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WESSELSBRON DISEASE: A CAUSE OF CONGENITAL PORENCEPHALY AND CEREBELLAR HYPOPLASIA IN CALVES

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ABSTRACT

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Fifteen pregnant cows were inoculated subcutaneously and intravenously between 101–147 days of gestation with the wild-type Wesselsbron disease virus. In addition, 2 foetuses were injected directly through the uterine wall after surgical exposure of the pregnant horn. The clinical symptoms, viraemia and serology in the cows are reported, as also the gross- and histopathology and the virological and serological results of the calves and foetuses.

Abortion was not an important manifestation of experimental Wesselsbron disease in cows, as it occurred in 3 animals only. Apart from a short temperature reaction in some cows no other clinical symptoms were recorded.

A viraemia was not always present in these cows and, when detected, was of low magnitude and short duration.

One cow, in which the foetus was inoculated at 115 days of gestation, aborted at 231 days. The foetus showed marked porencephaly and cerebellar hypoplasia.

Résumé

SUR LA MALADIE DE WESSELSBRON COMME CAUSE DE PORENCÉLHALIE CONGÉNITALE ET D'HYPOPLASIE CÉRÉBELLAIRE CHEZ LE VEAU

On a inoculé quinze vaches gestantes entre le 101*e et le* 147*e jour avec le virus type sauvage de la maladie de Wesselsbron, par voies sous-cutanée et intraveineuse. En outre on a procédé à une injection directe dans deux foetus à travers la paroi utérine après intervention chirurgicale pour mettre à nu la trompe gestante. On rend compte ici des symptômes cliniques, de la virémie et de la sérologie chez les vaches, ainsi que de la pathologie générale, de l'histopathologie et des résultats virologiques et sérologiques chez les veaux et les foetus.*

L'avortement n'a pas été une manifestation importante de la maladie de Wesselsbron expérimentale chez les vaches, car il ne s'est produit que chez 3 *animaux. On n'a pas noté d'autres symptômes cliniques, sinon une brève réaction thermique chez certaines vaches.*

Il n'y avait pas toujours de virémie chez ces vaches et, lorsqu'on en a décelé, elle était de faible amplitude et de courte durée.

Une seule vache, dont on avait inoculé le foetus au 115*e jour de la gestation, a avorté au* 231*e jour. Le foetus montrait une porencéphalie et une hypoplasie cérébellaire marquées.*

INTRODUCTION

Hydranencephaly, porencephaly, micrencephaly, hydrocephalus and cerebellar hypoplasia in new-born animals are well known malformations which can be the result of various congenital viral infections during the critical period of foetal brain development. Although in the past these teratogenic defects were frequently thought to have a hereditary background, in recent years convincing evidence has been presented that transplacental viral infection can cause such anomalies (Urman & Grace, 1964; Jubb & Kennedy, 1970; Hartley, De Saram, Della-Porta, Snowdon & Shephard, 1977).

Wesselsbron disease (WBD) virus was first isolated in the Republic of South Africa from an 8-day-old lamb (Weiss, Haig & Alexander, 1956). Apart from the original work on non-pregnant animals of various species and on pregnant sheep (Weiss *et al.*, 1956; Weiss, 1957), no experimental work had been done with this virus on pregnant cows. Coetzer & Barnard (1977), however, recently reported a syndrome of *hydrops amnii* in sheep associated with hydranencephaly and arthrogryposis in lambs inoculated with the attenuated Rift Valley fever (RVF) virus, attenuated WBD virus and with the wild-type WBD virus. In view of these findings it was decided to investigate the effect of the wild-type WBD virus on the pregnant cow and foetus.

