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HYDROPS AMNII IN SHEEP ASSOCIATED WITH HYDRANENCEPHALY AND ARTHROGRYPOSIS WITH WESSELSBRON DISEASE AND RIFT VALLEY FEVER VIRUSES AS AETIOLOGICAL AGENTS

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ABSTRACT

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 viruses as aetiological agents. *Onderstepoort Journal of Veterinary Research* 44 (2), 119–126 (1977).

During the 1974/75 lambing season numerous reports were received from various parts of the Republic of South Africa and South West Africa of severe abdominal distension in ewes after vaccination with the attenuated Rift Valley fever and/or attenuated Wesselsbron disease vaccine. The ewes were vaccinated at different stages of gestation in spite of recommendations to the contrary, the syndrome being especially obvious in ewes immunized with one or both of these vaccines during the first trimester of pregnancy. In some of the flocks *hydrops amnii* was recorded in as many as 15% of the ewes. Many of the ewes so affected showed a prolonged gestation of up to 6–7 months and, towards the end of gestation, were unable to rise or walk. They eventually died of ketosis, hypostatic pneumonia and complications due to dystocia.

The foetuses examined were malformed and larger than normal with a mass of 3,6–6,7 kg. They usually showed arthrogryposis, brachygnathy inferior, hydranencephaly, hypoplasia or segmental aplasia of the spinal cord and neurogenic muscular atrophy.

The amnion contained 8,0–18,0 l of amniotic fluid, the endometrium was oedematous, and cystic tube-like dilatations, 1–10 mm in diameter, filled with a clear fluid, were scattered in the endometrium.

No definite conclusions as to the aetiology of the syndrome could be drawn from serological tests performed on the ewes, lambs or foetuses.

Preliminary experimental work confirmed previous observations that the attenuated Wesselsbron disease vaccine virus is responsible for this syndrome and that the wild-type virus is also implicated. In addition, the attenuated Rift Valley fever vaccine virus was found to the responsible for arthrogryposis and hydranencephaly without *hydrops amnii* and for micrencephaly and arthrogryposis associated with *hydrops amnii* in the ewe.

Résumé

Hydrops amnii *chez le mouton, associé à l'hydranencéphalie et l'arthrogrypose, avec pour facteurs étiologiques les virus de la maladie de Wesselsbron et de la fièvre de la Rift Valley.*

Pendant la saison d'agnelage 1974/75 *de nombreux rapports ont été reçus, de différents endroits de la République d'Afrique du Sud et du Sud-Ouest africain, signalant une grave distension abdominale chez des brebis après leur vaccination par les virus atténués de la fièvre de la Rift Valley et/ou de la maladie de Wesselsbron. Malgré les recommandations faites, les brebis avaient été vaccinées à divers stades de leur gestation et le syndrome était particulièrement frappant chez les brebis qui avaient été immunisées au moyen d'un de ces vaccins, ou des deux, pendant le premier trimestre de leur gestation. Dans certains troupeaux l'*hydrops amnii *avait affecté jusqu'à 15% des brebis. Beaucoup de ces animaux ont eu une gestation prologée, allant jusqu'à 6–7 mois et, vers la fin de la gestation, ils étaient incapables de se redresser ou de marcher. A la fin ils sont morts de cétose, pneumonie hydrostatique et complications dues à la dystocie.*

Les foetus examinés étaient mal formés et d'une grosseur anormale, pesant de 3,6 à 6,7 kg. Ils montraient généralement de l'arthrogrypose, du brachygnathisme inférieur, de l'hydranencéphalie, de l'hypoplasie ou de l'aplasie segmentaire de la moëlle épinière et de l'atrophie musculaire neurogénétique.

L'amnios contenait de 8 à 18 l de liquide amniotique, l'endomètre était oedémateux et parsemé de dilatations cystiques tubiformes d'un diamètre de 1 à 10 mm, pleines d'un liquide clair.

Des tests sérologiques effectués sur les brebis, les agneaux ou les foetus n'ont pas livré de conclusions nettes quant à l'étiologie de syndrome.

