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An investigation of flavivirus infections of cattle in Zimbabwe Rhodesia with particular reference to Wesselsbron virus

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SUMMARY

A three-part epidemiological investigation was made on flaviviruses:

1. As a preliminary to tests on cattle sera from the field, the antigenic cross-reactivity of Wesselsbron, Spondweni, Usutu, Banzi, West Nile and yellow fever flaviviruses was studied in antisera prepared in guinea pigs. As described earlier for flaviviruses, sera were found to be highly cross-reactive in haemagglutination-inhibition (HAI) tests, less cross-reactive in complement-fixation (CF) tests and were virtually monospecific in microneutralization (NT) tests in Vero cell cultures.

2. Infection with Wesselsbron (WSL) virus produced mild febrile illness and viraemia in 5 out of 6 newborn calves, 3 out of 4 pregnant heifers and 3 out of 4 ewes. One heifer produced a weak calf which died soon after birth with WSL antibodies in its serum, indicating that infection had occurred in utero. The 3 other heifers produced healthy calves which lacked antibody in pre-colostral serum. Pathological changes occurred in the foetus in 2 out of 3 pregnant ewes and the ewe produced a healthy lamb which had antibodies to WSL virus in pre-colostral serum.

Unlike the situation in guinea pigs, cattle sera were monospecific for WSL virus in CF tests, but sheep sera cross-reacted with Banzi and yellow fever viruses. Re-infection of the cattle with Banzi, West Nile, Spondweni and Usutu viruses failed to induce marked antibody responses. The results suggest that antibodies to WSL virus in cattle sera from the field can be distinguished from those induced by other flaviviruses by quantitative serologial tests.

3. HAI antibodies to WSL virus were detected in 2648/14395 cattle sera tested over 11 years from 1967 to 1978 in the course of investigation of abortion, infertility and other diseases. Results of quantitative HAI, CF and NT tests with six flaviviruses on 409 selected sera confirmed that infection was due to WSL virus. Serological evidence failed to implicate WSL virus as a cause of abortion in cattle. In a prospective study, abortion occurred in only one out of 21 heifers observed to gain WSL infection during pregnancy in the field, but abortion also occurred in five out of 207 heifers which did not become infected with WSL. No histopathological lesions diagnostic of WSL disease were observed in 1998 specimens from cattle, sheep and goats examined over 44 months prior to October 1972, and WSL virus was isolated once, from the organs of a cow, out of 2106 specimens from cattle sheep and goats tested virologically over six years from October 1972

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to September 1978. HAI antibodies to WSL virus were detected in one out of 374 sera from aborted cattle foetuses. It was concluded that WSL virus is not an important cause of disease in cattle, despite widespread occurrence of infection.

INTRODUCTION

During investigation of abortion and infertility of cattle, it was found that haemagglutination–inhibition (HAI) antibodies to Wesselsbron (WSL) virus are common in the sera of cattle in Zimbabwe Rhodesia (Swanepoel *et al.* 1975; Swanepoel, 1976). Although the pathogenicity of the virus for sheep is well documented, evidence is lacking that it produces disease in cattle. In order to investigate the role played by the virus in the field, it is necessary to determine whether antibodies demonstrated in sera are due to infection with WSL virus or some other flavivirus. Members of the genus are grouped together on the basis of their cross-reactivity in the HAI test (Casals & Brown, 1954). Antisera are less cross-reactive in the complement-fixation (CF) test and show greatest specificity for individual viruses in the neutralization (NT) test, but cross-reactivity in all tests is greatly increased where consecutive infection occurs with two or more members of the group (Casals, 1957, 1961, 1963).

It is recommended that all of the known flaviviruses of a region should be included in tests intended to determine the occurrence of antibody to a member of the group (Theiler & Casals, 1958; Theiler & Downs, 1973). Primary infections are recognized by the sharply specific and relatively high titre of antibody detected, while secondary infections are marked by high heterologous antibody titres.

Five flaviviruses are known to be prevalent in southern Africa; WSL, Spondweni (SPO), Banzi (BAN) and Usutu (USU) were isolated initially in the region and the fifth, West Nile (WN), was described from Uganda originally but has also been isolated in southern Africa (Weiss, Haig & Alexander, 1956; Kokernot *et al.* 1957; Smithburn *et al.* 1959*b*; McIntosh, 1967; Kokernot & McIntosh, 1959). These viruses have been included in various studies of flaviviruses in the past (Casals, 1957, 1961, 1963; Theiler & Casals, 1958; Henderson *et al.* 1970; Theiler & Downs, 1973; De Madrid & Porterfield, 1974), but it was considered necessary to observe the cross-reactions which occur in HAI, CF and NT tests in antisera prepared in guinea pigs, to demonstrate whether antibodies to individual viruses could be distinguished in cattle sera from the field.

The need to perform NT tests in mice has always been a barrier to the conducting of quantitative rather than qualitative screening tests in surveys of antibodies to arthropod-borne viruses in the past, and the development of a convenient NT test in cell cultures was a further object of the preliminary study.

The experiments performed in the next stage of the investigation were intended to serve two purposes. The pathogenicity of WSL virus for cattle was tested by infecting six young calves and four pregnant heifers. Secondly, the cross-reactivity for flaviviruses of the antibodies produced in response to WSL infection was monitored to provide a basis for interpreting titres found in sera from the field. WSL infection was also studied in four ewes and limited observations were made on infections with flaviviruses other than WSL.

The third aspect of the investigation involved field studies on WSL infection in cattle in which: (*a*) quantitative serological tests were applied to sera from the field; (*b*) heifers in the field were monitored serologically to relate the occurrence of WSL infection to outcome of pregnancy; and (*c*) attempts were made to isolate WSL virus from pathological specimens from the field and to demonstrate antibodies in the sera of aborted foetuses.

MATERIALS AND METHODS

Viruses

The viruses obtained from the South African Institute for Medical Research, Johannesburg, comprised WSL strain H177, SPO strain AR94, BAN strain H336, USU strain AR1776 and WN strain H442. The type species of the flaviviruses, yellow fever (YF) virus, is not known to occur in southern Africa, but it was included in the study in the form of the 17D vaccine strain as an additional check on the specificity of antibodies to individual viruses. In pathogenicity tests, cattle and sheep were infected with the third mouse brain passage of WSL virus strain AR 11190 isolated from mosquitoes in Zimbabwe Rhodesia (McIntosh, 1972) to simulate natural infection.

Guinea Pig Antisera

The antisera were produced by intracerebral (i.c.) inoculation of guinea pigs with 0·1 ml of a 10% suspension of infected mouse brain followed by an intraperitoneal boost 14 days later in the instances of WSL, WN and BAN viruses, and by exsanguination on the twenty-first day. In the instances of YF, SPO and USU viruses, the booster dose was administered intracardially because it was found that intraperitoneal boosts produced inadquate antibody responses. Three guinea pigs were used per virus and their pooled sera were freeze dried in 0·5 ml volumes and stored at 4 °C.

Antigens

The antigens used in CF and HAI tests were produced by sucrose-acetone extraction of infected suckling mouse brain (Clarke & Casals, 1958).

HAI tests

Acetone-extraction of sera for removal of non-specific inhibitors of haemagglutinin, and performance of the HAI tests, followed the technique of Clarke & Casals (1958) except that HAI screening tests on field sera were performed by a microtechnique described elsewhere (Swanepoel *et al.* 1978).

CF tests

The CF tests were based on the procedure of Bradstreet & Taylor (1962) adapted to a microtechnique using 0·025 ml test volumes with Microtiter equipment (Cook Eng. Co., Alexandria, Va., U.S.A.). Antigens were used at optimal dilution and three lytic doses of complement were used. Sera were inactivated at 59 °C for 30 minutes and complement was fixed by overnight incubation at 4 °C. End-

points were recorded as the highest dilutions of serum producing complete fixation of complement.

NT tests

Ten per cent suspensions of infected mouse brain were used to infect Vero cell cultures (Yasamura & Kawakita, 1963). The cells were disrupted ultrasonically when approximately 75% showed cytopathic effect and the culture fluids were clarified by centrifugation for 5 minutes at 800 *g* and 5 °C. Dimethyl sulfoxide was added to produce a concentration of 10% and the culture fluids were stored in small volumes at −20 °C as a source of virus for NT tests. SPO virus was subcultured thrice in Vero cells before it produced cytopathic effect in the 3 to 4 days taken by other viruses. Sera were inactivated for 30 min at 59 °C and doubling dilutions from 1/4 to 1/4096 were prepared in 0·1 ml volumes of medium in Microtitre culture plates. Equal volumes of virus suspension containing a calculated 100 TCID50 of virus were added to each well and the serum–virus mixtures were incubated for 30 min at 22 °C before seeding with 4×10^4 Vero cells in 0·025 ml of medium (Eagle, 1959) plus 10% tryptose phosphate broth, 7·5% fetal calf serum, 400 i.u. per ml penicillin and 200 μg per ml streptomycin. The tests were read after 6 days incubation at 36 °C. Viruses were titrated in quadruplicate and end-points were estimated by the method of Kärber (1931). Sera were tested in duplicate and end-points were recorded as the highest dilution at which neutralization occurred in both replicates.

Immunoglobulin determinations

Quantitative tests for IgM and IgG in sera and colostral whey samples were based on the radial diffusion technique of Mancini, Carbonara & Heremans (1965). Concentrations of IgG and IgM in test samples were read in mg/100 ml against diameter of precipitation on standard curves.

Pathogenicity tests

Four 20-month-old Friesian heifers in their fourth to seventh month of pregnancy, six Friesian calves three to seven days old and four Dorper ewes aged two to three years, three of which were in mid to late pregnancy, were infected with $10^{4 \cdot 7}$ mouse i.c. LD50 of WSL virus strain AR 11190. Calves were infected subcutaneously (s.c.) and heifers and ewes intravenously. The calves had been left with their mothers for the first 24 hours of life to suck colostrum and were fed milk from buckets thereafter. All animals lacked NT antibody to WSL virus immediately before the experiment.

Temperatures and clinical features were monitored from the day before experiment to day 14 after infection. Viraemia was tested by inoculating 0·02 ml volumes of 10-fold dilutions of blood i.c. in litters of two- to six-day-old mice.

At calving and lambing, new born animals were bled before feeding and at intervals after the first day. A sample of colostrum was collected from each quarter of the mother's udder, pooled and treated with rennin to obtain whey. A calf which died shortly after birth and a lamb killed *in extremis* at birth, were examined

post mortem. Specimens were taken for virological, serological and histological examination. Two ewes which failed to lamb were killed for examination of the genital tract when the sheep experiment was terminated eight weeks after infection.

All serum and whey samples were stored at $-20°$ and tested together for HAI, CF and NT antibodies to the full range of flaviviruses.

Twenty-five months after the initial experiments, two of the original calves, one of the heifers and two susceptible three-year-old indigenous Mashona cows were inoculated s.c. with $10^{6\cdot1}$ mouse i.c. LD50 of BAN virus. Viraemia and temperature were monitored for 14 days and the animals were bled six weeks after inoculation for serological tests. Eight weeks after the second inoculation one of the original calves received $10^{5\cdot6}$ mouse i.c. LD50 of USU virus s.c., the other calf received $10^{4\cdot4}$ mouse i.c. LD50 of WN virus s.c. and the heifer received $10^{4\cdot6}$ mouse i.c. LD50 of SPO virus s.c. Viraemia and temperature were monitored for 14 days and the animals were bled six weeks after the third inoculation for serological tests.

Antibody tests on field sera

Batches of cattle sera submitted to the laboratory from April 1967 to September 1972 in connection with surveys of Rift Valley fever (RVF) and certain other diseases were tested for HAI antibodies to WSL virus. From 1 October 1972 onwards the HAI tests was applied routinely to a proportion of the cattle sera submitted to the laboratory for investigation of abortion, infertility and other disease, or for tests in connection with sale or export. Sheep and goat sera were also tested, as were sera collected for survey purposes from game animals, mainly during culling operations.

