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RESEARCH COMMUNICATION

FLAVIVIRUSES IN SOUTH AFRICA: PATHOGENICITY FOR SHEEP

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ABSTRACT

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Sheep are susceptible to at least 5 of the 10 flaviviruses known to be present in South Africa. Sheep, 7–9 months of age, injected with Wesselsbron, West Nile, Banzi, Uganda-S and AR 5189 (an unidentified virus related to Banzi and Uganda-S), responded with a moderate febrile reaction, a low grade viraemia of short duration and the production of virus neutralizing antibodies. The most pronounced manifestations of infection were encountered in pregnant ewes. Infection with West Nile, Banzi and AR 5189 resulted in abortion, stillbirth and neonatal death, characterized by congenital abnormalities of the brain.

INTRODUCTION

Ten flaviviruses are known to be present in South Africa: Wesselsbron (WSL), Bagaza (BAG), Turkey meningo encephalitis (TME), Ntaya (NTA), Banzi (BAN), Uganda-S (UG-S), West Nile (WN), Usutu (USU), Spondweni (SPO) and AR 5189 (an unidentified virus related to BAN and UG-S) (Barnard & Voges, 1986), but only WSL is regarded as a cause of disease in sheep. WSL has a marked predilection for embryonic tissues and has been identified as an aetiological agent of abortion in sheep (Weiss, Haig & Alexander, 1956) that may also be associated with congenital malformations (Coetzer & Barnard, 1977). Neutralizing antibody surveys showed a country-wide distribution, with a high prevalence along the Natal coast (Smithburn, Kokernot, Heymann, Weinbren & Zentkowsky, 1959). Routine diagnostic procedures seldom yielded virus, but serological investigation indicated that flaviviruses, other than WSL, may be involved in some cases of abortion and neonatal death associated with congenital malformations. Experimental work with flaviviruses is reported on and discussed in this paper.

MATERIAL AND METHODS

Experimental animals and their susceptibility to infection

Dorper lambs, 7–9 months old, reared under insect-free conditions, were used to determine the susceptibility to the 10 viruses. No virus neutralizing antibody to the different viruses could be detected at the time of infection. Two lambs were used for each virus and were each injected intravenously with an infective mouse brain suspension, containing a calculated dose of approximately $5 \times 10^{3,5}$ mouse LD_{50}.

Temperatures and clinical features were monitored daily for 18 days after infection. Viraemia was determined by inoculating 0,02 mℓ of tenfold dilutions of blood intracerebrally into each of 2 litters of 1-day-old mice. The lambs were bled at regular intervals, the serum being separated and tested in a microneutralization test (Barnard & Voges, 1986). A rise in body temperature, demonstrable viraemia and antibody response were considered indications of infection in sheep.

Pathogenicity

The pathogenicity of WN, BAN, AR 5189 and USU was determined in pregnant Dorper ewes. Ewe lambs were reared under insect-free conditions up until 18 months of age, when their oestrus cycle was synchronized with Repromap (Medroxy progesterone acetate)* intravaginal sponges and they were hand mated. Ewes that dit not come on heat after mating were regarded as being pregnant. On Days 46, 52 and 60, respectively, after mating, the ewes were inoculated intravenously with a virus suspension containing approximately 10^5 mouse LD_{50}, according to the schedule in Table 1.

Temperatures were monitored daily, and the ewes were under regular observation. Blood and colostrum were collected from ewes at the time of lambing and blood from foetuses and lambs before suckling. Serum was separated for use in microneutralization tests. Autopsies were performed on foetuses and on lambs that died within 3 weeks of birth. Brain, liver and spleen were collected for virus isolation in 1 day old mice and embryonated hen's eggs.

RESULTS

Temperature reactions and viraemia of sheep inoculated with the different viruses are shown in Fig. 1. A moderate biphasic temperature occurred in lambs injected with WSL, WN and BAN. Lambs injected with AR 5189 showed a slight febrile response for 1 day only. One lamb injected with USU showed an elevated temperature on 2 consecutive days. Viraemia was detected only in lambs inoculated with WSL and WN. Lambs inoculated with BAG, ITM, NTA and SPO failed to exhibit fever or viraemia (not shown). No clinical signs of disease was seen in any lamb.

Results obtained in the microneutralization tests are depicted in Fig. 2. Antibodies were observed only in lambs that had shown a febrile response and in lambs inoculated with UG-S. Lambs, inoculated with WSL and WN in which viraemia could be demonstrated, mounted a slightly better antibody response than the others.