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MATERIAL AND METHODS

Animals

Eighteen artificially inseminated cross-bred Africander cows were used in the experiment. As soon as pregnancy was confirmed, the cows were moved to an insect-free stable where they were kept under daily observation. The cows or foetuses were infected with WBD virus at different stages of gestation. Five cows were inoculated subcutaneously (s.c.), 10 cows intravenously (i.v.), while 2 foetuses were injected through the uterine wall after surgical exposure of the pregnant horn. One cow was used as a control. The temperatures of the cows were taken twice daily and any clinical signs were recorded for the first 12–14 days following inoculation. After this the animals were inspected once daily for the duration of the experiment (Table 1).

Surgical procedure

The pregnant horn was determined by rectal palpation. The animal was then sedated with 3 mℓ of a 2% solution of Xylacine* HCl given intramuscularly and the operation site was locally infiltrated with a 2% solution of lignocaine with adrenalin. After surgical exposure of the uterus the foetus was located and inoculated intramuscularly through the uterine wall with the WBD virus. The muscles and skin were opposed, using chromic gut and silk, respectively.

Source of virus inoculum

The WBD virus (Misc 27/74) used as the inoculum had undergone 3 intracerebral passages in day-old suckling albino mice. The brains of the sick and dead

* Rompun V Bayer, Agro-Chem.

TABLE 1 Gestation, route of inoculation with WBD virus, result of infection and serology of the cows, calves and foetuses

No. of cow	Stage of gestation when inoculated (days)	Route of inoculation	Temperature reaction	HI titre to WBD virus of cows before inoculation	HI titre to WBD virus of cows three weeks after inoculation	Birth of a normal calf	Abortion	Foetus with porencephaly and cerebellar hypoplasia	Precolostral HI titre to WBD virus in calves or foetuses
7106.....	101	s.c.	+	1:10	1:2560	+			1:10
8758.....	102	s.c.	+	1:10	1:1280	+			1:10
8877.....	135	s.c.		1:10	1:2560	+			1:10
8166.....	144	s.c.		1:10	1:640	+			1:5120
6031.....	147	s.c.		1:10	1:2560	+			1:10
9574.....	104	i.v.	+	1:10	1:1280	+			1:10
8146.....	108	i.v.	+	1:10	1:320	+			N/D
6803.....	110	i.v.	+	1:10	1:1280	+			1:10
9374.....	110	i.v.	+	1:10	1:640		+		1:10
9180.....	111	i.v.		1:10	1:1280	+			1:10
210.....	113	i.v.	+	1:10	1:160	+			1:2560
7940.....	119	i.v.		1:10	1:80	+			1:10
9617.....	121	i.v.	+	1:10	1:1280	+			N/D
8172.....	123	i.v.		1:10	1:640	+			1:10
9319.....	144	i.v.		1:10	1:80	+			N/D
9600.....	113	F		1:10	N/D		+		N/D
8865.....	115	F		1:10	1:160		+	+	1:10

s.c.=subcutaneously
i.v.=intravenously
F=direct foetal
N/D=not done

mice were harvested, homogenized and made up as a 10% suspension in buffer lactose peptone (BLP**). Ampoules with 0,5 mℓ of the suspension were lyophilized and sealed. The titre of this virus was $3\times10^{5,8}$ MLD_{50}/mℓ. After the contents of the ampoules had been reconstituted for inoculation to the original volume with distilled water, the cows were injected i.v. and s.c. with 3,0 mℓ and the foetuses with 1,0 mℓ of this suspension.

Haemagglutination inhibition assay

Blood for the serological assay was collected in sterile 10 mℓ vacuum tubes from each of the cows before infection and again 3 weeks after inoculation with the WBD virus. Precolostral sera were collected from the calves and foetuses shortly after birth or abortion.

The haemagglutination inhibition (HI) technique described by Clark & Casals (1958) was used to determine antiviral titres in the sera of the cows, calves and aborted foetuses. A sucrose-acetone extract of WBD infected mouse brain was used as antigen and the test was performed at pH 6,4.

Virus isolation

For viraemic studies, blood from 3 cows was collected in 10 mℓ heparinized vacuum tubes every 10–12 hours for the first 6–8 days after inoculation.