A proportion of the cattle sera submitted during 1974 was selected for quantitative HAI, CF and NT tests against the full range of flaviviruses: 207 WSL HAI-positive sera were selected from 73 herds in 32 localities of Zimbabwe Rhodesia and 112 HAI-negative sera were selected from eight of the same herds. A further 90 sera were selected from eight HAI-negative herds. Selection of the sera was governed by the intention of: (*a*) obtaining geographic cover of Zimbabwe Rhodesia and (*b*) testing both WSL HAI-positive and HAI-negative sera. In the instance of WSL HAI-positive sera it was considered sufficient to test one or two sera per herd merely to confirm that infection had been due to WSL and not another flavivirus. In the instance of WSL HAI-negative sera from herds in which HAI-positive sera had been detected, the intention was to test several sera per herd in order to guage the full extent of WSL infection in these herds. Likewise, several sera per herd were tested from herds where no WSL HAI-positive sera had been found, to determine whether or not, and to what extent, WSL infection had taken place in such herds.

Sero conversion study of pregnant heifers in the field

Pregnant heifers in five herds were bled shortly after the breeding season early in 1976, when pregnancy was confirmed by rectal examination. They were bled subsequently at approximately three monthly intervals over seven to nine months

Table 1. *HAI cross-reactions in sera of guinea-pigs infected with the known flavivirus of southern Africa plus YF virus. Titres are shown as reciprocals of serum dilution*

Serum from guinea pigs infected with	Antigens					
	WSL	WN	BAN	USU	SPO	YF
WSL	**320**	40	40	320	80	40
WN	80	**160**	40	320	40	40
BAN	160	80	**320**	640	40	80
USU	40	80	10	**160**	20	20
SPO	20	10	10	20	**160**	20
YF	20	—	—	10	—	**160**

until calving had taken place, and the sera were screened for NT antibody to WSL virus. Outcome of pregnancy was recorded.

Virological examination of field specimens

Before October 1972 pathological specimens submitted to the laboratory were usually examined bacteriologically and sometimes histologically, but from the start of a RVF epizootic in February 1969 onwards, it became routine to examine specimens from cattle, sheep and goats, including aborted fetuses, histologically as well as by other methods. From 1 October 1972, pathological specimens submitted to the laboratory for investigation of abortion or other disease in cattle, sheep and goats, were also tested routinely for presence of WSL and other arthropod-borne viruses. Approximately 10% suspensions of pooled organs, usually brain, spleen, liver, lung and kidney, and also placenta when available, were prepared by homogenizing the tissues in sucrose-phosphate buffer, pH 7·2, and centrifuging at 300 *g* and 4 °C for 20 min. The supernatant was inoculated i.c. in litters of one- to six-day-old mice which were observed for 14 to 21 days. Specimens were also routinely inoculated into calf testis cell cultures. Serum from heart-blood of aborted cattle fetuses was tested for presence of immunoglobulins as described above and screened for HAI antibody to WSL virus.

RESULTS

Cross-reactivity of flaviviruses

The results of the guinea-pig study are presented in Tables 1, 2 and 3. The titres of virus obtained in cell culture ranged from $10^{5\cdot5}$ infective doses (TCID50) per 0·1 ml and the actual dose used in NT tests with each virus ranged from 30 to 300 TCID50.

Pathogenicity tests

Combined curves for temperature, viraemia and WSL CF, HAI and NT antibody responses in the groups of calves, heifers and ewes following WSL infection, are presented in Figs. 1, 2 and 3. Most animals developed moderate fever and a mild degree of listlessness, hyperpnoea and inappetance. Viraemia titres are shown in Tables 4, 5 and 6. One each of the calves, heifers and ewes failed to

Table 2. *CF cross-reactions in sera of guinea-pigs infected with known flaviviruses of southern Africa plus YF virus. Titres are shown as reciprocals of serum dilution*

Serum from guinea-pigs infected with	Antigens					
	WSL	WN	BAN	USU	SPO	YF
WSL	**128**	32	8	16	16	16
WN	16	**128**	4	8	8	8
BAN	8	4	**64**	4	4	T*
USU	—	16	—	**32**	T	T
SPO	—	—	—	—	**32**	—
YF	4	4	4	**16**	T	**128**

* T = trace reaction

Table 3. *NT cross-reactions in sera of guinea-pigs infected with the known flaviviruses of southern Africa plus YF virus. Titres are shown as reciprocals of serum dilution*

Serum from guinea-pigs infected with	Viruses					
	WSL	WN	BAN	USU	SPO	YF
WSL	**1024**	—	—	—	—	—
WN	—	**1024**	—	—	—	—
BAN	—	—	**32**	—	—	—
USU	—	8	—	**16**	—	—
SPO	—	—	—	—	**32**	—
YF	—	—	—	—	—	**256**

exhibit fever, viraemia or illness. Antibody responses were weakest and attainment of maximum titres was delayed in the animals which failed to exhibit viraemia. Of these animals, calf 6 failed to develop demonstrable HAI antibodies and heifer 9 failed to develop CF antibodies. One year after infection HAI titres in three calves remaining on experiment were 1/40, 1/20 and 1/20 while NT titres were 1/64, 1/128 and 1/32.

Heifers 9, 11 and 12 gave birth to normal full-term calves which lacked antibodies to WSL or other flaviviruses at birth, but which gained antibodies from colostrum (Table 7). The calf of heifer 11 was prevented from sucking colostrum for eight hours after birth and consequently showed deficient uptake of colostral antibodies into its circulation. Heterologous antibody activity waned rapidly in the calves but WSL titres persisted for up to 16 weeks (Table 8).

Heifer 22, five and a half months pregnant at infection, produced a weak calf at full-time which died 4 h after birth. The calf failed to feed and the presence of WSL HAI and NT antibodies in its serum indicates that it was infected *in utero*. The calf had congestion, oedema and haemorrhages of the meninges, brain, larynx, trachea, lungs, myocardium, spleen and mucosa of the small intestine. There was proliferation of bile ducts and fibrous tissue in the portal tracts with radiation into the lobules of the liver, and moderate haemosiderosis. No virus or bacterium could be isolated from lung, liver, spleen, kidney, or brain. The findings suggest that the immediate cause of death was circulatory failure.

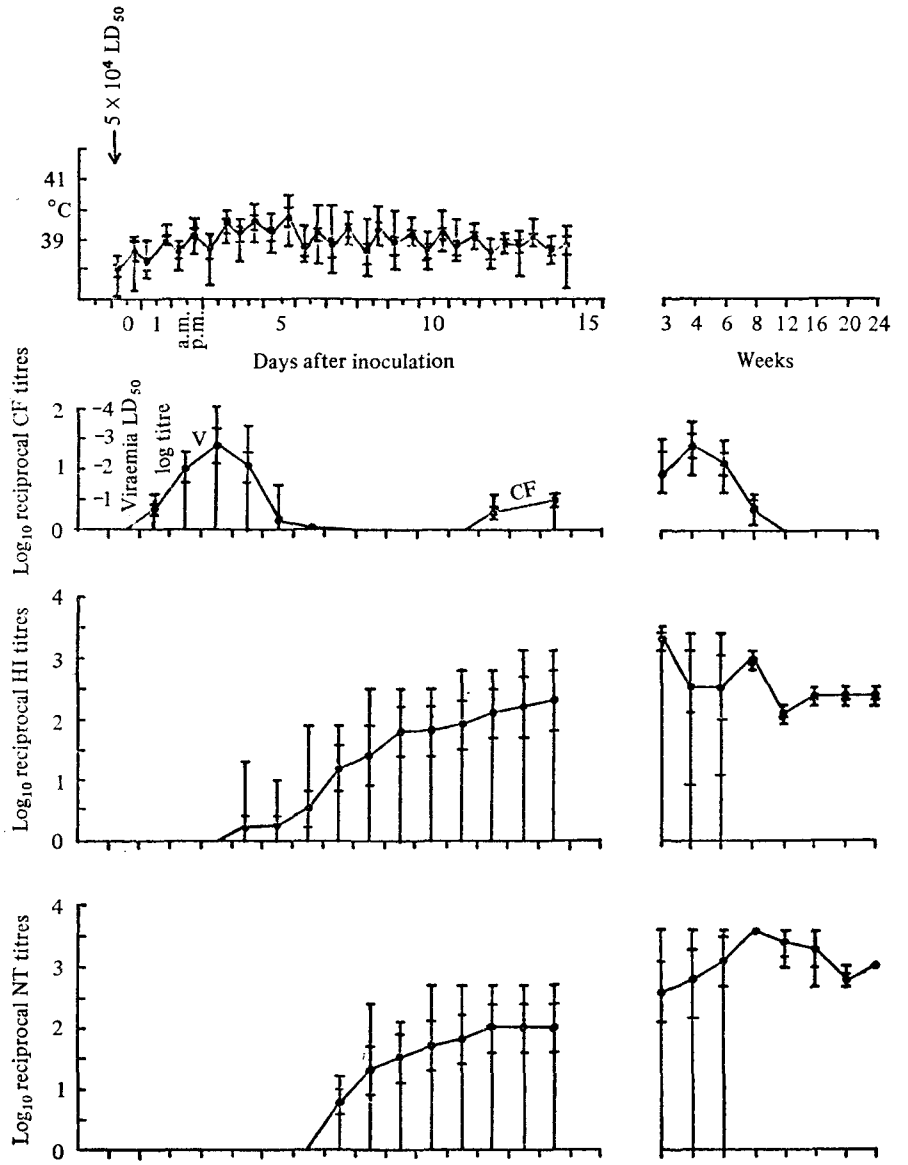

Fig. 1. Response of six calves to infection with WSL virus.

Ewe 930 produced twin lambs at full term eight weeks after infection. One was healthy but the other was moribund and was killed soon after birth. This lamb showed marked atrophy of musculature and arthrogryposis. No virus or bacterium could be isolated and the only antibody activity in the serum of the killed lamb was a minimal WSL HAI titre of 1/10. Its twin lacked antibody and acquired colostral immunity (Table 9). Ewe 983 produced a healthy lamb eight weeks after infection but had septic mastitis and had to be killed. It did not feed the lamb and presence of antibody activity in the serum of the lamb indicates that infection occurred *in utero* (Table 9). Ewe 984 was confirmed to be non-pregnant when