Twelve of the 16 infected ewes produced lambs (Table 1). Two ewes infected with WN and one each infected with AR 5189 and USU were either probably not pregnant or had aborted unnoticed shortly after infection. Viraemia was detected in only 1 ewe infected with WN. The same ewe also showed a moderate rise in body temperature 2 days after infection. Two other ewes, No. 1 infected with AR 5189 and No. 1 infected with USU, showed moderate temperature reactions on Day 13 and Days 2, 3 and 6, respectively. No clinical signs of infection were observed at any time, but all the ewes tested

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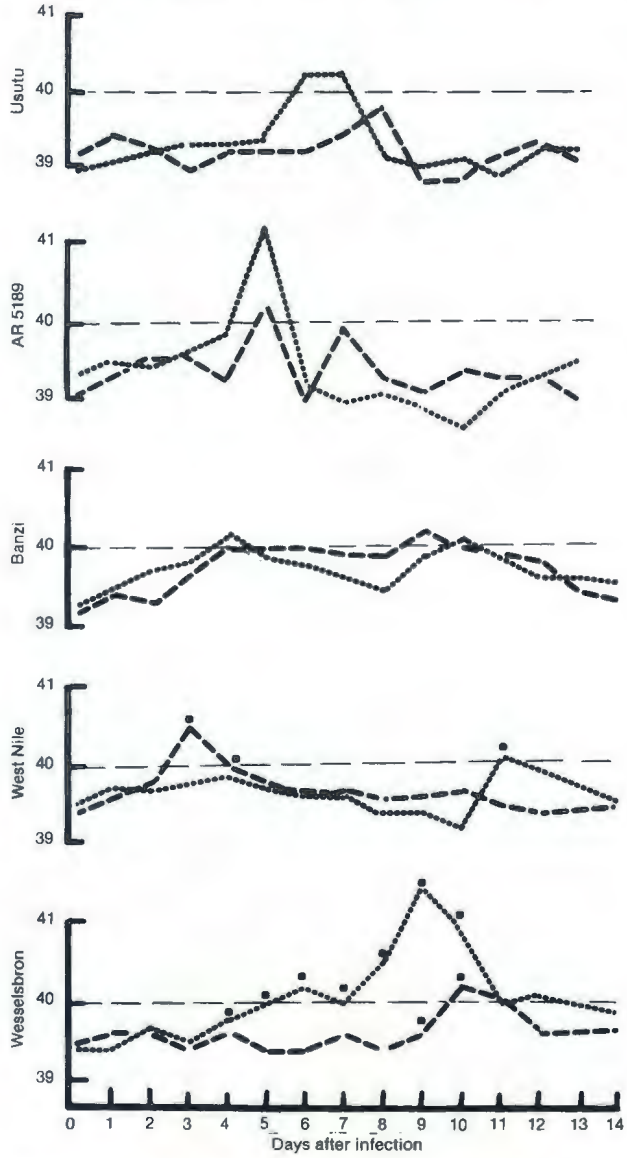

FIG. 1. Temperature reactions and duration of viraemia (▬) in lambs experimentally infected with flaviviruses. Each line represent one of two experimental animals injected with each virus.

responded by the production of virus neutralizing antibodies (Table 1). Antibody titres in colostrum were significantly higher than those of serum.

All the ewes, except one, lambed 146–149 days after mating. This is normal for Dorper ewes. Ewe No. 2, infected with AR 5189, expelled a small (±7 cm) badly macerated foetus 68 days after infection. The size of the foetus indicated that it had died shortly after the ewe became infected.

Abnormalities were observed only in lambs of ewes infected with WN, BAN and AR 5189. Internal hydrocephalus was seen in one lamb (Lamb No. 1, Ewe No. 3 infected with BAN) which appeared normal at birth but died 21 days later. Porencephaly and hydranencephaly were observed in weak and dumb lambs (Lamb No. 2, Ewe No. 1 infected with WN; Lamb No. 1, Ewe No. 2 infected with BAN; Lamb No. 2, Ewe No. 1 infected with AR 5189), and in one stillborn lamb (Lamb No. 2, Ewe No. 3 infected with BAN).

Four twins were born. In each of 2 twins one lamb was normal and the other one affected. No virus was isolated from foetal specimens nor could we demonstrate virus neutralizing antibody in precolostral serum.