** BLP=Final concentration of 1% peptone and 5% lactose in 1/10 M phosphate buffer.

At autopsy, liver, spleen, brain, kidney and lung were collected in sterile bottles from the calves and aborted foetuses for virus isolation. These specimens were ground and made up to a 10% suspension in BLP and injected into suckling albino mice by the intracerebral route. The brains of the sick and dead mice were harvested and suspended in BLP and the virus was then identified by means of a serum neutralization test, using the serial virus dilution constant serum technique (Cunningham, 1960). The organ suspensions were titrated, using 10-fold dilutions in BLP, and families of day-old albino mice were then infected intracerebrally with each dilution. The MLD_{50}/0,03 mℓ was calculated by the method of Reed & Muench (1938).

The blood collected from the 3 cows for viraemic studies was treated in the same way as the above specimens, except that the MLD_{50} was not determined.

Pathology

Autopsies were performed as soon as possible after birth or abortion on all the calves and foetuses. A wide range of tissues was collected in 10% buffered formalin. The formalin-fixed tissues were routinely processed and embedded in paraffin wax. Sections were cut at 3–4 μm and stained with haematoxylin and eosin (HE). Coronal sections 3–4 mm thick were made from the whole brain which was embedded in agar, and examined grossly, and suitable blocks selected for microscopical examination.