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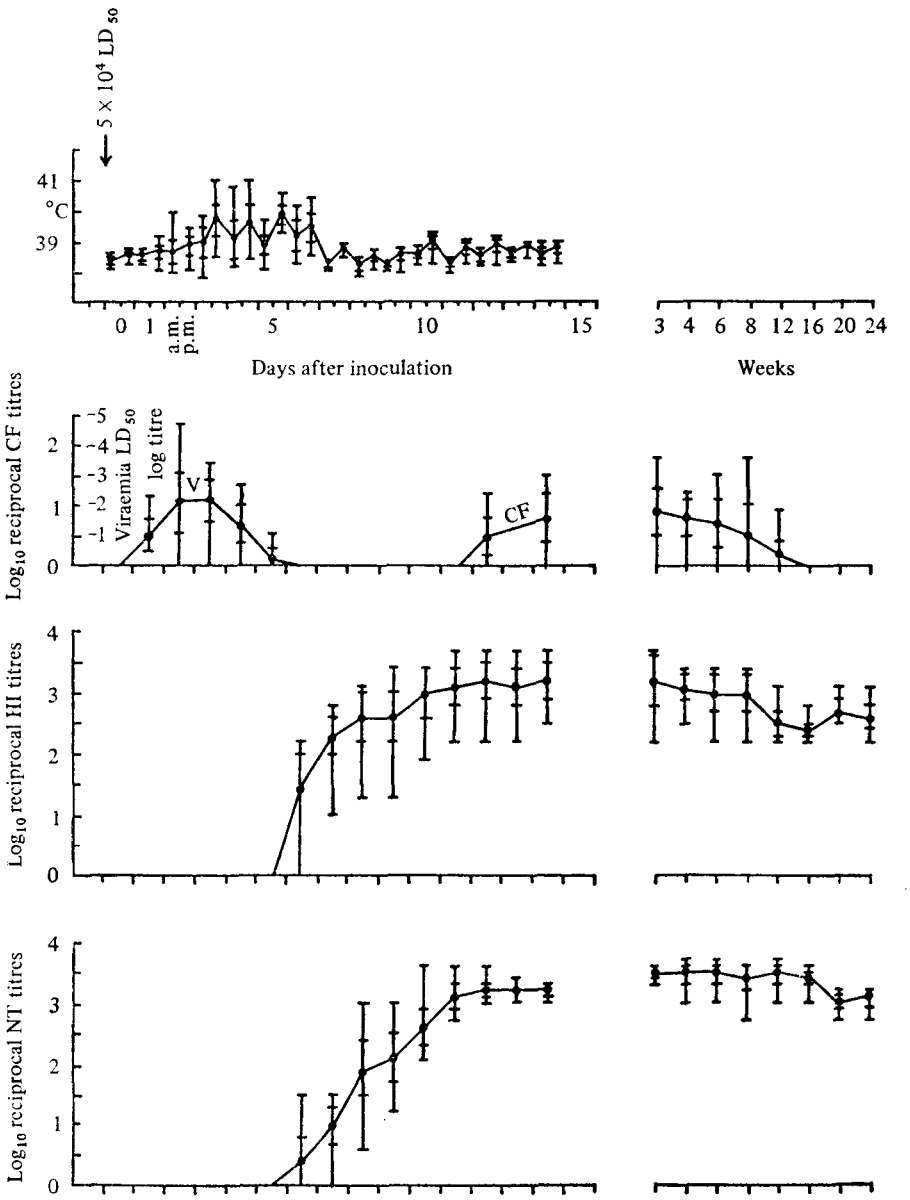

Fig. 2. Response of four heifers to infection with WSL virus.

slaughtered eight weeks after infection, and ewe 988 was found to contain a mummified fetus which was too autolysed for histological or microbiological examination. From its crown-rump length, 10 cm, the fetus can be estimated to have been about 57 days old (Joubert, 1956) and from the date of breeding of the ewe, it can be estimated that fetal death occurred within a week of infection of the ewe. Thus, in three pregnant ewes infected with WSL virus, pathological changes in the fetus occurred in two and fetal infection without apparent ill effect occurred in the third.

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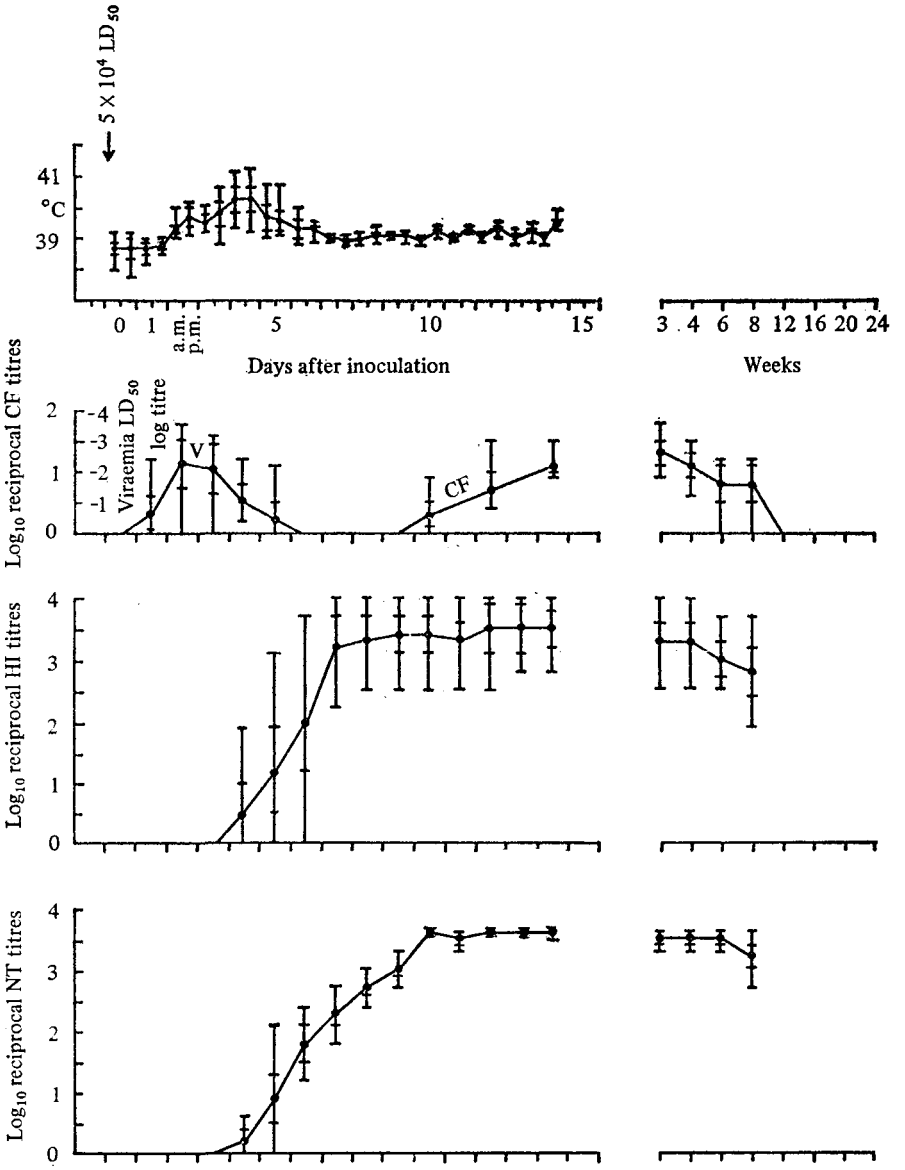

Fig. 3. Response of four ewes to infection with WSL virus.

Most calves, heifers and ewes showed slight transient leucopaenia, mainly neutropaenia, around days three to five of infection. There were no marked changes in packed cell volumes, prothrombin times, alanine transaminase (GPT) and aspartate transaminase (GOT) levels in serum.

Cross-reactivity for flaviviruses during WSL antibody response

Combined curves showing the cross-reactivity for flaviviruses of the antibodies produced in response to WSL infection in calves, heifers and ewes, are presented in Figures 4, 5 and 6. Heterologous antibody activity was lower-titred and tended

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Table 4. *Viraemia in six calves following infection with WSL virus*

Calf number	Log $_{10}$ mouse ic LD50 per 0·02 ml blood (day) Day ... 1	2	3	4	5	6	7 to 14
1	1·1	3·6	4·0	2·8	—	—	—
2	1·2	2·6	4·0	2·4	—	—	—
3	0·6	2·5	3·1	1·2	—	—	—
4	1·1	1·6	3·3	3·5	0·3	0·1	—
5	0·5	2·2	2·3	2·5	1·5	—	—
6	—	—	—	—	—	—	—
Mean	0·75	2·1	2·8	2·1	0·3	0·02	—
S.E.	±0·2	±0·5	±0·6	±0·5	±0·2	±0·02	

Table 5. *Viraemia in four pregnant heifers following infection with WSL virus*

Heifer number	Log_{10} mouse ic LD50 per 0·02 ml blood. Day ... 1	2	3	4	5	6 to 14
9	—	—	—	—	—	—
11	0·3	2·3	2·8	2·7	1·1	—
12	1·5	1·3	2·2	1·4	—	—
22	2·3	4·8	3·4	1·3	—	—
Mean	1·0	2·1	2·1	1·4	0·3	—
S.E.	±0·5	±1·0	±0·7	±0·6	±0·3	

Table 6. *Viraemia in four ewes following infection with WSL virus*

Ewe number	Log_{10} mouse ic LD50 per 0·02 ml blood. Day ... 1	2	3	4	5	6 to 14
930	—	—	—	—	—	—
983	0·1	3·6	3·2	2·4	2·1	—
984	—	2·0	1·8	1·7	—	—
988	2·4	3·4	3·2	—	—	—
Mean	0·6	2·3	2·1	1·0	0·5	—
S.E.	±0·6	±0·8	±0·8	±0·6	±0·5	

to be more transient than homologous WSL antibody activity. Homologous WSI HAI titres were at least 16-fold greater than heterolgous titres during the first eight weeks following infection and homologous NT titres were at least 128-fold greater than heterologous titres, but differences between homologous and heterologous titres decreased somewhat as antibody levels declined. HAI antibodies were most broadly cross-reactive with heterologous flaviviruses and CF antibody response were more specific than NT responses, with only the sheep exhibiting heterologous CF activity. The highest degree of cross-reaction occurred with YF and BAN viruses in all three groups of animals, but the sera of calves showed less cross-reactivity than did those of heifers and ewes.

Table 7. *IgM, IgG and antibody titres in serum and colostrum of heifers on day of calving, and in serum of calves prior to sucking colostrum on the following day. Titres are expressed as reciprocals of serum dilution*

Sample	mg % IgM	mg % IgG	HAI titres						NT titres					
			WSL	WN	BAN	USU	SPO	YF	WSL	WN	BAN	USU	SPO	YF
Heifer 9														
Serum	360	2200	1280	40	80	80	—	—	256	—	16	—	—	8
Colostrum	1300	12000	10240	160	80	80	40	40	1024	—	32	—	—	16
Calf Serum														
Pre-colostral	70	400	—	—	—	—	—	—	—	—	—	—	—	—
Day 1	170	1000	1280	40	80	40	20	20	1024	—	—	—	—	—
Heifer 11														
Serum	520	1500	640	—	20	—	—	—	2048	—	8	—	—	4
Colostrum	950	6000	2560	—	20	—	—	—	4096	—	32	—	—	8
Calf serum														
Pre-colostral	40	150	—	—	—	—	—	—	—	—	—	—	—	—
Day 1*	190	1500	20	—	—	—	—	—	32	—	—	—	—	—
Heifer 12														
Serum	400	2800	2560	80	40	40	20	40	4096	—	8	—	—	4
Colostrum	540	4600	1280	40	40	80	20	20	4096	—	16	—	—	4
Calf serum														
Pre-colostral	22	10	—	—	—	—	—	—	—	—	—	—	—	—
Day 1	400	2000	320	—	20	20	—	—	1024	—	4	—	—	—
Heifer 22														
Serum	225	2000	320	—	20	—	—	10	4096	—	8	—	—	4
Calf serum														
Pre-colostral	70	170	1280	80	320	80	80	40	8192	—	32	—	—	16

* Calf of heifer 11 prevented from sucking colostrum for 8 h after birth.

