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WESSELSBRON DISEASE: VIROLOGICAL AND SEROLOGICAL STUDIES IN EXPERIMENTALLY INFECTED SHEEP AND GOATS

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ABSTRACT

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Adult sheep and goats and new-born lambs and kids were experimentally infected with a Wesselsbron disease virus. The viraemia in lambs commenced approximately 27 h after infection and lasted on the average for 50 h. A febrile reaction, which was mostly biphasic, commenced several hours after the viraemia and outlasted it by 50 h. The viraemia in adult animals began about 50 h after infection and lasted for 30 h. The fever usually commenced several hours after the viraemia and, as in 3 cases out of 4 in lambs, it outlasted the viraemia by at least 30 h. The virus could be reisolated in mice from every tissue examined in lambs, although it has previously been shown that pathological lesions are restricted to the liver and lymphatic tissues.

Résumé

MALADIE DE WESSELSBRON: ÉTUDES VIROLOGIQUES ET SÉROLOGIQUES SUR DES MOUTONS ET DES CHÈVRES INFECTÉS ARTIFICIELLEMENT

Des chèvres et des moutons adultes ainsi que des agneaux et chevreaux nouveau-nés ont été infectés artificiellement avec le virus de la maladie de Wesselsbron. La virémie chez les agneaux commença approximativement 27 heures après l'infection et dura en moyenne pendant une période de 50 heures. Une fièvre, principalement biphasique, commença plusieurs heures après la virémie et persista plus longtemps que celle-ci pour une période de 50 heures. La virémie chez les animaux adultes commença environ 50 heures après l'infection et elle dura pendant 30 heures. La fièvre commença habituellement plusieurs heures après la virémie et, comme dans trois cas sur quatre, chez les agneaux, elle persista plus longtemps que la virémie pour une période d'au moins 30 heures. Le virus peut être ré-isolé dans des souris à partir de chaque tissu examiné dans les agneaux, malgré la observation faite antérieurement que les lésions pathologiques sont limitées au foie et aux tissus lymphatiques.

INTRODUCTION

The virus of Wesselsbron (WSL) disease belongs to the subgroup Flavivirus of the family Togaviridae (Fenner, 1976) and was isolated for the first time from a new-born lamb in the Wesselsbron district in the Orange Free State (Weiss, Haig & Alexander, 1956). Subsequent work showed that WSL virus mainly causes mortality amongst new-born lambs and kids, is an inapparent disease in adult sheep, goats and cattle, and may give rise to abortions in pregnant ewes (Weiss *et al*, 1956; Weiss, 1957; Coetzer, Theodoridis & Van Heerden 1978; Coetzer, Theodoridis, Herr & Kritzinger, 1979; Coetzer & Theodoridis, unpublished observations). In addition to these effects, the virus may also be responsible for teratology in the developing foetus in sheep and pregnant cattle (Coetzer & Barnard, 1977; Coetzer *et al.*, 1979).

The emphasis in the present study is on the replication and distribution of the virus in experimentally infected lambs and kids and in adult sheep and goats by determining the virus concentration in the blood and tissues over a period.

Serological studies were also done on the animals that recovered from the infection.

MATERIALS AND METHODS

Virus

The WSL virus, isolated in mice during the 1973–74 outbreak (Coetzer *et al.*, 1978) and kept lyophilized at −20 °C, was used to infect the experimental animals. The virus had been passaged 3 times in mouse brain and had a titre of $10^{6,6}$ mouse LD_{50}/mℓ.

Infection of experimental animals

Dorper sheep and goats were used. All the adult animals were tested for haemagglutinating antibodies to WSL virus and only the susceptible ones were infected. The new-born lambs were kept with their mothers in a reasonably insect-free stable. Lambs and kids not older than 3 days were injected intradermally with 0,5 mℓ of virus suspension, and adult sheep and goats subcutaneously with 0,5 mℓ of the suspension.

Virus assay in the blood and tissues

Blood in an anticoagulant (heparin) was periodically collected from the infected lambs and kids according to the time schedule shown in Fig. 1. Four infected lambs were sacrificed at 24 h intervals after infection and samples of various tissues were removed aseptically. Three ewes and 2 goats were killed after infection at the time intervals shown in Fig. 2. In most instances these samples had to be stored for a couple of days at −20 °C before being tested. The blood samples were diluted 1:10 in buffered lactose peptone (BLP*) containing penicillin (500 IU/mℓ) and streptomycin (500μg/mℓ), and the tissues were macerated and ground to a homogenate. A 10% suspension in BLP was prepared containing the same concentration of antibiotics as was used for the blood suspension. The supernatant was used for the titration. Tenfold serial dilutions of the above suspensions were prepared in BLP and one litter of day-old mice was injected intracerabrally with 0,025 mℓ of the dilutions. Deaths were recorded for 10 days, the mouse LD_{50} being calculated by the method of Reed & Muench (1938).

Serological tests

To identify the reisolated virus with WSL positive sheep serum, the serum-virus neutralization test was carried out in day-old mice, using the constant serum-tenfold virus dilution method of Cunningham (1960). At the same time, sera of some of the experimental animals that recovered were tested for neutralizing antibodies against WSL virus.