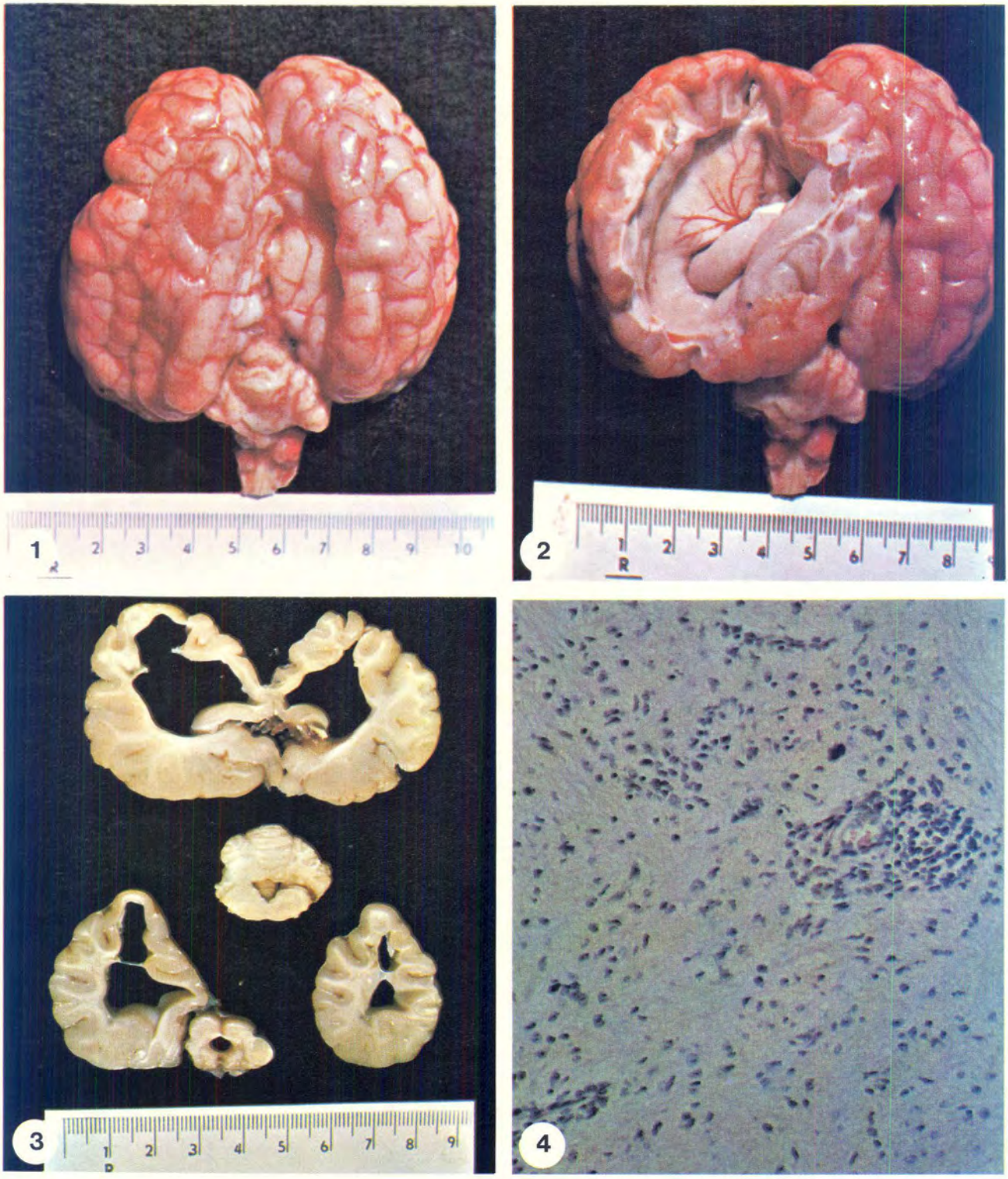

FIG. 1 Cerebral hemispheres flabby and partially collapsed. Note cerebellar hypoplasia
FIG. 2 Ventricles markedly dilated
FIG. 3 Cavitation of the cerebral subcortical white matter
FIG. 4 Perivascular mononuclear cell cuffing and mineralization in the brain. HE×75

Results

Temperature reactions and clinical symptoms

Of the 15 cows inoculated only 1 showed a significant rise in temperature (40,0–40,8 °C), 7 developed a slight temperature (39,4–40,0 °C), while the body temperature of the rest remained normal (Table 1). The temperature reaction occurred approximately 44–125 h after infection and usually lasted for 12–24 h only. No other clinical signs were noted in any of the cows. Because of the surgical procedure which preceded inoculation, the temperature reaction of the 2 cows in which the foetus had been inoculated was not taken into account.

Three cows aborted during the course of the experiment, while the rest gave birth to normal calves (Table 1). Cow 9374 was infected i.v. at 110 days of gestation and aborted 1 month later. Since autolytic changes in this foetus were advanced, no histopathological examination or virus isolation was done. In cows 9600 and 8865 the foetuses were inoculated directly. The foetus of cow 9600 was injected at 113 days of age and a badly decomposed foetus was aborted 8 days later. No specimens of this case were collected for further examination. The foetus of cow 8865, inoculated at 115 days of gestation, was aborted, apparently alive, at 231 days of gestation, but died shortly after. This foetus showed various brain abnormalities (*vide infra*).

Haemagglutination inhibition assay

The serum for antiviral HI titres of the pregnant cows was collected before inoculation and again 3 weeks later. All the cows showed a rise in HI antibodies, the titres recorded ranging from 1:80 to 1:2560 (Table 1).

Only 2 of the calves, one born out of cow 8166, infected at 144 days of gestation and the other out of cow 210, infected at 113 days of gestation, contained HI antibodies to WBD virus (Table 1).

Gross pathology

Fourteen clinically normal calves were born from the cows infected with WBD virus. No lesions were seen macroscopically in any of these calves. Gross lesions were seen only in the aborted foetus of cow 8865. This foetus was smaller than normal, appeared slender and more bony, with the head slightly domed.

The cerebral hemispheres were flabby and partially collapsed (Fig. 1), while the brain did not fill the whole of the calvarium. The gyri were well-developed except for a few of them located in the dorsal part of the occipital lobes of the cerebral hemispheres, which were more flattened. Over the greater part of the cerebral hemispheres the grey and white matter (especially the latter) was reduced to a layer 4–6 mm thick, while the ventricles were markedly dilated and filled with a clear watery fluid (Fig. 2). The frontal and occipital lobes of the cerebral hemispheres were slightly better developed and cavities of varying size were located in the subcortical white matter. These were more marked in the frontal and occipital lobes and were usually separated from the ventricles by a thin membrane (Fig. 3). Occasionally these cavities communicated with the ventricles, probably as a result of the tearing of the membrane during the preparation of the coronal sections.