Table 8. *The persistence of colostrally acquired antibodies in three calves from heifers infected with WSL virus. Titres are expressed as reciprocals of serum dilution*

		HAI titres						NT titres					
Calf	Week	WSL	WN	BAN	USU	SPO	YF	WSL	WN	BAN	USU	SPO	YF
Calf of heifer 9	1	640	20	40	40	—	—	256	—	—	—	—	—
	2	320	20	40	40	—	—	256	—	—	—	—	—
	6	80	10	20	10	—	—	64	—	—	—	—	—
	8	80	—	—	—	—	—	32	—	—	—	—	—
Calf of heifer 11	1	40	—	—	—	—	—	64	—	—	—	—	—
	2	40	—	—	—	—	—	16	—	—	—	—	—
	6	20	—	—	—	—	—	16	—	—	—	—	—
	8	20	—	—	—	—	—	4	—	—	—	—	—
Calf of heifer 12	1	320	—	20	20	—	—	1024	—	4	—	—	—
	2	80	—	20	10	—	—	512	—	—	—	—	—
	4	80	—	—	—	—	—	256	—	—	—	—	—
	6	40	—	—	—	—	—	128	—	—	—	—	—
	8	40	—	—	—	—	—	64	—	—	—	—	—
	12	20	—	—	—	—	—	32	—	—	—	—	—
	16	10	—	—	—	—	—	4	—	—	—	—	—
	20	—	—	—	—	—	—	—	—	—	—	—	—

Table 9. *Antibody titres in serum and colostrum of ewes on day of lambing, and in serum of lambs prior to sucking colostrum and subsequently. Titres are expressed as reciprocals of serum dilution*

	HAI titres						NT titres					
Sample	WSL	WN	BAN	USU	SPO	YF	WSL	WN	BAN	USU	SPO	YF
Sheep 930												
Serum	1280	80	160	40	—	80	512	—	16	—	—	—
Colostrum	1280	80	320	160	—	80	512	—	4	—	—	4
Lamb 1												
Pre-colostral	10	—	—	—	—	—	—	—	—	—	—	—
Lamb 2												
Pre-colostral	—	—	—	—	—	—	—	—	—	—	—	—
Day 1	40	20	40	—	—	—	32	—	8	—	—	8
Lamb of 983 – 6 weeks	320	20	80	20	20	20	64	—	16	—	—	—

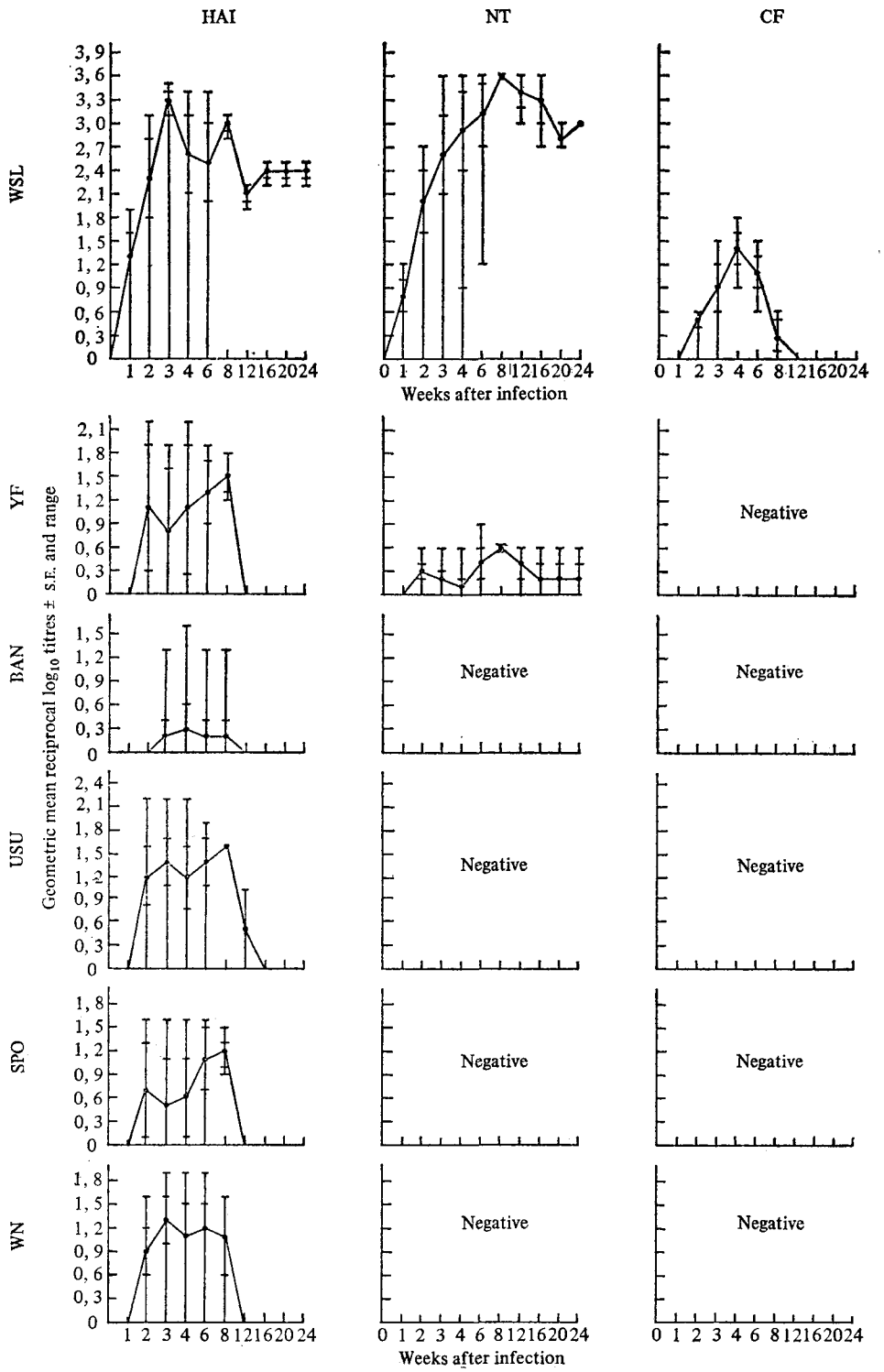

Fig. 4. Cross-reactivity for flaviviruses of antibodies induced in six calves by infection with WSL viruses.

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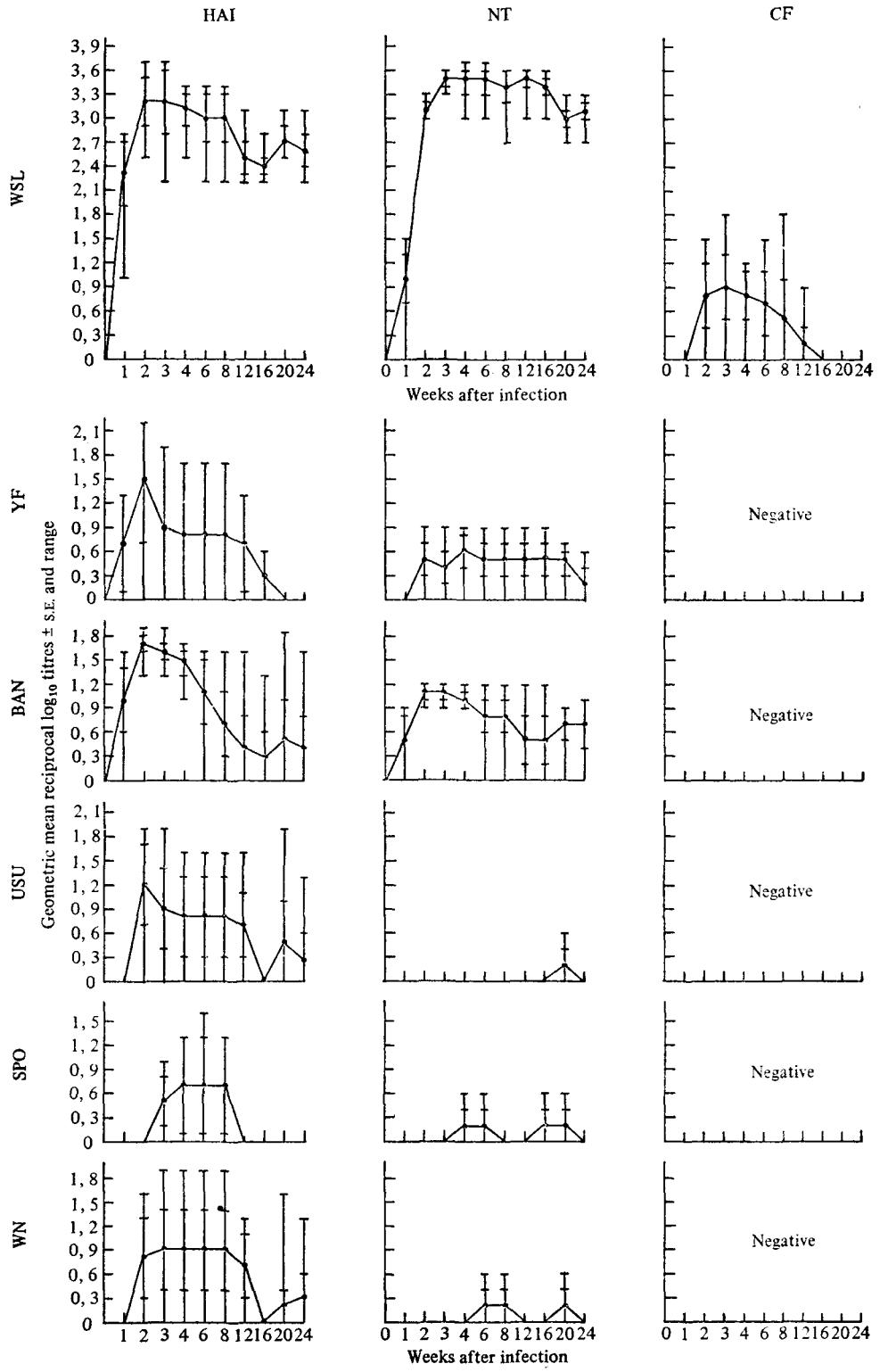

Fig. 5. Cross-reactivity for flaviviruses of antibodies induced in four heifers by infection with WSL virus.

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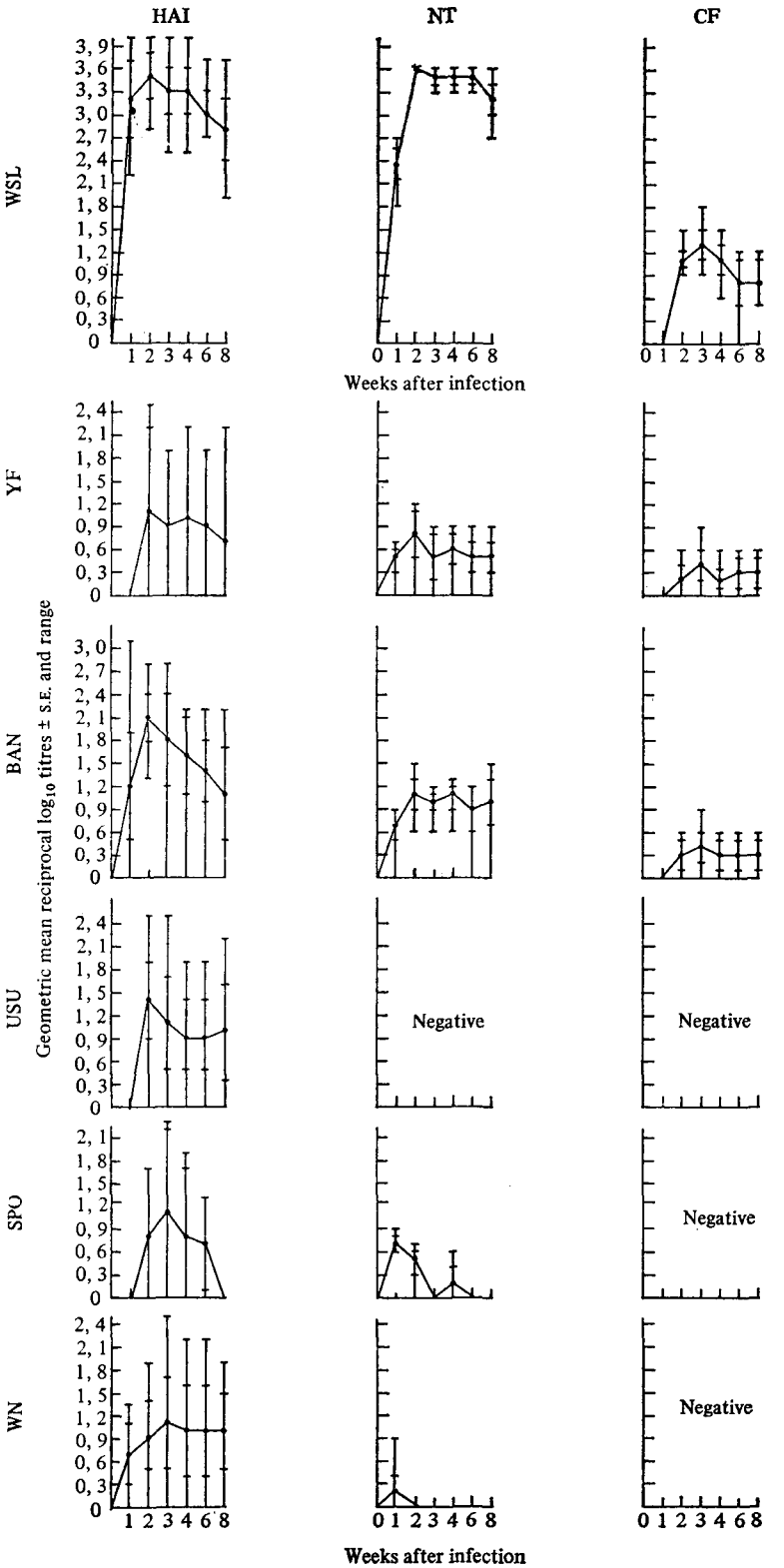

Fig. 6. Cross-reactivity for flaviviruses of antibodies induced in four ewes by infection with WSL virus.