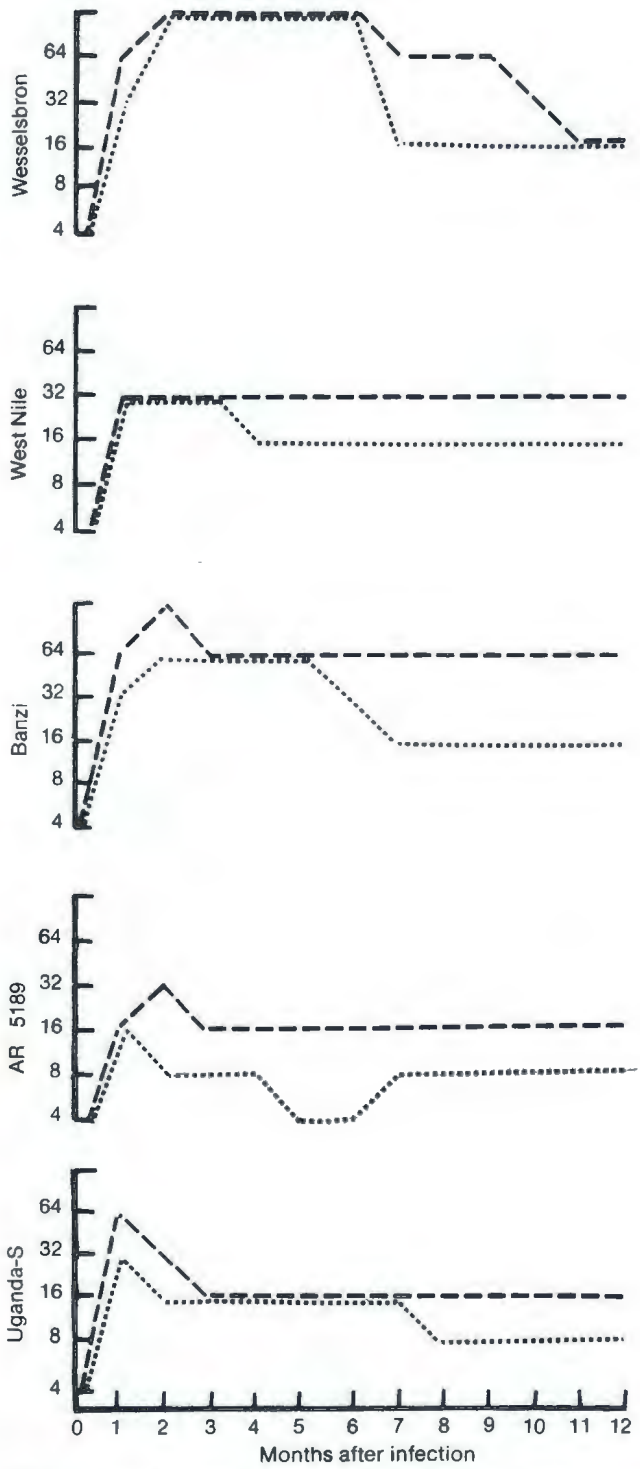

FIG. 2. Antibody titres of lambs 7–9 months old infected with 5 different flaviviruses. Each line represent one of two experimental animals injected with each virus.

DISCUSSION

In planning the present investigation, we assumed that infection with other members of the flaviviruses would follow a pattern similar to that of WSL infection. Experience gained with WSL was therefore taken as a guide-line.

WSL virus was initially isolated almost accidentally from a lamb that died during an investigation performed on ewes aborting after Rift Valley fever vaccination (Weiss, Haig & Alexander, 1956). Subsequent serological investigation indicated a widespread occurrence of WSL and the involvement of the virus in abortions, although isolation of the virus from field cases was rarely

TABLE 1 Reaction of pregnant Dorper ewes to experimental infection with WN, BAN, AR 5189 and USU virus

Virus	No.	Ewes[1]				Lambs			
		Viraemia	Fever	VNA[2]		No.	Abortion stillborn, weak, normal	Died on day	Developmental abnormalities
				Serum	Colostrum				
West Nile									
46[3]	1	+	+	16	64	1	Normal	—	None observed
						2	Weak	2	Hydranencephaly
60	4	—	—	64	1024	1	Normal	—	None observed
Banzi									
46	1	—	—	128	1024	1	Normal	—	None observed
46	2			16	1024	1	Weak	4	Porencephaly
52	3	—	—	32	512	1	Normal	21	Internal hydrocephalus
						2	Stillborn	—	Hydranencephaly
60	4	—	—	8	512	1	Normal	—	None observed
						2	Stillborn		NA
AR 5189									
46	1	—	+	8	32	1	Normal	—	None observed
						2	Weak	1	Hydranencephaly
46	2	—	—	32	ND[4]	1	Abortion	—	Decomposed
60	4	m241	—	32	128		Normal	—	None observed
Usutu									
46	1	—	+	ND	ND	1	Normal	—	None observed
52	3	—	—	ND	ND	1	Normal	—	None observed
60	4	—	—	ND	ND	1	Normal	—	None observed

