not done.

Table 4. *Summary of existing information on reactions between RVF antiserum and African phleboviruses or antigen preparations*

	Virus or antigen					
Test	RVF	SAF	GOR	AMT	GF	Extracted from
HAI	> 640	320	ND*	ND	ND	Shope, Peters & Walker, 1980
IF†	ND	32	ND	< 4	ND	Shope *et al.* 1981
IF†	> 512	64	ND	8	ND	Shope *et al.* 1981
IF	2048	32	< 4	< 4	32	Tesh, Peters & Meegan, 1982
PRNT	5120	< 10	< 10	< 10	< 10	Tesh, Peters & Meegan, 1982
CF	32	ND	< 4	ND	ND	Shope, Peters & Walker, 1980
CF	32	ND	< 4	< 4	< 4	Shope, *et al.* 1981

* ND, test not done.
† Two different RVF antisera were used in IF tests by the same authors.

virus isolated in circumstances where the disease is not suspected. An unidentified virus which has a haemagglutinin can be screened against pools of group-reactive antisera in HAI tests and the final identification of the virus within a serogroup can then be made by ID or neutralization, which give monospecific reactions (Shope & Sather, 1979). In practice, unidentified viruses are more commonly screened by CF against pools of reference antisera.

GOR and SAF were the only two phleboviruses to cross-react in the CF and ID tests performed with mouse reagents (Tables 2 and 3). These two viruses were isolated from the same individual wild gerbil in the Central African Republic (Digoutte, 1975), and it is possible that genetic reassortment occurred. Members of the family *Bunyaviridae* have three-segmented RNA genomes which are capable of reassortment (Gentsch & Bishop, 1976) and there is evidence that this occurs in nature (Klimas *et al.* 1981; Ushijima, Clerx-Van Haaster & Bishop, 1981), although this may not be true of the genus *Phlebovirus* (Bishop, 1985). Nevertheless, the relationship between GOR and SAF viruses would seem to merit more detailed investigation.

In the pathogenicity experiments with phleboviruses in sheep we were aware that RVF virus itself, despite its virulence in natural outbreaks, frequently fails to induce overt disease following peripheral administration in the laboratory. The task of evaluating the protective effect of vaccines is often complicated by the fact

that even unvaccinated control sheep develop what is apparently mild infection following challenge with ostensibly virulent RVF virus (unpublished records, Veterinary Research Institute, Onderstepoort). Hence serum enzyme and haematological values were monitored in addition to temperature and viraemia, to increase the sensitivity with which the pathogenic effects of virus infection could be detected. The sheep which were infected with RVF virus developed fever and viraemia, but manifested only transient depression and anorexia. Yet the marked changes which occurred in leucocyte, platelet and serum enzyme values (Figs. 1 and 2) suggested that the mild signs shown by the sheep did not reflect the severity of the underlying disease. This was confirmed by finding extensive necrotic lesions in the liver of the sheep which was killed for examination on day 4 following infection with RVF virus. It can be concluded that AST, SDH and GLDH are useful enzymes for demonstrating liver damage, which appears to be a regular feature of RVF infection in susceptible animals, but elevated AST values due to liver damage must be distinguished from a muscle-derived component (Duncan & Prasse, 1977). The findings confirm that the ALT test is of no value in herbivores (Duncan & Prasse, 1977), and the lack of change in GGT values indicates that the biliary tree is not primarily affected in RVF (Schmidt & Schmidt, 1976).

None of the African phleboviruses other than RVF appeared to induce disease in sheep. Apart from the mild fevers of less than 24 h duration recorded in the sheep inoculated with AMT virus, there was no clear indication that the viruses caused infection in sheep. Homologous antibody responses were weak by comparison with the homologous response in RVF-infected sheep and it is possible that in some instances the antigenic stimulus was provided solely by the inoculum, rather than by replication of virus. None of the other viruses induced in the sheep antibodies which cross-reacted with RVF antigen in HAI tests, and the few cross-reactions observed in CF and CPENT tests with RVF antigen or virus were so weak as to be negligible. It can be concluded that it is unlikely that the antibodies induced by other African phleboviruses would cause confusion in the diagnosis of RVF.

In contrast to the other phleboviruses, RVF induced antibodies in sheep which were widely cross-reactive in HAI tests, although reactions tended to be more specific in CF and CPENT tests (Fig. 4). At present, the cross-reactivity of the antibodies induced by RVF virus can be regarded as being of little practical consequence in sub-Saharan Africa. This would obviously change if another phlebovirus were to be found causing disease of man or livestock in the region.

Following the discovery that RVF is a phlebovirus, there was speculation that non-pathogenic members of the genus could be used to confer immunity to RVF, and it was shown that immunization with GOR and AMT viruses protected a proportion of mice against peripheral infection with RVF virus (Shope *et al.* 1981). The method used for immunizing mice was not specified, but we found that a single administration of any of the African phleboviruses failed to immunize sheep against infection with RVF virus (Fig. 5; Table 2). A further possibility was that genetic reassortment could be used to produce a hybrid vaccine virus from RVF and one of the other phleboviruses (Eddy *et al.* 1981). The lack of pathogenicity of the African phleboviruses for sheep, demonstrated in the present study, would render them suitable for use in producing such a vaccine virus. However, technical

difficulties may preclude production of a suitable hybrid virus (Eddy *et al.* 1981; Bishop, 1985).

RVF is mainly encountered as a pathogen of sheep, cattle and man. The present observations on the other African phleboviruses were confined to sheep, and extrapolations to cattle may not be valid. Nevertheless, the fact that the African phleboviruses lack pathogenicity for sheep suggests that they differ fundamentally from RVF virus. Moreover, extensive field studies on RVF in cattle (Davies, 1975; Swanepoel *et al.* 1975; Swanepoel, 1976 & 1981) failed to produce evidence which would suggest that the other phleboviruses cause infection or disease of cattle in Africa.

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