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* BLP: Final concentration of 1% peptone and 5% lactose in 0,1 M phosphate buffer

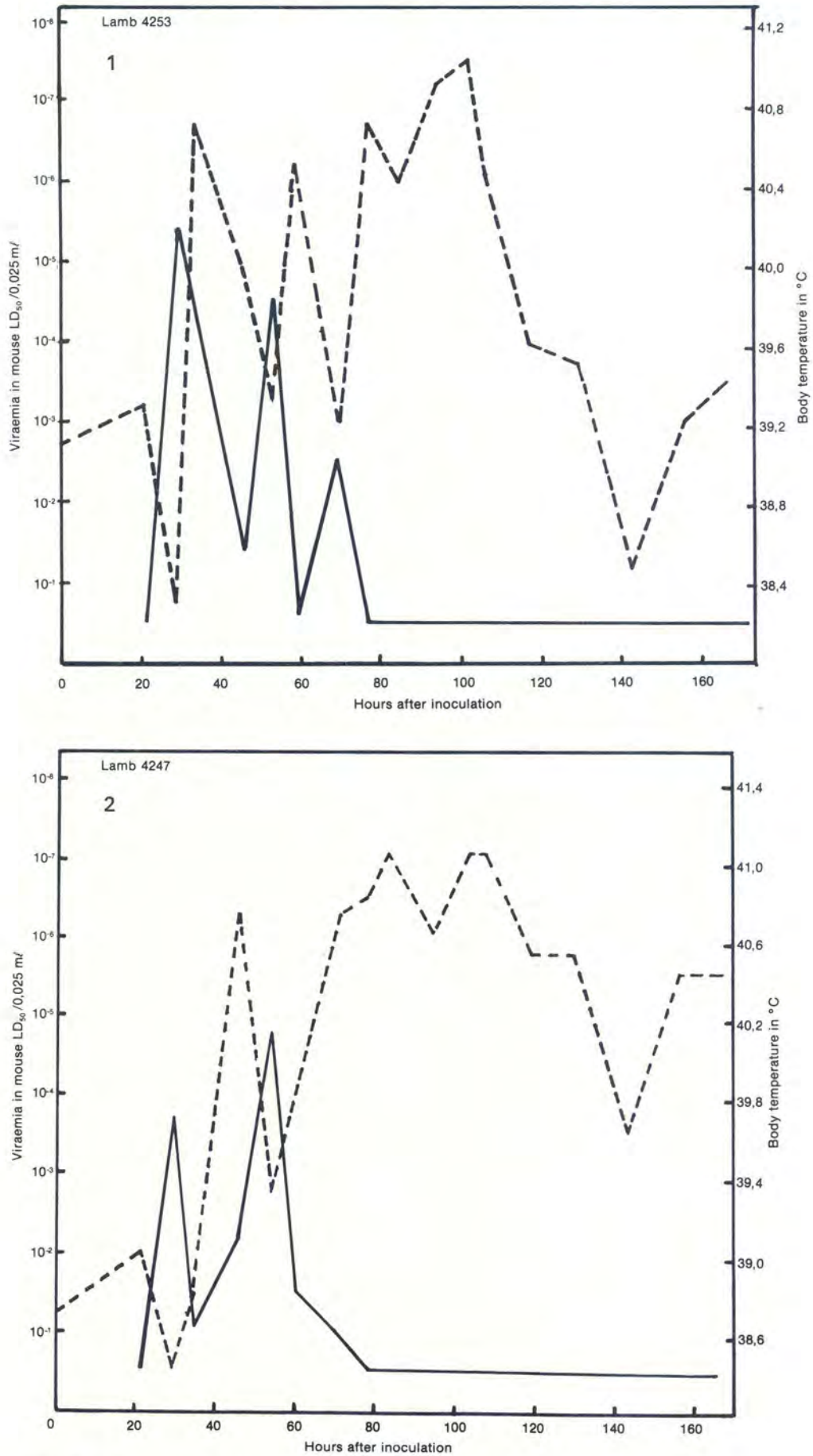
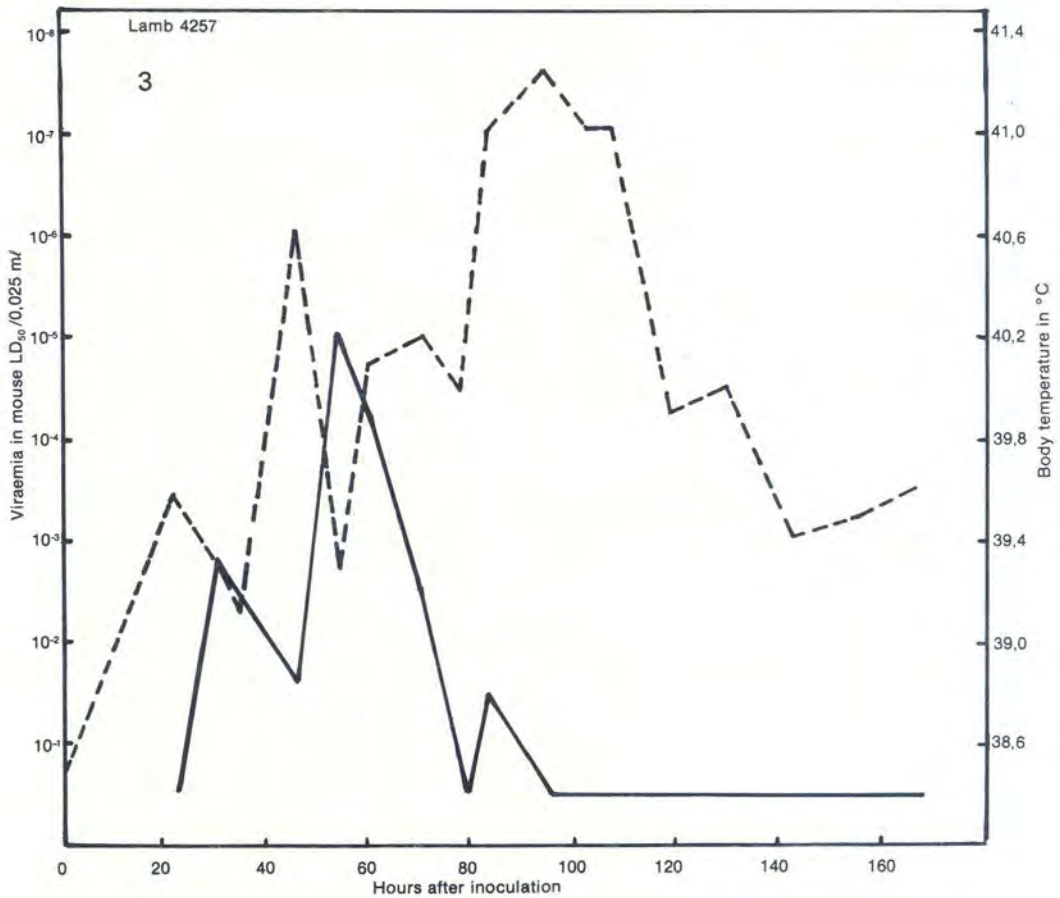
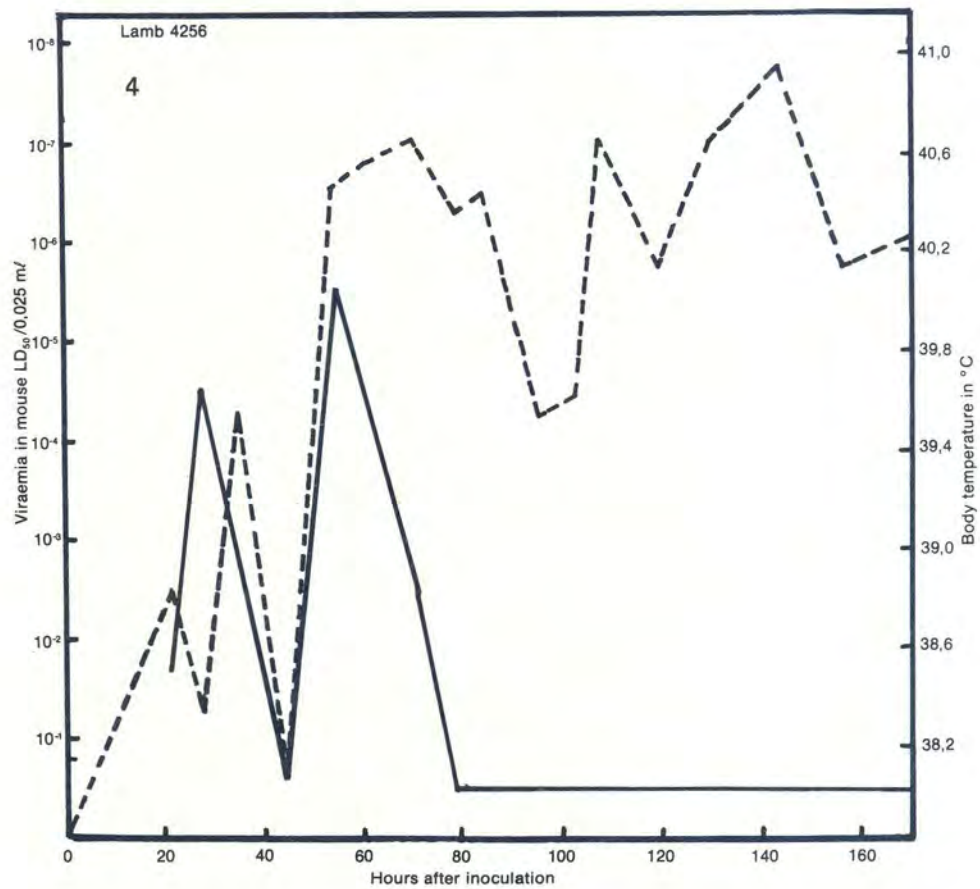