[1] Only results of ewes that aborted or lambed are shown
[2] Antibody titre expressed as reciprocal of serum dilution
[3] Day of pregnancy on which virus was injected
[4] ND—not done

achieved (Weis *et al.*, 1956; Coetzer & Barnard, 1977; Fagbambi, 1980; Blackburn & Swanepoel, 1980). Experimental infection of pregnant sheep with WSL may result in abortion with isolation of the virus from foetuses aborted 1–2 weeks after infection (Weiss *et al.*, 1965 and Fagbambi, 1980) and/or congenitally malformed foetuses from which virus cannot be isolated (Coetzer & Barnard, 1977).

In this study there was a striking similarity between the reactions of sheep infected with WN, BAN, UG-S and AR 5189 and of those infected on previous occasions, with WSL (Weiss *et al.*, 1956; Coetzer & Barnard, 1977). No clinical signs were seen in adult sheep, but they showed a mild febrile reaction of short duration (Fig. 1). Viraemia was detected only in some sheep. All sheep showing a rise in body temperature also responded by the production of virus neutralizing antibodies.

The absence of any reaction in lambs infected with ITM, BAG, SPO, NTA and the absence of abnormalities in lambs from ewes infected with USU is no proof that sheep are not susceptible to these viruses, as the viruses used may have lost their pathogenicity during passage in mice.

The response in pregnant ewes (Table 1) is very similar to that seen in ewes infected with WSL (Coetzer & Barnard, 1977). Among the 13 lambs produced by ewes infected with WN, BAN and AR 5189, 1 abortion and 2 stillbirths occurred. Three lambs were weak and dumb at birth and died within 4 days. One lamb, which appeared normal, died 21 days later. Externally, all the lambs appeared normal. On autopsy, however, congenital abnormalities, including hydranencephaly, porencephaly and internal hydrocephalus, was seen in 5 lambs. The question arises as to how many similar cases have hitherto gone unnoticed. The lesions which were limited to the brain could possibly be overlooked in extensive farming practice, since foetuses are not always found and farmers are inclined to ascribe weak lambs to a number of other causes.

Although all the ewes infected with WN, BAN and AR 5189 responded by the production of humoral and colostral virus neutralizing antibody, no antibody was detected in the precolostral serum of lambs. This indicates either an insufficient stimulation, or more probably, foetal incompetence at the time of infection, as enhanced antibody response occurs only during the second half of gestation (Fahey & Morris, 1974).

The identification of additional neurotropic viruses, such as WN, BAN, AR 5189 and possibly UG-S (which were not injected into pregant ewes), increases the number of virological agents to be considered in cases of abortion, stillbirth and neonatal death. The inability to demonstrate flaviviruses in congenitally malformed foetuses is a tremendous hindrance in efforts to determine the aetiology and to evaluate the importance of the viruses involved. As WSL, WN and BAN are zoonoses, they may also affect the human foetus. Extensive epidemiological investigations seem necessary to elucidate the problem.

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REFERENCES

BARNARD, B. J. H. & VOGES, S. F., 1986. Flaviviruses in South Africa: Diagnostic procedures. *Onderstepoort Journal of Veterinary Research, 181–185.*

BLACKBURN, N. K. & SWANEPOEL, R., 1980. An investigation of flavivirus infection of cattle in Zimbabwe/Rhodesia with particular reference to Wesselsbron virus. *Journal of Hygiene,* 85, 1–33.

COETZER, J. A. W. & BARNARD, B. 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,* 45, 93–106.

FAGBAMBI, A. H., 1980. Susceptibility of West African Dwarf sheep to indigenous Wesselsbron virus. *British Veterinary Journal,* 136: 57–61.

FAHEY, K. J. & MORRIS, B., 1978. Humoral immune responses in foetal sheep. *Immunology,* 35, 651–661.

SMITHBURN, K. C., KOKERNOT, R. H., HEYMANN, C. S. WEINBREN, M. P. & ZENTKOWSKY, D., 1959. Neutralizing antibodies for certain viruses in the sera of human beings resident in northern Natal. *South African Medical Journal,* 33, 555–561.

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, 183–195.

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