Des expériences préliminaires ont confirmé des observations antérieures d'après lesquelles le virus atténué du vaccin contre la maladie de Wesselsbron est responsable de ce syndrome; le virus de type sauvage y est aussi impliqué. En outre le virus attênuê du vaccin contre la fièvre de la Rift Valley a été reconnu responsable de l'arthrogrypose et de l'hydranencéphalie sans hydrops amnii *ainsi que de la micrencéphalie et de l'arthrogrypose associées à l'*hydrops amnii *chez la brebis.*

INTRODUCTION

It is well known that certain viruses have a special predilection for the developing foetal central nervous system (CNS) and may provoke various congenital malformations and inflammatory lesions in the immature foetal brain. These abnormalities in the foetal CNS may be associated with arthrogryposis in the young and/or *hydrops amnii* in the mother.

Natural cases of arthrogryposis and hydranencephaly of the foetus or lamb, associated with *hydrops amnii* in the ewe, are described in this report. The attenuated Rift Valley fever (RVF) and/or attenuated Wesselsbron disease (WBD) vaccines and, in addition, the wild-type WBD virus were suspected of being the cause of these manifestations.

Preliminary experimental work with these agents was conducted in pregnant sheep in an attempt to reproduce the syndrome. The results of the experiments are briefly recorded and will be discussed in detail in a subsequent paper.

TERMINOLOGY

For the sake of clarity some of the terms used to describe anomalies of the foetal brain and foetal membranes are briefly defined.

Hydranencephaly: According to Osburn, Silverstein, Prendergast, Johnson & Parshall (1971), the cerebral hemispheres are reduced, in whole or in part, to membranous fluid-filled sacs. Hydranencephaly can

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be distinguished from hydrocephalus and anencephaly in that the head of the animal is usually of normal size. Many authors, when referring to hydranencephaly, use the terms hydrocephalus, ox-bladder brain, Rinderblasen-porencephalie, hydromicrocephaly, porencephaly or schizencephaly.

Porencephaly: This condition is characterized by cystic defects in the cerebrum which may or may not communicate with the ventricles or subarachnoid space or with both (Naef, 1958).

Arthrogryposis: Arthrogryposis denotes congenital deformities of the limbs, accompanied by muscle atrophy and flexion and fixation of the joints. It may be accompanied by a cleft palate and *spina bifida.*

Hydrops amnii or *Hydramnios:* In this condition there is a gradual enlargement or filling of the amniotic cavity associated with a genetic or congenitally defective foetus (Roberts, 1971).

Hydrallantois: Hydrallantois is the accumulation of allantoic fluid in the allantois and is usually associated with a diseased uterus (Roberts, 1971).

History

During the 1974/75 lambing season numerous reports were received from the north-western Cape Province, southern parts of South West Africa and Western Transvaal of abortions or ewes developing *hydrops amnii* with resultant dystocia, a syndrome which occurred in 15% of the flocks on certain farms.

Various deformities were observed in lambs that died shortly after birth. Because of the expanse and relative isolation of the areas concerned, it was not possible to investigate all the outbreaks in detail and, in addition, many farmers were unable to provide exact dates of matings, vaccinations, etc. The detailed information from a number of farms, however, is summarized in Table 1.

It soon became evident from the histories provided that, in all instances where *hydrops amnii* occurred, pregnant ewes had been vaccinated with the attenuated RVF vaccine, and sometimes, in addition, with the attenuated WBD vaccine, in spite of recommendations to the contrary, evidently because outbreaks of the natural disease of RVF and WBD were prevalent in the areas at the time.

The most obvious clinical finding in the ewes was the marked abdominal distension (Fig. 1). Most of the ewes had difficulty in walking and some towards the end of pregnancy also had difficulty in rising. As a result, many ewes died from secondary ketosis or hypostatic pneumonia and many of the affected ewes were in a poor or even emaciated condition.

Arthrogryposis in the lambs caused dystocia and subsequent death in a very high percentage of ewes.

The lambs were apparently alive until the commencement of parturition but died because of the abnormal positioning of the limbs and head during the delayed birth process. Some of the lambs were born alive but unable to stand, as their front legs were flexed and crooked, and, in one case, convulsions and circling movements were reported.

Prolonged gestation periods of up to 6–7 months were recorded in some flocks, and one farmer reported regression of udder development in 150 ewes, apparently due to foetal resorption or early abortion.

Materials and Methods

Investigation of field material

Twenty-two (14 Karakul, 6 Merino, 2 Dorper) ewes were examined. During transportation 3 ewes died and 5 aborted, with the result that in these cases measurement of the amniotic and allantoic fluids was not possible, neither could the foetal membranes be examined. A caesarian section was performed on one ewe because of her high breeding potential, and complete autopsies were done on the remaining 13 ewes and foetuses after euthanasia, the contents of the amnion and allantois being measured and mass of the foetuses determined. The specific gravity (S.G.) of the amniotic and allantoic fluids was determined with a chemical balance.