FIG. 1–4 Viraemia and body temperature of lambs infected with Wesselsbron disease virus
(– – – Temperature)
(——— Viraemia)


Lamb 4257
3
Viraemia in mouse LD50/0,025 ml
Body temperature in °C
Hours after inoculation
0
20
40
60
80
100
120
140
160
41,4
41,0
40,6
40,2
39,8
39,4
39,0
38,6


Lamb 4256
4
Viraemia in mouse LD50/0,025 ml
Body temperature in °C
Hours after inoculation
0
20
40
60
80
100
120
140
160
41,0
40,6
40,2
39,8
39,4
39,0
38,6
38,2

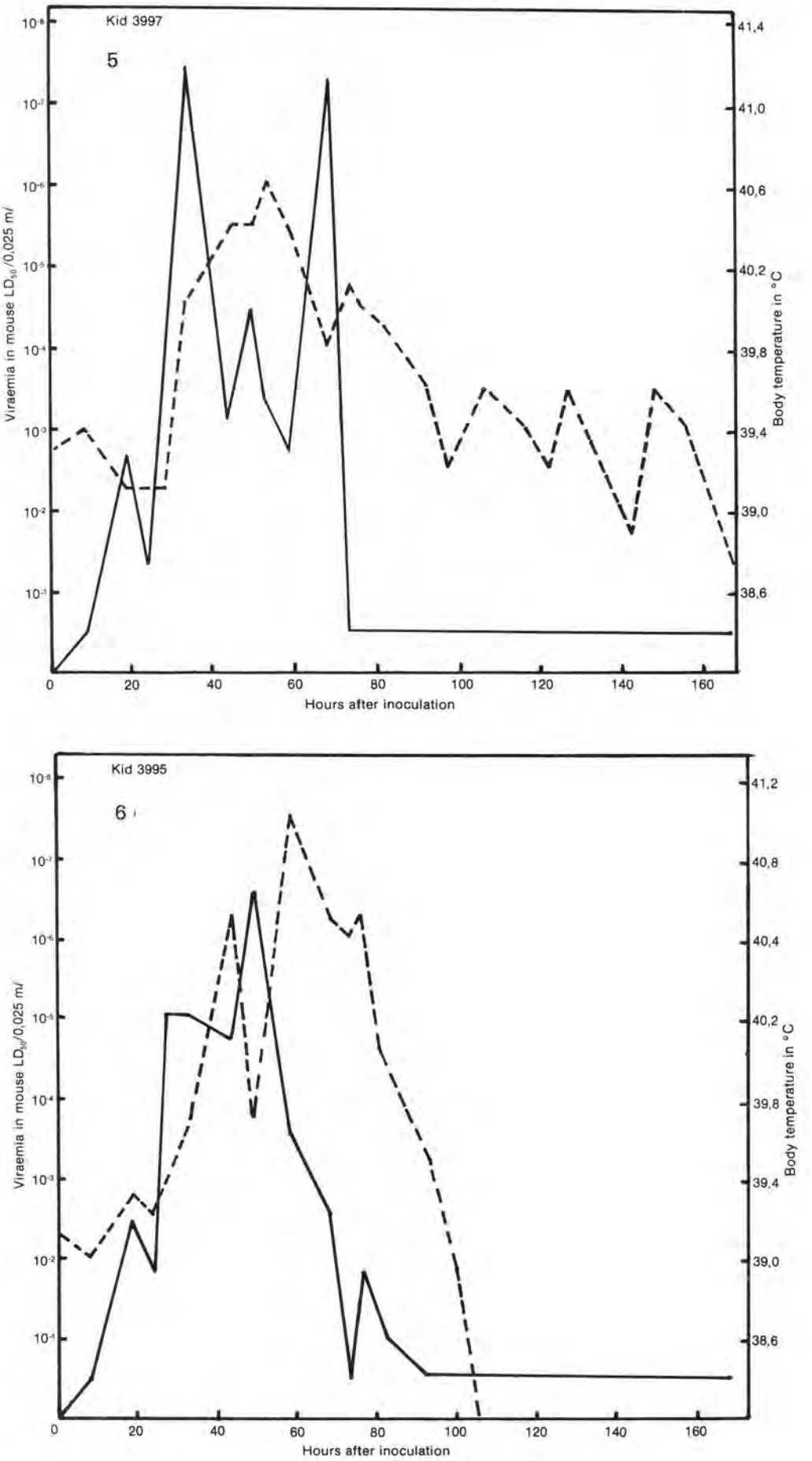
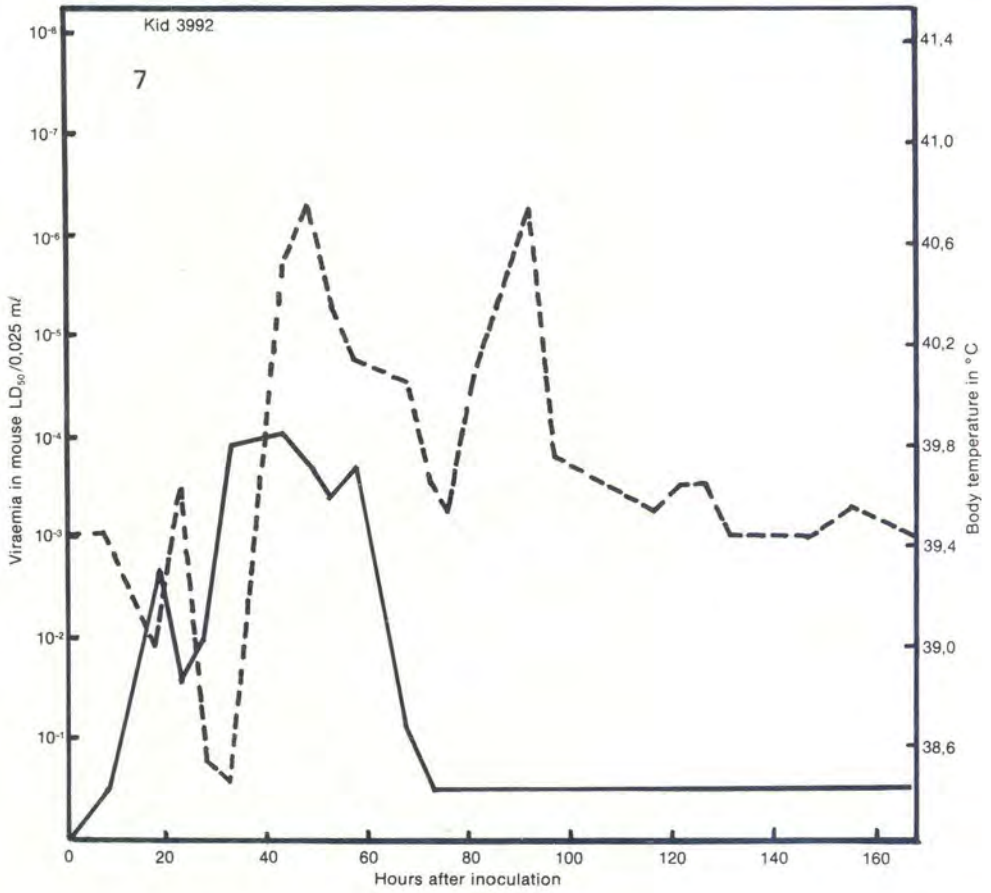
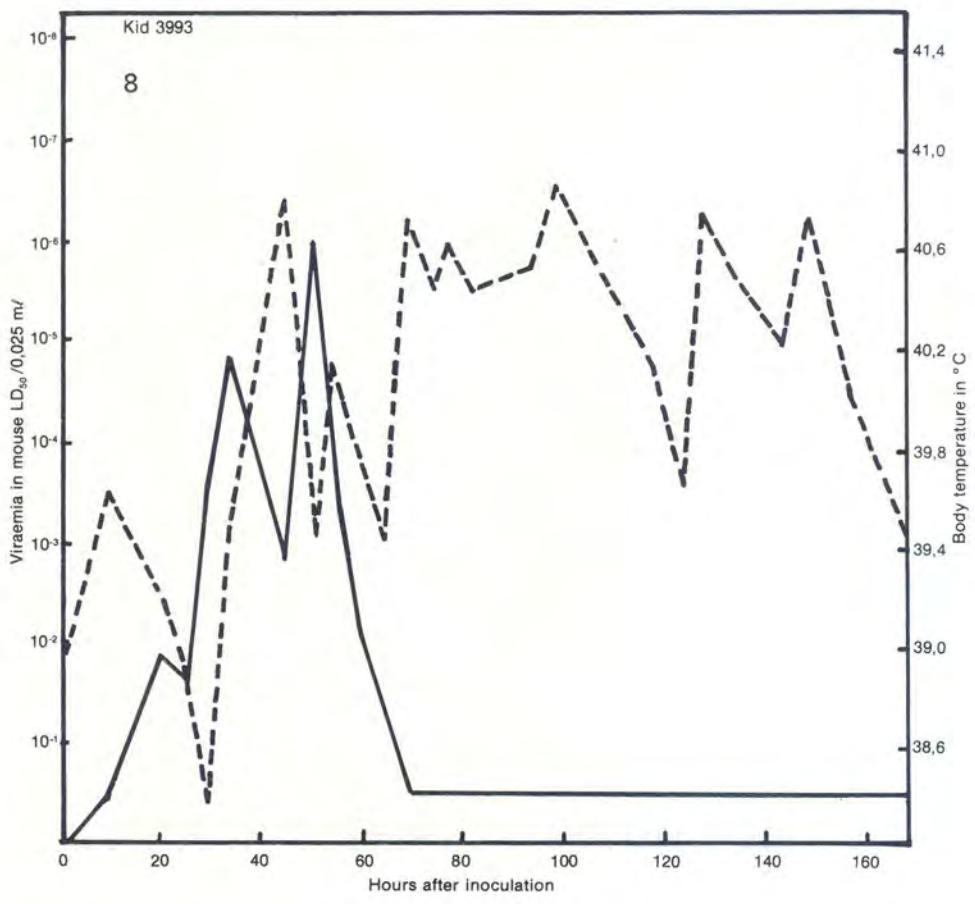