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Twenty-five months after the original experiments, two of the original calves, now grown, had monospecific NT titres to WSL virus of 1/4 and 1/8 while one of the original heifers had WSL NT titres of 1/16 and a YF titre of 1/4. These three cattle plus two susceptible cows failed to develop fever, viraemia or an antibody response following infection with BAN virus. The calves and heifer failed to develop fever or viraemia following a third infection, with WN, USU and SPO viruses respectively, but in the calf given WN virus, the WSL NT titre increased from 1/4 to 1/8 and a WN NT titre of 1/32 appeared. USU infection in the second calf, boosted a WSL NT titre from 1/8 to 1/16 but no other antibody response occurred. In the heifer, given SPO virus, WSL and YF NT titres remained at 1/16 and 1/4, and a titre of 1/4 appeared against WN virus. There were no HAI responses.

Antibody tests on field sera

The results of WSL HAI tests on 14395 cattle sera received from the field over 11 years was summarized in Table 10 by agricultural year, which runs from 1 October each year to 30 September in the succeeding year. Over the same period, WSL HAI antibodies were detected in 18/360 sheep sera and 3/56 goat sera. The WSL antibodies occurred in all parts of Zimbabwe Rhodesia where livestock are kept, as well as in the extreme south-east, north and north-west of the country where there are no livestock but where antibodies were detected in sera from game animals.

Although the incidence of WSL HAI-positive sera varied from year to year, analysis of monthly figures fails to reveal any tendency for the incidence of positive sera or for mean titres to fluctuate in a definite pattern with season, suggesting that a degree of virus activity occurs throughout the year. However, there was a marked surge in incidence of positive cattle sera with the exceptionally heavy rains which fell in the early months of 1978.

The 14395 cattle sera tested over the 11 year period were received as 1438 batches of samples from individual herds and WSL HAI antibody was detected in 935 batches, which suggests that antibodies occurred in 65·0% (935/1438) of herds. However, this is only an approximate estimate since some batches of sera represented repeated testing of the same herds and in certain other instances too few sera were tested to allow accurate assessment of the presence or absence of antibodies in the herd.

Few paired sera were received and in no instance was it possible to associate the occurrence of abortion with evidence of recent WSL infection through demonstrating a diagnostic rise in antibody titre in such acute and convalescent phase sera.

Detailed analysis was made of the sera received during the twelve calendar months of 1974. The 3316 cattle sera received during this period came from 237 herds and WSL HAI antibodies were detected in 61·6% (146/237) of the herds. Of the 3316 sera tested during 1974, 2327 sera came from the herds in which HAI antibodies were detected and 19·8% (461/2327) of these sera had WSL HAI antibodies while 80·2% (1866/2327) lacked antibodies. The remaining 989 sera

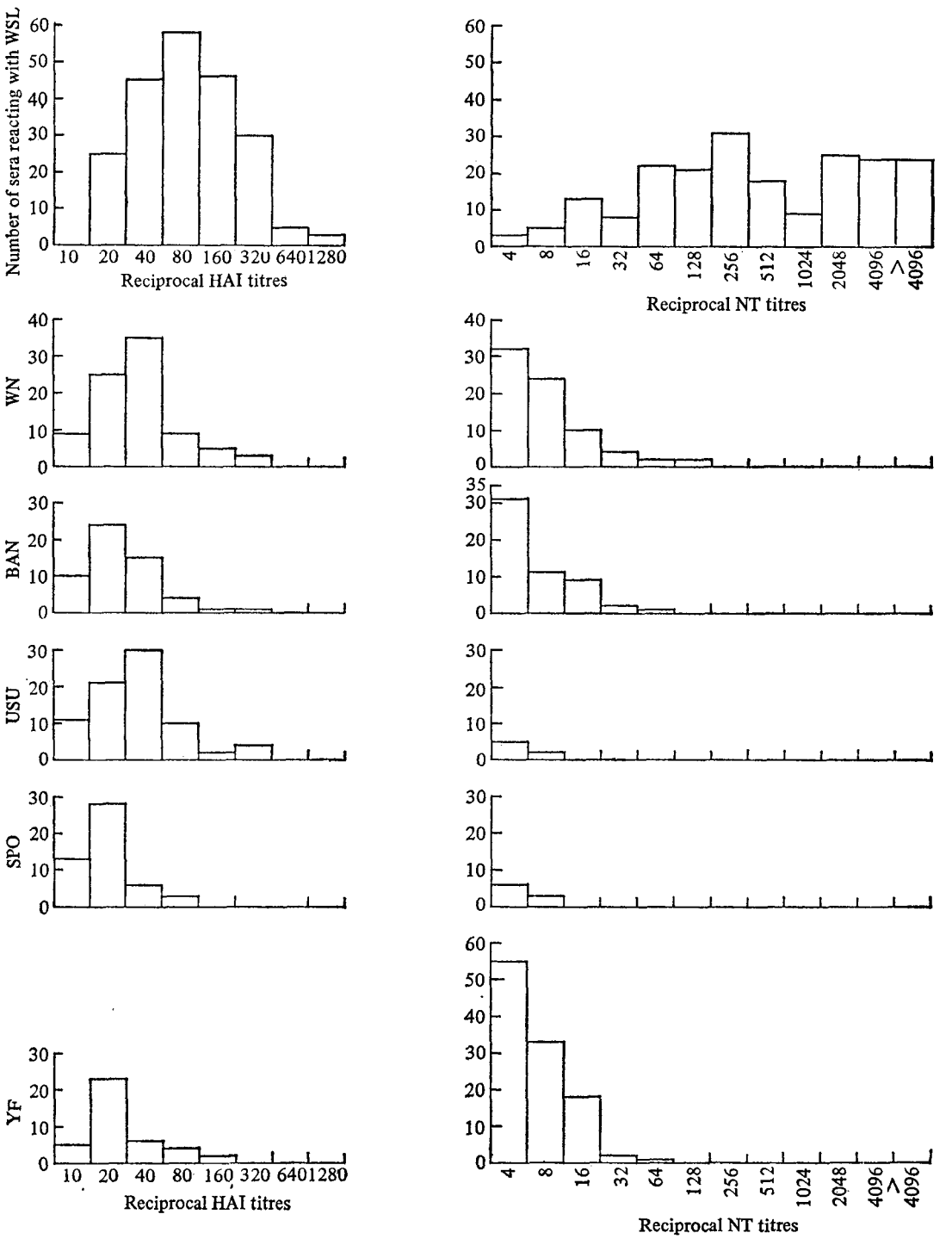

Fig. 7. HAI and NT titres to flaviviruses in 207 WSL HAI-positive cattle sera.

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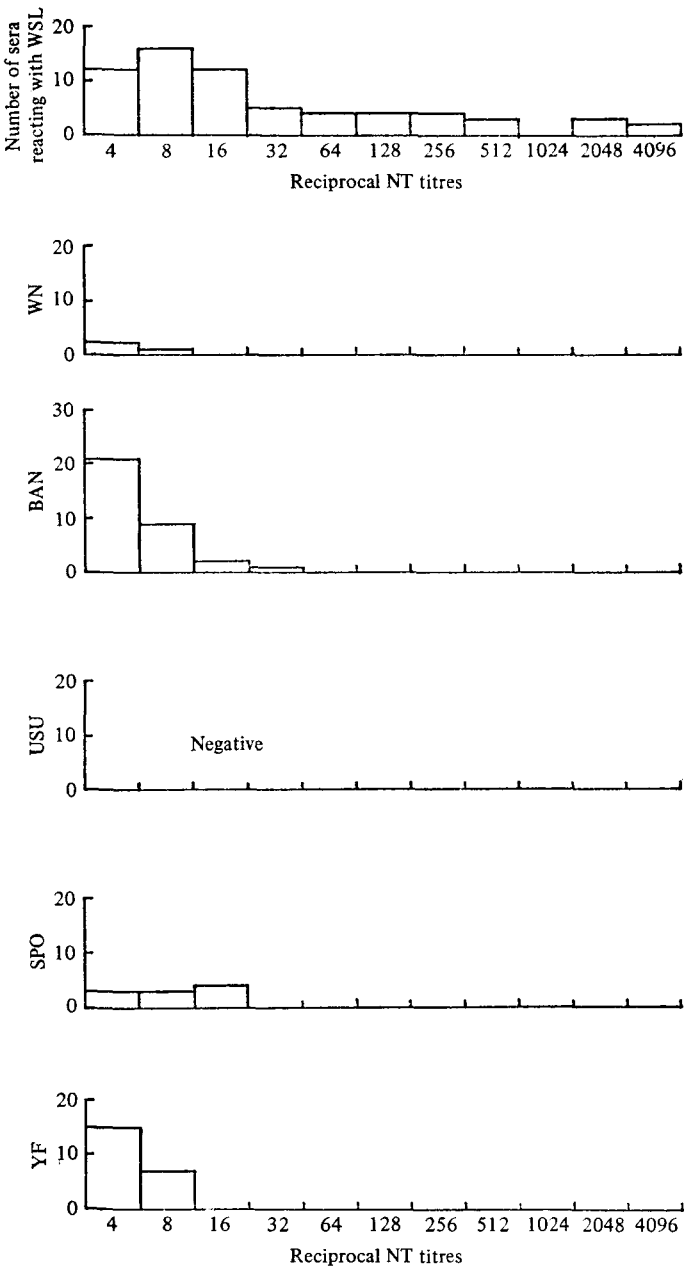

Fig. 8. NT titres to flaviviruses of 112 WSL HAI-negative sera from HAI-positive herds. None of the sera were HAI-positive with any virus.

tested during 1974 came from the 38·4% (91/237) of herds in which no HAI antibodies were detected.

The results obtained with the 409 sera selected for quantitative CF, HAI and NT tests with the full range of flaviviruses are summarized in histogram form in Figs. 7, 8 and 9. The cross-reactions exhibited by individual sera are summarized

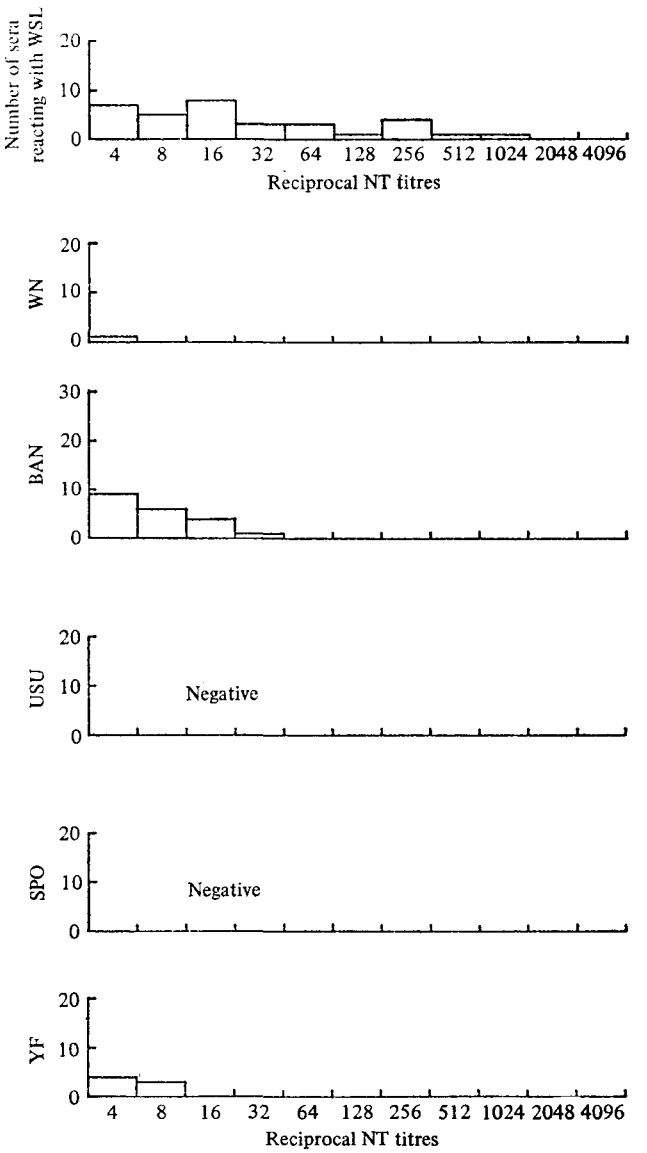

Fig. 9. NT titres to flaviviruses of 90 sera from WSL HAI-negative herds. None of the sera were HAI-positive with any virus.

in Tables 11 and 12 and the proportions of sera in each of the three groups which reacted with the various antigens and viruses are shown in Tables 13 and 14 together with geometric means titres and standard errors of the means.