Apart from porencephaly, cerebellar hypoplasia was the most prominent feature in this foetus (Fig. 1 & 2). The folia were still discernible but were markedly diminished in size. The basal parts of the brain, the spinal cord, peripheral nerves as well as the other organs seemed normal.

Histopathology

Few histopathological changes were observed in the brain of the porencephalic foetus. In general, the neuropil throughout the brain appeared to be less cellular because of the loss of neurones and glial cells. Notwithstanding the gross cerebellar hypoplasia, the architecture and histological ratio of the different cell layers in the cerebellum were within the normal range. There was no indication of gliosis or perivascular mononuclear cell cuffing in either the cerebrum or cerebellum. The perivascular spaces, and especially those in the vicinity of the ventricles, were markedly dilated.

Although the calf of cow 210 appeared clinically normal at birth and no gross lesions were seen in the brain, it had a single focal area of encephalomalacia with mononuclear cell cuffing (mainly lymphocytes) of the adjacent blood vessels in the thalamic area. Small foci of mineralization were noted in the affected part of the brain and a few macrophages containing yellow-brown pigments could be detected in the perivascular spaces surrounding this area of encephalomalacia (Fig. 4).

Apart from the brain lesions described for these 2 calves, no other histopathological lesions were observed in the tissues of any of the calves examined.

Virus isolation

Of the 3 cows (8172, 9617, 7940) on which viraemic studies were made, only cow 9617 developed a measurable viraemia 77–101 h post-inoculation. This viraemia was of low magnitude and of short duration and was accompanied by a temperature reaction of 40,0–40,8 °C. No rise in body temperature was recorded in the other 2 cows.

No virus could be isolated from the blood, brain, liver, spleen, lung, kidney or lymph nodes of any of the calves or aborted foetuses.

Discussion

Many cases of hydranencephaly in new-born calves in the Republic of South Africa have been observed during the past 5–6 years (J. G. Pienaar & J. A. W. Coetzer 1978, unpublished observations). These calves were either aborted, stillborn, or alive. When alive they were frequently recumbent and unable to rise, and showed nystagmus, head tremors, opisthotonus, galloping movements with the legs and an inability to suckle without assistance. Others walked around aimlessly or were ataxic, showing little interest in their surroundings, though they could suckle to some extent. They were usually in a poor and dehydrated condition.

Sound evidence has been presented that various congenital brain defects such as hydranencephaly, porencephaly, micrencephaly and cerebellar hypoplasia in cattle could result from clinical or subclinical viral infection of the dam. The viruses incriminated include the following: both the modified and wild-type blue-tongue (BT) virus (McKercher, Saito & Singh, 1970; Richards, Crenshaw & Bushnell, 1971; Barnard & Pienaar, 1976), Akabane virus (Inaba, Kurogi & Omori, 1975; Hartley, De Saram, Della-Porta, Snowdon & Shephard, 1977), and bovine viral diarrhoea-mucosal disease (BVD-MD) virus [Kahrs, Scott &

De Lahunta, 1970 (a, b, c); Brown, De Lahunta, Scott, Kahrs, McEntee & Gillespie, 1972; Scott, Kahrs, De Lahunta, Brown, McEntee & Gillespie, 1972]. It is clear from the results obtained during this study that the wild-type WBD virus should also now be added to the list of viruses which may have a deleterious effect on the bovine foetal brain.