FIG. 5–8 Viraemia and body temperature of kids infected with Wesselsbron disease virus
(– – – – Temperature)
(——— Viraemia)


Kid 3992
7
Viraemia in mouse LD_{50}/0,025 m/
Body temperature in °C
Hours after inoculation


Kid 3993
8
Viraemia in mouse LD_{50}/0,025 m/
Body temperature in °C
Hours after inoculation

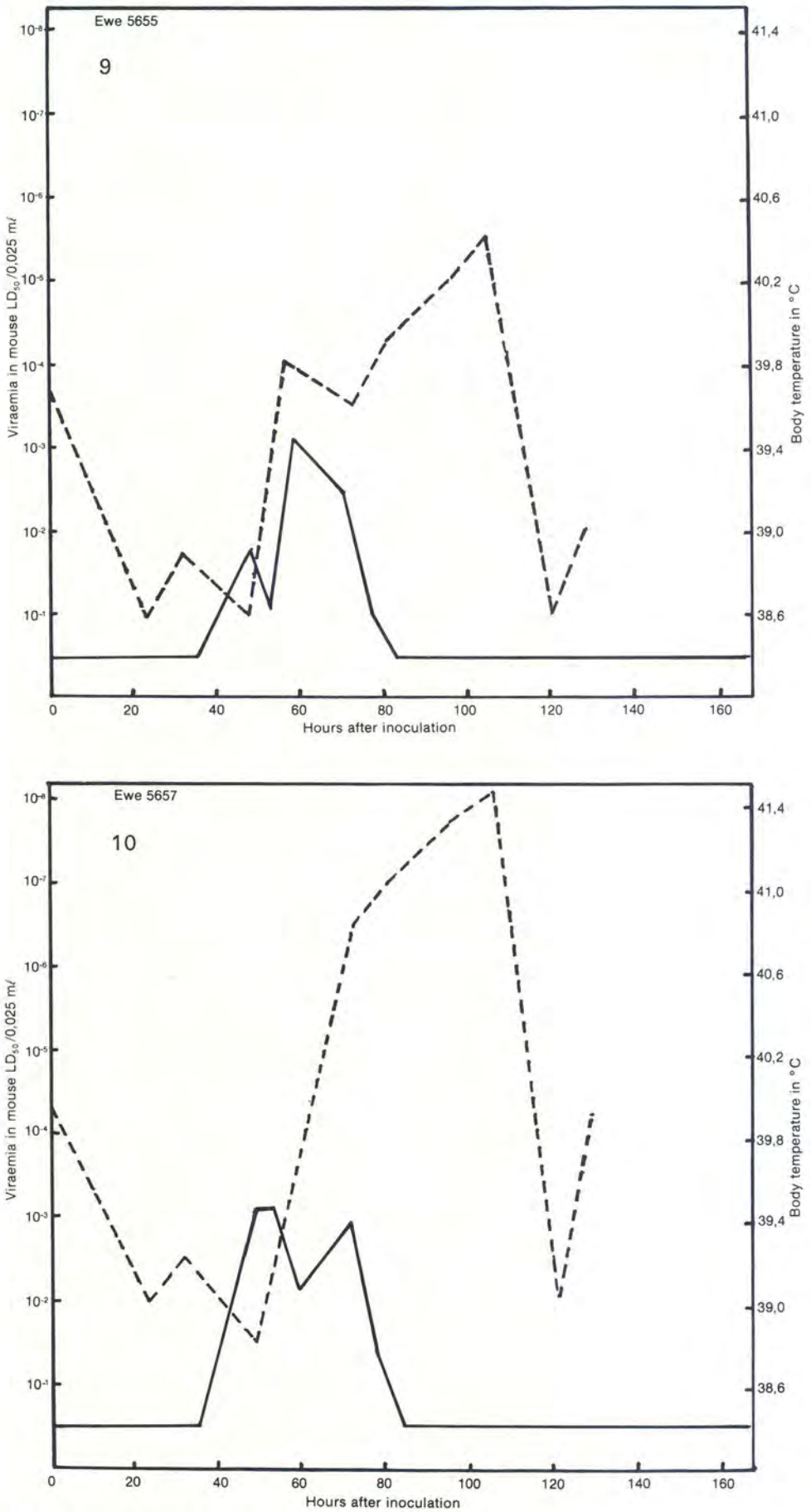
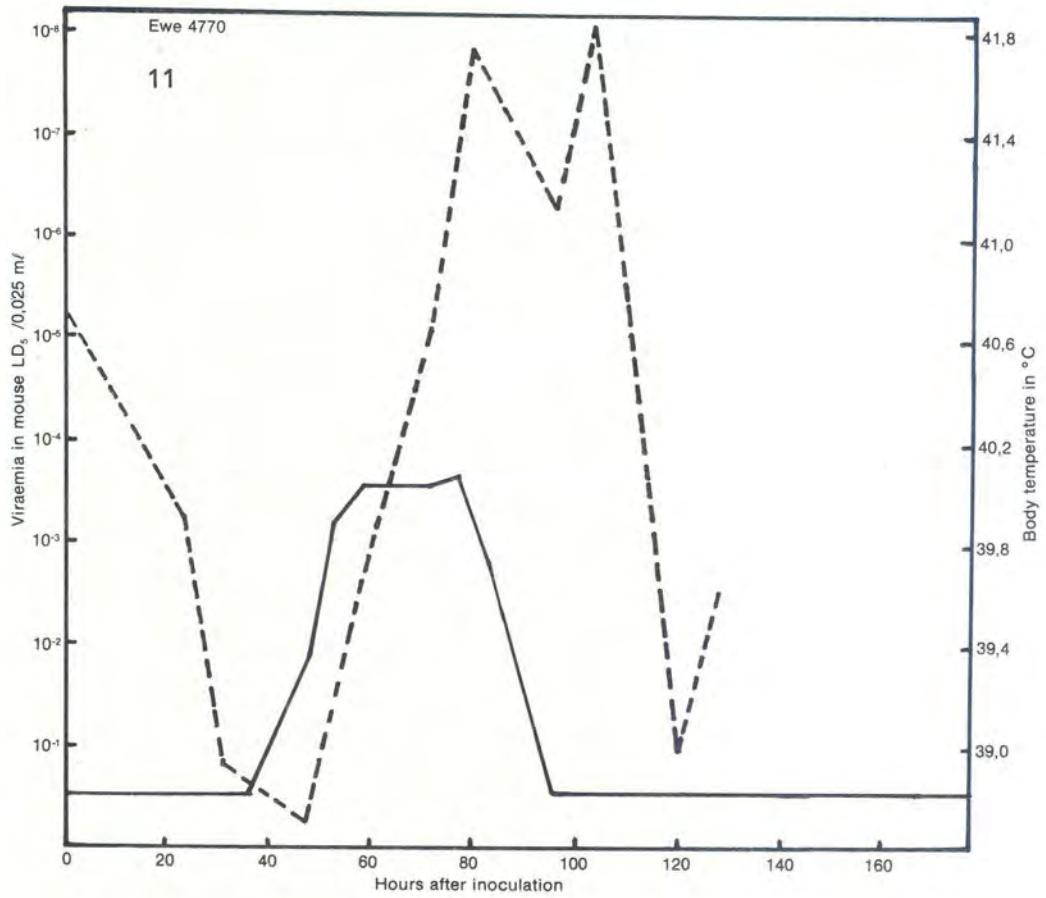
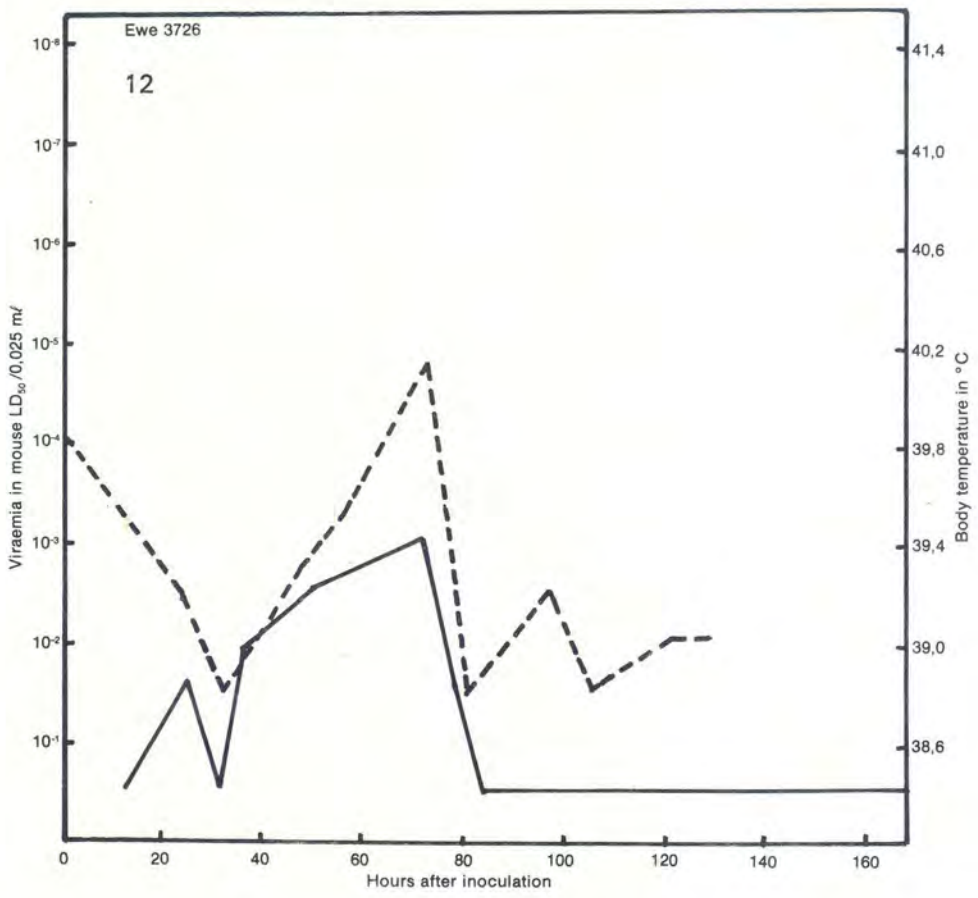