Only one of the 409 sera reacted in the CF test, with a titre of 1/8 with WSL antigen only, and this serum had monospecific WSL HAI and NT titres of 1/80 and 1/4096 respectively. None of the sera in the two groups which lacked HAI antibody to WSL virus, had HAI antibodies to any of the other flaviviruses tested. HAI titres to WSL virus were higher or at least equal to titres to other viruses in

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Table 10. *Results of HAI tests for antibody to WSL virus on cattle sera over an eleven-year period*

Agricultural year	Number of sera	Number of sera positive	Per cent positive
1967	132	9	6·8
1968	0	0	—
1969	1108	206	18·6
1970	890	150	16·9
1971	114	7	6·1
1972	61	5	8·2
1973	906	81	8·9
1974	3194	501	15·7
1975	3358	360	10·7
1976	1618	357	22·1
1977	700	139	19·9
1978	2314	833	36·0
Total	14395	2648	18·4

every instance. WSL NT titres occurred in all of the HAI-positive herds and in seven of the eight HAI-negative herds; sera from the eighth HAI-negative herd lacked NT titres to any of the flaviviruses tested here. NT titres to WSL virus were higher than NT titres to other viruses in: (*a*) 96·1 % (199/207) of the HAI-positive sera, (*b*) 52·7 % (59/112) of HAI-negative sera from HAI-positive herds and (*c*) 34·4 % (31/90) of sera from HAI-negative herds; titres of $\geqslant$ 1/4096 being recorded frequently with WSL virus, while the highest NT titre recorded with another virus was 1/128 with WN virus in two sera. In the few sera where NT titres to other viruses equalled or exceeded WSL titres, the levels of antibody were very low, the highest NT titre to any virus ranging from 1:4 to 1:32 in such sera. It was BAN and YF titres which equalled or exceeded WSL titres in these sera, although WN titres also did so in six instances and an SPO titre did in one instance. Two of the HAI-positive sera, from different localities, were unique in that they exhibited HAI titres of up to 1/80 with most of the flaviviruses yet lacked NT activity to any virus.

Of the 409 sera studied in detail, 125 came from cows which were known to have aborted or calved normally, while individual histories were not known for the remaining 284 cattle. NT titres excess of 1/1024, indicative of recent WSL infection, occurred in 23·3 % (17/73) of cows known to have aborted and in 21·1 % (11/52) of cows known to have calved normally. Alternative diagnosis of Rift Valley Fever (RVF) or brucellosis were established in eight of the 17 cows which aborted with high WSL titres indicative of recent infection.

Sero-conversion in pregnant heifers

Out of 228 heifers monitored for WSL NT antibody during pregnancy, abortion occurred in: (*a*) three out of 86 which had WSL antibodies at the outset and remained sero-positive throughout the period of observation; (*b*) one out of 25 heifers which had antibodies at the outset but in which titres fell below 1/8, the lowest dilution tested, during pregnancy; (*c*) one out of 96 heifers which remained

Table 11. *Flavivirus cross-reactions in* 207 *HAI-positive field sera*

No. of sera reacting in HAI tests with the following antigens:	No. of sera reacting in NT tests with the following viruses																					HAI totals	
	Nil	WSL only	WSL WN	WSL BAN	WSL USU	WSL YF	WSL WN BAN	WSL WN USU	WSL WN SPO	WSL WN YF	WSL BAN YF	WSL SPO YF	WSL WN BAN USU	WSL WN BAN SPO	WSL WN BAN YF	WSL WN USU YF	WSL WN SPO YF	WSL BAN USU YF	WSL BAN SPO YF	WSL WN BAN USU YF	WSL WN BAN SPO YF		
WSL only	—	42	2	2	1	33	6	—	—	4	5	—	—	—	6	1	—	—	—	—	1	103	103
WSL WN	—	3	1	—	—	1	—	—	—	2	—	—	—	—	1	—	—	—	—	—	—	8	24
WSL BAN	—	1	—	—	—	—	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—	3	
WSL USU	—	1	1	—	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	4	
WSL SPO	—	1	—	—	—	3	—	—	—	2	—	—	—	—	1	—	—	—	1	—	—	8	
WSL YF	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	
WSL WN BAN	—	1	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	3	21
WSL WN USU	—	6	2	1	—	—	—	—	—	3	1	—	—	—	1	—	—	—	—	—	—	14	
WSL WN SPO	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	
WSL USU YF	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	2	
WSL WN BAN USU	—	1	—	—	—	1	—	—	—	—	—	—	—	—	1	—	—	1	—	—	—	4	14
WSL WN BAN SPO	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	1	
WSL WN USU SPO	—	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	
WSL WN USU YF	—	2	—	—	—	2	—	—	—	—	—	—	1	—	1	—	—	—	—	—	—	6	
WSL WN BAN USU SPO	—	1	—	—	—	2	—	—	—	1	—	—	—	—	4	—	—	—	—	—	1	9	19
WSL WN BAN USU YF	—	2	1	—	—	—	—	—	—	1	—	—	—	—	4	—	—	—	—	—	1	9	
WSL WN USU SPO YF	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1	
WSL WN BAN USU SPO YF	2	5	2	—	—	2	3	—	1	—	3	1	—	2	1	1	1	—	—	1	1	26	26
NT Totals	2	69	11	3	1	47	9	1	1	17	11	1	1	2	21	2	1	1	1	1	4	207	
	2	69	62				40						29							5			

Table 12. *Flavivirus cross-reactions in NT tests with 112 WSL HAI-negative sera from WSL HAI-positive herds and 90 sera from WSL-HAI negative herds*

	Sera reacting in NT tests with the following viruses														
	Monospecific				Two-way crossing					Three-way crossing			Four-way crossing	Total
HAI negative sera from	Neg. only	WSL only	BAN only	YF only	WSL WN	WSL BAN	WSL SPO	WSL YF	BAN YF	WSL BAN YF	WSL BAN SPO	WSL WN SPO	WSL BAN SPO YF	
WSL HAI-positive herds	34	27	6	6	—	14	6	4	1	10	1	2	1	112
		39			25					13				
WSL HAI-negative herds	43	22	8	4	1	9	—	1	2	—	—	—	—	90
		34			13					0				

Table 13. *Proportions of 207 WSL HAI-positive sera reacting with flaviviruses in HAI and NT tests, plus reciprocals of geometric mean titres and standard errors of means of sera reacting in the tests*

	Sera reacting in HAI tests			Sera reacting in NT tests		
Antigen/ virus	No. of sera reacting	Per cent sera reacting	Reciprocal of geometric mean HAI titre ± S.E.	No. of sera reacting	Per cent sera reacting	Reciprocal of geometric mean NT titre ± S.E.
WSL	207	100	91·2 ± 1·1	205	99·0	333·1 ± 1·1
WN	86	45·1	4·4 ± 1·1	71	34·3	2·0 ± 1·1
BAN	55	26·5	2·4 ± 1·1	54	26·1	1·6 ± 1·1
USU	78	37·7	3·8 ± 1·1	7	3·4	1·0 ± 0·1
SPO	50	24·2	2·0 ± 1·1	10	4·8	1·1 ± 1·0
YF	45	21·7	2·0 ± 1·1	107	51·7	2·6 ± 1·1
Nil	—	—	N/A*	2	1·0	N/A*

* Not applicable.

Table 14. *Proportions of WSL HAI-negative sera reacting with flaviviruses in NT tests, plus reciprocals of geometric mean titres and standard errors of means of sera reacting in the tests*

	112 sera from WSL HAI positive herds			90 sera from WSL HAI-negative herds		
Virus	No. of sera reacting	Per cent sera reacting	Reciprocal of geometric mean NT titre ± S.E.	No. of sera reacting	Per cent sera reacting	Reciprocal of geometric mean titre ± S.E.
WSL	65	58·0	6·9 ± 1·2	32	35·6	3·2 ± 1·2
WN	2	1·8	1·0 ± 0·1	1	1·1	0 1 ± 0·1
BAN	33	29·5	1·6 ± 1·1	21	23·3	1·5 ± 1·1
USU	—	—	N/A*	—	—	N/A*
SPO	10	8·9	1·2 ± 1·1	—	—	N/A*
YF	22	19·6	1·4 ± 1·1	7	7·8	1·1 ± 1·0
Nil	34	30·4	N/A*	42	46·7	N/A*

* Not applicable.

sero-negative throughout and (*d*) one out of 21 heifers which gained antibodies to WSL virus during pregnancy.

Virological examination of field specimens

From February 1969 to September 1972, 1998 specimens from cattle, sheep and goats, including aborted fetuses, were examined bacteriologically and histologically. No lesions indicative of WSL infection were seen, but RVF was diagnosed histologically in 259 instances.

Over the six years from October 1972 to September 1978, 2106 pathological specimens from cattle, sheep and goats were tested in mice for isolation of arthropod-borne viruses (Table 15). WSL virus was isolated on one occasion only, from a

Table 15. *Pathological specimens tested for arthropod-borne viruses, October* 1972 *to September* 1978

	Fetuses, placentas and new born animals	Animals older than 7 days
Cattle	1102	700
Sheep	117	164
Goats	18	5
	1237	869

cow which died in February 1978. Histopathological lesions in the cow, disseminated small foci of necrosis in liver, kidney and spleen, were not consistent with WSL lesions described in sheep (le Roux, 1959; Coetzer, Theodoridis & van Heerden, 1978). There were 167 isolations of other viruses in mice, including 133 isolations of RVF virus over the six years.

Immunoglobulins were found in 162 out of 374 sera from aborted cattle fetuses, but only one out of the 374 sera had HAI antibody to WSL virus, at a titre of 1/40. The fetus failed to yield virus and no histopathological lesions were seen in its liver.

DISCUSSION

The degree of serological cross-reaction between the known flaviviruses of southern Africa was examined in a simple system with guinea pig sera possessing maximum specificity for individual viruses. Casals (1957) established that (*a*) antibodies are least cross-reactive early in infection and become progressively more cross-reactive and (*b*) antibodies are most specific for individual viruses following primary infection and become more cross-reactive following re-infection, even with the same virus. The most specific serum should therefore result from a single administration of virus and collection of serum early in infection. It was found necessary, however, to administer a second dose of virus and to collect serum at 21 days in order to obtain significant antibody titres. Nevertheless, the results indicated that antigenic relationships among southern African flaviviruses conform to the general pattern described for the group by Casals (1957) in that the sera were broadly cross-reactive in the HAI test, less cross-reactive in the CF test and were virtually monospecific in the NT test (Tables 1–3). The implication was that primary infections with WSL virus could be recognized readily by performing quantitative NT tests in cell culture, but before examining specimens from the field, further tests were conducted on the sera of experimentally infected cattle.