Certain foetal, maternal and viral factors must be taken into account when interpreting the severity and incidence of lesions or deformities in the foetus or new-born animal. Many of the malformations are seen only several months after the acute stage of the disease in the dam when the conceptus is aborted or when the malformed animal is born. Fucillo & Sever (1973), in a discussion of various important factors related to transplacental infections, indicated that, if morphogenesis is incomplete at the time of foetal infection, the pregnancy might terminate in stillbirth or abortion, with malformation or degenerative lesions in the foetus. It is well known that the central nervous system (CNS) of the foetus is especially vulnerable to infection and teratogenic defects during the critical period of CNS development. This critical period varies from one animal species to another and is linked with the duration of gestation (Osburn, Silverstein, Prendergast, Johnson & Parshall, 1971; Barnard & Pienaar, 1976; Coetzer & Barnard, 1977; Parsonson, Della-Porta & Snowdon, 1977).

Other factors which render the foetus more susceptible to infections are the degree of immune competence, underdeveloped lymphoid tissue, the rate of clearance by the reticuloendothelial (RE) system and decreased interferon production (Fucillo & Sever, 1973; Schultz, 1973).

The abnormal foetus reported here showed porencephaly and cerebellar hypoplasia. It is of interest to note that certain congenital viral infections in cattle such as BT and Akabane disease result in hydranencephaly, porencephaly and micrencephaly, whereas in BVD-MD cerebellar hypoplasia is the prominent lesion. Hoewever, in some viral infections (WBD and RVF viruses) in sheep, the cerebellum as well as the cerebrum can show teratogenic defects (Coetzer & Barnard, 1977). The selective destruction of rapidly multiplying cells in the developing CNS by different viruses might explain these anomalies. It was shown by Osburn, Johnson, Silverstein, Prendergast, Jochim & Levy, (1971) that the modified BT virus affects mainly the cells in the ventricular and subventricular zones. They postulate that the arrest of cell migration from these zones to the cerebral cortex results in the formation of hydranencephaly or porencephaly. On the other hand, cerebellar hypoplasia follows after the destruction of the cells in the granular layer of the cerebellum (Brown *et al.*, 1972).

Johnson & Mims (1968) indicated that the duration and magnitude of viraemia were critical in the invasion of the CNS and that growth and seeding of virus from extraneural sites might be just as important in this respect. These findings were corroborated for wild-type WBD virus in the experimental work on sheep where it was found that, apart from its specific hepatotropic properties (Coetzer, Theodoridis & Van Heerden, 1978), the virus also possessed marked latent neurotropic properties (Coetzer & Barnard, 1977). In the present study it was shown that a temperature reaction and viraemia were not always present after experimental infection in the cow or that, when detected, they were of low magnitude and of short duration.

The same phenomenon was also described by Coakley, Pini & Gosden (1967) in experimental infection of bovines with RVF virus. The relationship between maternal and foetal infection is complex because of the presence of the placenta, which changes during the course of gestation (Winsatt, 1950), and it is thus possible that, with a low viraemia, as found in this study, the chances of transplacental infection are reduced.

Where the cows were infected between 101–147 days of gestation, abortion was not an important manifestation of WBD in our study. The abortion which did occur in cow 9600 could probably be ascribed to the surgical procedure or trauma which followed the manipulation of the foetus. Swanepoel (1976) reported that it would appear that WBD virus is widespread in Rhodesia but that it does not seem to be a cause of serious disease or abortion in cattle. No important clinical symptoms were recorded by us other than a short temperature reaction in some of the cows.

Two of the calves (from cow 8166 and cow 210) contained antibodies to WBD virus. Both of them were clinically normal and showed no symptoms of CNS disturbance. While no histopathological lesions could be detected in the CNS or any other organ of the calf born from cow 8166, focal encephalomalacia and other inflammatory changes were seen in the thalamic area of the other calf. As both of them responded immunologically to WBD virus it is clear that they were transplacentally infested, though the infection could only be correlated with brain lesions in one of them.

The negative serological results in the other calves and foetuses might be explained by the fact that transplacental infection had not taken place either because of low viraemia or the absence of viraemia in the mother or that, although infected, the foetus was not immunologically competent at that stage of development.

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