FIG. 9–12 Viraemia and body temperature of ewes infected with Wesselsbron disease virus
(– – – – Temperature)
(——— Viraemia)


Ewe 4770
11
Viraemia in mouse LD50 /0,025 ml
Body temperature in °C
Hours after inoculation


Ewe 3726
12
Viraemia in mouse LD50 /0,025 ml
Body temperature in °C
Hours after inoculation

The haemagglutination inhibition test (HAI) was carried out with all available sera from recovered animals by the technique of Clarke & Casals (1958). A sucrose-acetone extract of WSL virus-infected mouse brain was used as antigen and the test performed at pH 6,4.

Results

Viraemia and body temperature in lambs and kids

The viraemia in all 4 lambs commenced at about 27 h after infection and was detectable for 50 h (Fig. 1–4), 2 main peaks being observed at 30 h and 50 h. The body temperature started rising between 35–45 h in 3 lambs (Fig. 1–3), dropped to normal at 55 h and rose again within hours. The fever continued for at least 90 h in 2 of the lambs (Fig. 1 & 2) and for about 110 h in the other two (Fig. 3 & 4).

WSL virus could be demonstrated within 18 h after inoculation in the blood of all 4 kids (Fig. 5, 6, 7 & 8). Viraemia reached a peak at about 35 h after infection in 3 of the kids (Fig. 5, 7 & 8), while in the 4th kid it was higher at 50 h (Fig. 6). At about 70 h the viraemia dropped in all four. The body temperature rose between 35 and 45 h after infection in all 4 kids, registered 2 main peaks (Fig. 6, 7 & 8) and became normal at about 120 h in 3 of them (Fig. 5, 6 & 7), whereas in the 4th kid (Fig. 8) it rose once more at 125 h.

Virus distribution in tissues of lambs

At 24 h after infection no virus could be detected in the tissues of the lamb that was killed (Table 1). At 48 h and 72 h respectively, virus was present in most tissues, the highest concentration being in the blood and then in the adrenal and liver (Table 1). The virus was present in almost every tissue of the lamb that died at 96 h after infection, although the lesions were restricted mainly to the liver and lymphatic tissue (Coetzer, Theodoridis & Van Heerden, 1978).

TABLE 1 Wesselsbron disease virus concentration in logs 10 mouse LD_{50}/0,025 mℓ in the tissues of experimentally infected lambs

	Lamb X11	Lamb 9981	Lamb 9996	Lamb* 9982
Killed hours post-inoculation	24	48	72	96*
Blood	<0,5	5,0	5,0	6,5
Liver	<0,5	1,5	2,5	2,5
Spleen	<0,5	<0,5	<0,5	1,6
Heart	<0,5	<0,5	<0,5	2,2
Adrenal	<0,5	3,6	3,5	1,5
Brain	<0,5	1,4	<0,5	2,5
Lung	<0,5	2,5	2,6	5,5
Kidney	<0,5	1,0	<0,5	3,7
Prescapular lymph node	<0,5	<0,5	2,2	2,5
Mesenteric lymph node	<0,5	<0,5	1,6	2,5

* Died shortly before post-mortem examination

Viraemia and temperature reaction in ewes

WSL virus could be demonstrated in the blood at about 50 h in 2 ewes (Fig. 10 & 11), at 58 h in the 3rd ewe (Fig. 9) and at 35 h in the 4th ewe (Fig. 12). Viraemia ceased at about 80 h after infection, giving an average duration of 30 h in 3 ewes, and 50 h in the fourth (Fig. 12). The body temperature started to rise at about 70 h (Fig. 10, 11 & 12) in 3 ewes, and 55 h in the fourth (Fig. 9). The highest temperature was recorded at about 100 h in 3 animals (Fig. 9, 10 & 11) and at 70 h in the fourth (Fig. 12). The fever outlasted the viraemia by at least 30 h in 3 cases out of 4 (Fig. 9, 10 & 11).