In the earliest surveys of antibodies to flaviviruses in southern Africa, sera were screened for neutralizing activity against a single member of the group, namely, WN or WSL virus (Weinbren, 1955; Kokernot *et al.* 1956; Weiss *et al.* 1956). In subsequent studies, sera were screened against a range of 3 to 5 flaviviruses and only sera possessing monospecific neutralizing activity were considered to be diagnostic of infection with the virus concerned (Smithburn *et al.* 1959*b*; Kokernot *et al.* 1960; Kokernot, Smithburn & Kluge, 1961). Mouse NT tests are impracticable

for large numbers of specimens and in the most recent surveys, sera were screened for HAI antibodies to a range of flaviruses and only HAI-positive sera were subjected to NT screening against the relevant viruses (McIntosh *et al.* 1962*a*, *b*; Kokernot *et al.* 1965*a*, *b*; Dickinson *et al.* (1965). Again, this represented simple qualitative NT screening of sera and the specificity of sera-reacting with more than one virus could not be determined. Furthermore, there was no attempt to demonstrate NT activity in HAI-negative sera.

Elsewhere, there has been a trend towards the use of cell cultures for titration of arthropod-borne viruses and for performing NT tests (Buckley, 1959; Karabatsos & Buckley, 1967; Bergold & Mazzali, 1968; Stim, 1969; David-West, 1971; De Madrid & Porterfield, 1974). The method described here offered a simple and practical way of performing quantitative NT tests routinely on specimens from the field.

The pathogenicity of WSL virus for sheep is well documented. Necrosis of the liver is the most common lesion and infection causes death in pregnant ewes, neonatal death, late abortion and congenital abnormalities (Weiss *et al.* 1956; Weiss, 1957; Belonje, 1958; le Roux, 1959; Coetzer & Barnard, 1977; Coetzer *et al.* 1978). Previous pathogenicity tests in cattle were limited to an experiment in which six non-pregnant cattle exhibited mild fever only (Weiss *et al.* 1956; Weiss, 1957) and to the observation that one out of six neonatal calves died following infection in a study of viraemia (McIntosh, 1972; McIntosh, 1974, personal communication).

In the present experiments, five out of six calves exhibited mild febrile illness and one out of four pregnant heifers produced a weak calf which died shortly after birth, while the remaining three heifers produced healthy calves. The presence of liver lesions does not prove conclusively that the death of the calf resulted from WSL infection, but the bile duct changes are similar to those described in WSL infection in neonatal lambs (Coetzer *et al.* 1978). It is nevertheless evident from the presence of antibodies in the serum of the calf that the virus is capable of crossing the placental barrier in cattle. However, failure to detect antibodies in pre-colostral serum of the three remaining calves born to infected mothers, indicates that the virus is not regularly invasive for the fetus in cattle, even when viraemia of several days duration occurs in the mother. From the fact that pathological changes occurred in the fetus in two out of three pregnant ewes and fetal infection was shown to have occurred in the third, it appears that the strain of virus used was virulent and invasive for the fetus in sheep. The arthrogryposis and muscular atrophy observed in one lamb are consistent with lesions described in congenital WSL infection in sheep (Coetzer & Barnard, 1977).

From the limited evidence of past and present experiments, therefore, it appears that WSL virus generally produces mild febrile illness in cattle, but may occasionally cause more serious disease and the infection may cross the placental barrier, possibly resulting in disease of the fetus. The limited observations in the laboratory were augmented by the observations made on WSL in the field.

Coetzer & Barnard (1977) demonstrated HAI antibody to WSL virus in the sera of sheep fetuses and the present observations on antibodies in pre-colostral

sera confirm the usefulness of the technique for diagnosing *in utero* infection. The present observations on colostral immunity suggest that immune cows can confer protection on their calves of up to four months duration.

By comparing the threshold intensity of viraemia required for infection of mosquitoes, $10^{2\cdot5}$ mouse IC LD50 in 0·002 ml (Simasthein & Olson, 1973) with the intensity of viraemia attained in cattle in the pathogenicity experiments, up to $10^{4\cdot8}$ mouse LD50 per 0·02 ml, it can be surmised that cattle can serve as a source of WSL virus for infection of mosquitoes, as was suggested by McIntosh (1972).

The pattern of antibody cross-reactions observed in cattle and sheep differed from that in guinea pigs. As in guinea pigs, the sera of cattle and sheep infected with WSL virus cross-reacted most with other flaviviruses in the HAI test, but the ratios of homologous titres to heterologous titres were much higher during early response than in guinea pigs. Contrary to the situation in guinea pigs, the sera of cattle and sheep were least cross-reactive in the CF test, with only sheep sera exhibiting heterologous CF activity, and broad cross-reaction occurred in NT tests, although heterologous NT titres were much lower than homologous titres during early response.

The sera of cattle and sheep showed greatest cross-reactivity for BAN and YF viruses following WSL infection, yet this relationship was not evident in the guinea pig experiments. There is similar variation between results obtained by other authors. Smithburn *et al.* (1959*a*) found weak cross-reactions in NT tests between WSL, BAN and YF viruses, and strong cross-reaction between BAN and Uganda S viruses with antisera prepared in guinea pigs. Henderson *et al.* (1970) found that WSL infection conferred protection against YF in monkeys, but BAN did not, while Theiler & Downs (1973) state, without citing evidence, that WSL, BAN and YF form a close antigenic group with Uganda S virus. De Madrid & Porterfield (1974) failed to demonstrate any particularly close relationship between WSL, BAN and YF in NT tests with antisera prepared in rabbits, but confirmed that Uganda S is closely related to BAN. Undoubtedly some of the differences in results are related to variation in immunization schedules and test procedures. It appears that the age or immunological maturity of the host may also influence the occurrence of cross-reaction during antibody response since the sera of the neonatal calves studied here were notably less cross-reactive than the sera of the heifers in NT tests.

From the results obtained in the later experiments it appears that consecutive infection with different flaviviruses does not necessarily induce enhanced antibody response as demonstrated by Casals (1957, 1961, 1963), possibly because partial cross-immunity conferred by one virus in some instances suppresses infection with a second virus. Failure of BAN virus to induce an antibody response even in two apparently susceptible cattle, suggests that antibodies to this virus demonstrated in sera from the field in the past (Kokernot, Smithburn & Kluge, 1961; McIntosh *et al.* 1962*a*, *b*) could represent heterologous activity of antibodies to some other flavivirus, probably WSL, but confirmatory tests on the susceptibility of cattle to BAN, SPO and USU viruses are necessary.

It is concluded from the present experiments that antibodies to WSL virus in

cattle sera can be distinguished from antibodies induced by other southern African flaviruses by quantitative serological tests. Although CF tests are more specific than are NT tests, the presence of CF activity in serum is too transient for this technique to be of use in surveys. Theoretically at least, the CF test would be useful for distinguishing recent from past infection. HAI titres also wane more rapidly than NT titres and use of the HAI test in conjunction with the NT test in surveys, would allow an estimate to be made of how recently infection took place.

The findings in quantitative HAI and NT tests on sera from the field leave no doubt that in most instances the antibodies to flaviviruses demonstrated in cattle resulted from infection with WSL virus. Antibodies to WN virus were possibly more numerous and of slightly higher titre among HAI-positive sera from the field (Fig. 7). Than could have been expected on the basis of heterologous antibody activity following WSL infection, but no sera were found with very high WN titres and low WSL titres, and it is concluded that the evidence for circulation of WN virus in cattle in Rhodesia is not convincing. This makes interesting contrast with the position in South Africa where WN virus is thought to be widely active, particularly in the highveld region (Weinbren, 1955; Kokernot, Smithburn & Weinbren, 1956; Smithburn *et al.* 1959*a*; McIntosh *et al.* 1962 *a*, *b*; 1964; Dickinson *et al.* 1965), and where a large WN epidemic was recently described in the northern Cape province (McIntosh *et al.* 1976).

The antibodies to BAN and YF viruses detected in sera from the field can readily be explained in terms of heterologous antibody activity following WSL infection, as observed in the pathogenicity tests with WSL virus. BAN virus has been isolated from mosquitoes in Zimbabwe Rhodesia (McIntosh, 1972) but the results of the present serum tests fail to provide definite evidence of circulation of the virus in cattle in this country. YF virus is not known to have occurred in Zimbabwe Rhodesia and the nearest reported presence of the virus in recent times was in Luanda, Angola, 1973 (Gear, 1977). It seems possible that widespread occurrence of WSL virus would interfere with circulation of YF virus in cattle in Zimbabwe Rhodesia.

Antibodies to SPO and USU viruses were sparse and of low titre and it is concluded that there is no evidence for circulation of these viruses in cattle in Zimbabwe Rhodesia. There appears to be little evidence for circulation in cattle in this country of a flavivirus unrelated to those tested for here. It could be expected that infection with such a virus would produce sera which would cross-react with the present flaviviruses in HAI tests and fail to react in NT tests, yet only two such sea, with low HAI titres, were encountered from separate localities.

If it is accepted that the occurrence in a serum of a higher NT titre to WSL virus than to other flaviviruses is diagnostic of past infection with WSL virus, it is possible to arrive at an estimate of the total proportion of adult cattle in Zimbabwe Rhodesia which had undergone WSL infection at the time of the tests in 1974. From the results that: (*a*) WSL HAI antibodies occurred in 61·6% of herds; (*b*) 19·8% of sera in these herds had HAI antibodies and (*c*) WSL NT titres exceeded other NT titres in 96·1% of the HAI-positive sera, it follows that the

HAI-positive cattle which are accepted as having definitely undergone WSL infection represent 11·7% (61·6 × 19·8 × 96·1) of all cattle tested. Similarly, HAI-negative cattle in HAI-positive herds which underwent WSL infection represent 26·1% (61·6 × 80·2 × 52·7) of all cattle, and cattle in HAI-negative herds which underwent WSL infection represent 11·6% (38·4 × 87·5 × 34·4) of all cattle; this last calculation taking into account that WSL NT antibodies occurred in 87·5% (7/8) of HAI-negative herds. This, it emerges that WSL infection had occurred in 49·4% (11·7 + 26·1 + 11·6) of all cattle. Likewise, WSL infection had taken place in 95·2% (61·6 + 38·4 × 87·5) of all herds tested in 1974.

There are obviously limitations to the reliance which can be placed on this type of extrapolation from limited observations, but the figures serve to show that WSL infection has taken place in a high proportion of cattle in a high proportion of herds in Zimbabwe Rhodesia during recent years. In fact, the estimate of WSL infection having occurred in 49·4% of cattle tested in 1974, agrees well with the incidence of NT antibody demonstrated in heifers in the field in 1976; 48·7% (111/228) had NT antibody early in pregnancy and 46·9% (107/228) at the end of pregnancy.

The presence of CF antibody in a serum would indicate that infection had been recent, yet CF antibody was detected in only one of the 409 sera of 1974 studied in detail despite the fact that high HAI and NT titres indicative of recent infection occurred in many of the sera. It seems possible that CF antibody activity, present at low titres, is labile and does not last as well in sera submitted from the field as it does in sera taken and stored under optimal conditions during experiments in the laboratory. Nevertheless, it can be accepted arbitrarily from observations in experimental infections that NT titres of 1/1024 or greater, accompanied by HAI titres, are indicative of infection within about six months before sampling, and NT titres lower than this, but still accompanied by HAI titres, are indicative of infection within a year or so before sampling. Applying these criteria to sera accepted as coming from cattle which definitely underwent WSL infection, indicates that 5·1% of all cattle (82/207 HAI-positive cattle) underwent WSL infection within six months before sampling and 11·7% of all cattle (199/207 HAI-positive cattle) underwent infection within a year or so before sampling. These figures agree well with sero-conversion from negative to positive which occurred in 9·2% (21/228) of heifers during seven to nine months of observation in 1976, but it appears from the surge in incidence of HAI titres that the challenge rate increased markedly in 1978.