Virus distribution (assay) in tissues

A study was made of virus concentration in the tissues of 3 sheep and 2 goats (Table 2). These animals were killed at various intervals after infection for the study of the pathology, and the same material was used for studying the virus concentration. The highest virus titres were demonstrated in the liver and blood, and then in the spleen, lung and brain. WSL virus was present in fewer tissues in Sheep 4668, which was killed earlier, than in Sheep 3721 which was killed 23 h later (Table 2).

TABLE 2 Wesselbron disease virus concentration in logs 10 mouse LD_{50}/0,025 mℓ in the tissues of experimentally infected sheep and goats

	Sheep			Goats	
	3731	4668	3721	5340	1371
Killed hours post-inoculation	54	64	77	94	94
Blood	2,6	3,1	3,5	2,5	—
Liver	2,5	2,5	5,0	2,5	3,5
Spleen	1,1	1,1	4,3	<0,5	1,6
Heart	1,2	<0,5	1,2	—	—
Adrenal	1,8	<0,5	2,2	<0,5	<0,5
Brain	<0,5	<0,5	1,5	1,5	1,0
Lung	<0,5	1,4	2,6	1,1	—
Kidney	<0,5	1,0	—	<0,5	<0,5
Mesenteric lymph node	—	—	—	1,5	1,3

Development of antibodies to WSL virus

All the viruses reisolated from the blood and tissue samples of the inoculated lambs were neutralized by known WSL positive sheep serum. All the sera from the inoculated animals tested at random neutralized the known WSL virus (rusults not shown).

With very few exceptions, the majority of the inoculated ewes, goats, lambs and kids showed high HAI titres in their sera when bled in the 3rd week (Table 3). The mean value of the titre was the highest in the lambs and kids and then in the ewes and the goats. The latter had the lowest titre (Table 3).

Discussion

The WSL virus isolated from sheep during the most recent outbreak of the disease in 1974 caused fever in the majority of the infected lambs, kids, ewes and goats. The mortality rate among lambs was 27% (Coetzer *et al.*, 1978) and nil among adult animals. Viraemia was present for some time before the onset of fever but ceased several hours before the fever subsided. However, the overlapping period was long enough to allow blood samples to qualify as a diagnostic medium. The graphs of this study can be used as a guide as to the optimum time for collecting blood for diagnostic purposes.

TABLE 3 Convalescent haemagglutination inhibition titres of experimentally infected animals. Sera were taken 3–4 weeks after infection

Ewes		Lambs		Goats		Kids		Mean value
Nos.	Titre	Nos.	Titre	Nos.	Titre	Nos.	Titre	
5385	1280	4407	5120	1349	640	3959	2560	Ewes
5386	250	4414	1280	1362	280	3957	5120	2436
5389	2560	4409	1640	2155	320	3962	2560	
5390	1280	4408	1280	2518	640	3944	2560	Lambs
5393	1280	4413	1280	2519	640	3960	5120	3670
5397	320	4131	2560	2535	1280	3943	640	
5399	2560	4132	2560	2644	320	3961	2560	Goats
5402	1280	4133	2560	5658	160	3958	640	529
5403	5120	4135	2560	2677	320	3964	5120	
5408	2560	4136	2560	2684	640	3963	2560	Kids
5411	1280	4138	5120	2830	640	3989	5120	3233
5413	5120	4139	5120	2852	320	3990	1280	
5420	640	4232	2560	3111	160	3992	2560	
5421	5120	4234	5120	3138	160	3993	5120	
4123	5120	4235	1280	6563	320	3994	5120	
4124	2560	4236	5120			3996	2560	
4130	640	4017	1280			4020	5120	
4137	2560	4254	5120			4019	2560	
4148	1280	4257	5120			4018	2560	
4149	2560	4256	5120					
4152	2280	4253	5120					
4231	1280	4247	5120					
4233	5120	4249	5120					

The maximum virus titre in the blood of adult sheep was $4 \times 10^{4,5}$ mouse $LD_{50}/m\ell$, with a mean value of $4 \times 10^{4,2}$. Although this value is low, sheep could still be the species responsible for the maintenance of the virus in the years of interval between epizootics. One should also keep in mind the fact that under natural conditions the environmental stress factors or serial passages of the virus in sheep may be responsible for higher virus concentration in the blood. However, studies on host-insect relationship are necessary to establish such a possibility. Once lambs become infected during the course of an epizootic, insect transmission would be easier, since they develop a higher level of viraemia than adult animals.

Serology data show that cattle acquire WSL virus infection between the epizootics without any clinical symptoms being observed. This was the case with the sera of a substantial number of cattle collected from all provinces during the years 1969–1970, while the WSL outbreak only started becoming noticeable during 1974 (Theodoridis, unpublished results). Similarly, 3 years after WSL had subsided among sheep, young cattle were positive to this virus. Weiss (1957) also found serological evidence of infection among cattle after an epizootic in sheep.

These serological data indicate that cattle can be suspected as a natural reservoir of WSL virus. However, this assumption is challenged by the fact that, while experimentally infected cattle failed to develop viraemia (one of 15) and clinical symptoms, they invariably seroconverted (Coetzer *et al.*, 1979).

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