It is notable that NT titres fell below 1/8 in 11% (25/228) of pregnant heifers during the period of observation, in 1976. From this, and from observations in experimental infections, it appears that serological immunity to WSL virus in cattle may wane to minimal or undetectable levels within two to three years after infection.

It is concluded that WSL has been the dominant flavivirus in cattle in Zimbabwe Rhodesia in recent years, that at any one time about half of the cattle may possess antibodies to the virus and that in the course of a year about one eighth of cattle (11·7%) may become infected.

Attempts to relate serological evidence to recent infection to occurrence of abortion in the present study, failed to produce convincing evidence that WSL infection is associated with abortion of cattle in the field. In a prospective experiment, abortion occurred in only one out of 21 heifers observed to gain infection during pregnancy, and abortion also occurred in five out of 207 heifers which failed to gain WSL infection during the experiment.

Histopathological lesions characteristic of those described in WSL disease in sheep, were not observed in 1998 specimens from cattle, sheep and goats examined over 44 months before October 1972. WSL virus was isolated from the organs of one cow out of 2106 pathological specimens from cattle sheep and goats, including 1237 specimens related to abortion and neonatal death, examined over six years from October 1972 to September 1978. A low WSL HAI titre was demonstrated in one out of 374 sera from aborted cattle fetuses tested over the same period. In South Africa, WSL virus was isolated from the blood, and subsequently from the organs, of a sick cow which died near Johannesburg (McIntosh, personal communication) and the virus was isolated from the organs of two young calves submitted for examination *post mortem* to the Veterinary Research Institute at Onderstepoort (Coetzer, personal communication).

Thus, it is concluded that, although WSL virus may on occasion cause pathological changes in the fetus, abortion and other disease in cattle, it does not do so on a significant scale despite widespread occurrence of infection. Coetzer *et al.* (1978) came to a similar conclusion with regard to sheep in South Africa; that the incidence of disease is low despite evidence of widespread occurrence of the virus. There are comparatively few sheep in Zimbabwe Rhodesia and in the limited observations made on this species, WSL virus was not implicated as a cause of disease in the field.

The Director of Veterinary Services has given permission for publication of this paper.

REFERENCES

Belonje, C. W. A. (1958). Field observations on Wesselsbron disease. *Journal of the South African Veterinary Medical Association* **29**, 1–12.

Bergold, G. H. & Mazzali, R. (1968). Plaque formation by arboviruses. *Journal of General Virology* **2**, 273–84

Bradstreet, C. M. & Taylor, C. E. D. (1962). Technique of complement-fixation tests applicable to the diagnosis of virus diseases. *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service* **21**, 96–104.

Buckley, S. M. (1959). Propagation, cytopathogenicity and haemagglutination-haemadsorption of some arthropod-borne viruses in tissue culture. *Annals of the New York Academy of Science* **81**, 172–87.

Casals, J. (1957). The arthropod-borne group of animal viruses. *Transactions of the New York Academy of Science, Series* 2, **19**, 219–35.

Casals, J. (1961). Procedures for the identification of arthropod-borne viruses. *Bulletin of the World Health Organization* **24**, 723–34.

Casals, J. (1963). New developments in the classification of arthropod-borne viruses. *In* Proceedings of the 7th International Congress on Tropical Medicine and Malaria. *Anais de Microbiologia* **11**, 13–34.

Casals, J. & Brown, L. V. (1954). Haemagglutination with arthropod-borne viruses. *Journal of Experimental Medicine* **99**, 429–49.

CLARKE, D. H. & CASALS, J. (1958). Techniques for haemagglutination and haemagglutination inhibition with arthropod-borne viruses. *American Journal of Tropical Medicine and Hygiene* **7**, 561–73.

COETZER, J. A. W. & BARNARD, J. H. (1977). *Hydrops amnii* in sheep associated with hydranencephaly and arthrogryposis with Wesselsbron disease and Rift Valley fever as aetiological agents. *Onderstepoort Journal of Veterinary Research* **44**, 119–26.

COETZER, J. A. W., THEODORIDIS, A. & VAN HEERDEN, A. (1978). Wesselsbron disease. Pathological, heamatological and clinic studies in natural cases and experimentally infected newborn lambs. *Onderstepoort Journal of Veterinary Research* **45**, 93–106.

DAVID-WEST, T. S. (1971). Propagation and cytopathogenicity of arboviruses in mouse embryo cell culture. *Journal of General Virology* **10**, 173–80.

DE MADRID, A. T. & PORTERFIELD, J. S. (1974). The flaviviruses (Group B arboviruses): a cross-neutralisation study. *Journal of General Virology* **23**, 91–6.

DICKINSON, D. B., MCGILLIVRAY, G. M., MCINTOSH, B. M. & WINTER, P. A. D. (1965). Antibodies against certain arboviruses in sera from human beings and domestic animals from the south-western and north-western regions of the Cape province of South Africa. *South African Journal of Medical Science* **30**, 11–18.

EAGLE, H. (1959). Amino acid metabolism in mammalian cell cultures. *Science* **130**, 432–7.

GEAR, J. H. S. (1977). Haemorrhagic fevers of Africa, an account of two recent outbreaks. *Journal of the South African Veterinary Association* **48**, 5–8.

HENDERSON, B. E., CHESHIRE, P. P., KIRYA, G. B. & LULE, M. (1970). Immunologic studies with yellow fever and selected African group B arboviruses in rhesus and vervet monkeys. *American Journal of Tropical Medicine and Hygiene* **19**, 110–18.

JOUBERT, D. M. (1956). A study of prenatal growth and development in the sheep. *Journal of Agricultural Science* **47**, 382–428.

KARABATSOS, N. & BUCKLEY, S. M. (1967). Suceptibility of a baby hamster kidney cell line (BKH21) to infection with arboviruses. *American Journal of Tropical Medicine and Hygiene* **16**, 99–105.

KÄRBER, G. (1931). Beitrag zur kollektiven Behandlung pharmakologischer Reihensversuche. *Archiv für Experimentelle Pathologie und Pharmakologie* **162**, 480–83.

KOKERNOT, R. H. & MCINTOSH, B. M. (1959). Isolation of West Nile virus from a naturally infected human being and from a bird. *Sylvieta rufescens* (Vieillot). *South African Medical Journal* **33**, 987–9.

KOKERNOT, R. H., SMITHBURN, K. C., GANDARA, A. F., MCINTOSH B. M. & HEYMANN, C. S. (1960). [Neutralising antibodies to arthropod-borne viruses in sera of residents of Mozambique]. Portuguese, Eng. summary. *Anais do Instituto de Medicina Tropical* **17**, 201–30.

KOKERNOT, R. H., SMITHBURN, K. C. & KLUGE, E. (1961). Neutralising antibodies against arthropod-borne viruses in the sera of domestic quadrupeds in Tongaland, Union of South Africa. *Annals of Tropical Medicine and Parasitology* **55**, 73–85.

KOKERNOT, R. H., SMITHBURN, K. C. & WEINBREN, M. P. (1956). Neutralising antibody to arthropod-borne viruses in human beings and animals in the Union of South Africa. *Journal of Immunology* **77**, 313–23.

KOKERNOT, R. H., SZLAMP, E. L., LEVITT, J. & MCINTOSH, B. M. (1965*b*). Survey for antibodies against arthropod-borne viruses in the sera of indigenous residents of the Caprivi Strip and Bechuanaland Protectorate. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **59**, 237–43.

KOKERNOT, R. H., SMITHBURN, K. C., MUSPRATT, J. & HODGSON, B. (1957). Spondweni virus, an agent previously unknown, from *Taeniorhynchus* (*Mansonoides*) *uniformis* Theo. *South African Journal of Medical Science* **22**, 103–12.

KOKERNOT, R. H., CASACA, V. M. R., WEINBREN, M. P. & MCINTOSH, B. M. (1965*a*). Survey for antibodies against arthropod-borne viruses in the sera of indigenous residents of Angola. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **59**, 563–70.

LE ROUX, J. M. W. (1959). The histopathology of Wesselsbron disease in sheep. *Onderstepoort Journal of Veterinary Research* **28**, 237–43.

MANCINI, C., CARBONARA, A. O. & HEREMANS, J. F. (1956). Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry* **2**, 235–54

MCINTOSH, B. M. (1967). Usutu virus in: *Catalogue of arthropod-borne viruses of the world*. United States Department of Health Education and Welfare Bethseda.

McIntosh, B. M. (1972). Rift Valley fever 1. Vector studies in the field. *Journal of the South African Veterinary Association* **43**, 391–5.

McIntosh, B. M., Dickinson, D. B., Serafini, E. T. & de Sousa, J. (1962*a*). Antibodies against certain arboviruses in sera from human beings and domestic animals from the South African highveld. *South African Journal of Medical Sciences* **27**, 87–94.

McIntosh, B. M., Jupp, P. G., Dos Santos, I. & Meenehan, G. M. (1976). Epidemics of West Nile and Sindbis viruses in South Africa with *Culex* (*C.*) *univittatus* Theo. as vector. *South African Journal of Science* **72**, 295–300.

McIntosh, B. M., McGillivray, G. M., Dickinson, D. E. & Malherbe, H. (1964). Illness caused by Sindbis and West Nile viruses in South Africa. *South African Medical Journal* **38**, 291–4.

McIntosh, B. M., Serafini, E. T., Dickinson, D. P. & Weinbren, M. P. (1962*b*). Antibodies against certain arboviruses in sera from human beings resident in the coastal areas of southern Natal and eastern Cape Provinces of South Africa. *South African Journal of Medical Sciences* **27**, 77–86.

Simasthein, P. & Olson, L. C. (1973). Factors influencing the vector potential of *Aedes aegypti* and *Culex quinquefasciatus* for *Wesselsbron virus*. *Journal of Medical Entomology* **10**, 587–90.

Smithburn, K. C., Kokernot, R. H., Heymann, C. S., Weinbren, M. P. & Zentkowsky, D. (1959*a*). Neutralizing antibodies for certain viruses in the sera of human beings resident in northern Natal. *South African Medical Journal* **33**, 555–61.

Smithburn, K. C., Paterson, H. E., Heymann, C. S. & Winter, P. A. D. (1959*b*). An agent related to Uganda S from man and mosquitoes in South Africa. *South African Medical Journal* **33**, 959–62.

Stim, T. B. (1969). Arbovirus plaquing in two simian kidney cells lines. *Journal of General Virology* **5**, 329–38.

Swanepoel, R. (1976). Studies on the epidemiology of Rift Valley fever. *Journal of the South African Veterinary Association* **46**, 93–4.

Swanepoel, R., Blackburn, N. K., Efastratiou, S. & Condy, J. B. (1978). Studies on Rift Valley fever in some African murids (*Rodentia:* Muridae). *Journal of Hygiene* **80**, 183–96.

Swanepoel, R., Blackburn, N. K., Lander, K. P., Vickers, D. B. & Lewis, A. R. (1975). An investigation of infectious infertility and abortion of cattle. *Rhodesian Veterinary Journal* **6**, 42–55.

Theiler, M. & Casals, J. (1958). The serological reactions in yellow fever. *American Journal of Tropical Medicine and Hygiene* **7**, 585–94.

Theiler, M. & Downs, W. G. (1973). *The Arthropod Borne Viruses of Vertebrates*. Yale University Press: New Haven.

Weinbren, M. P. (1955). The occurrence of West Nile virus in South Africa. *South African Medical Journal* **29**, 1092–7.

Weiss, K. E. (1957). Wesselsbron virus disease. *Bulletin of Epizootic Diseases of Africa* **5**, 459–65.

Weiss, K. E., Haig, D. A. & Alexander, R. A. (1956). Wesselsbron virus – a virus not previously described, associated with abortion in domestic animals. *Onderstepoort Journal of Veterinary Research* **27**, 183–95.

Yasamura, U. & Kawakita, Y. (1963). Studies on Sv40 in relationship with tissue culture. *Nippon Rhinsho* **21**, 1201–9.