Virus isolation

Foetal specimens from the liver, spleen, kidney, lung, brain, ascitic fluid, amniotic and allantoic fluids and placenta were collected for virus isolations. Some of these specimens were pooled but the majority were processed separately. Approximately 1,0 g of tissue was minced and mixed with 10 ml of a phosphate buffer containing 1,0% peptone and 5,0% lactose as well as 500 units penicillin and 500 microgrammes streptomycin. The suspension obtained was left at room temperature for 1–2 h. Isolation was performed in mice by intracerebral injection of 0,03 ml of the supernate into 2 families of 1–3-day-old albino mice. The mice were observed for 18 days. The brain material of mice that died was collected and treated in the same way as the original specimens.

TABLE 1 Information derived from the presence of *hydrops amnii*

Farm	Stage of pregnancy (days)	No. of ewes vaccinated	Vaccine used*	No. of *hydrops amnii* cases	No. of abortions
A	90	350	RVF	13	35
B	30–35	400	RVF WBD	50	—
C	30–45	150	RVF WBD	37	—
D	30–45	—	RVF	54	—
E	90–105	800	RVF	106	350

* RVF = Rift Valley fever
WBD = Wesselsbron disease
— = Unknown

Serological tests

Serum specimens from 18 ewes and 11 foetuses were available. The haemagglutination inhibition (HI) technique described by Clark & Casals (1958) was used to determine antiviral titres. A sucrose-acetone extract of RVF infected hamster liver and WBD infected mouse brain, respectively, were used as antigen. The HI test for RVF was performed at pH 6,1 and that for WBD at pH 6,4.

Experimental cases

Of the 33 pregnant Merino ewes used, 29 were 42–74 days pregnant. These were infected intravenously (I/V) or subcutaneously (S/C) or the foetus was injected through the uterine wall after exposure of the pregnant uterus (Table 4). Eleven ewes were infected with wild-type WBD virus, 9 ewes with the attenuated WBD vaccine and 9 ewes with the attenuated RVF vaccine. Four ewes were used as controls. The animals were kept in a reasonably insect-free stable and were observed daily.

Serum samples from ewes, lambs or foetuses were collected for serological tests after abortion, after the birth of an abnormal or normal lamb, or when the ewe was sacrificed for autopsy.

Virus isolations from foetal tissues were done as described above.

Results

Gross Pathology

Natural cases

(i) *Foetuses*

The main features of the syndrome are noted in Table 2.

Ten of the 22 foetuses examined were alive when removed from the uterus. Movements of the limbs were limited and respiration was extremely shallow. The pelts of some of the Karakul lambs that were alive had a hairy appearance, especially on the sacral region. A clear, yellow, watery to slightly gelatinous fluid was present subcutaneously and intramuscularly in most of the foetuses, being so abundant in some cases that the carcasses had a water-logged appearance. One foetus had a subcutaneous haematoma, 5–7 cm in diameter, on the lateral surface of the neck.

Postural abnormalities

Except in 1 case, all the lambs showed pronounced arthrogryposis (Fig. 2), the main postural abnormalities being: brachygnathy inferior, torticollis, sometimes with rotation round the long-axis of the spine, thoracolumbar lordosis and scoliosis (Fig. 3). The limbs were contracted to varying degrees and the joints fixed in a flexed position. In the live foetuses, movement of the mandible was very restricted.

Central nervous system

The developmental abnormalities observed in the foetal CNS are summarized in Table 3. These ranged from a virtual absence of the brain to hydranencephaly where all the main structures were present, but diminished in size. In 21 foetuses the cerebral hemispheres were either absent or reduced to membranous, semitranslucent sacs, or vesicles, containing a clear watery fluid (Fig. 4).

While most cerebral hemispheres were 1–2 mm thick, two foetuses had cerebral hemispheres 2–4 mm thick with shallow gyri and focal chalky, white areas 2–4 mm in diameter scattered throughout the hemispheres (Fig. 5), giving them a hard consistency after formalin fixation. Thirteen foetuses had aplasia of the cerebellum (Fig. 5 & 6) and, in 5, marked hypoplasia of the cerebellum was evident (Fig. 5). The different parts of the midbrain were either underdeveloped or absent. Although very rudimentary, the thalamus was as a rule recognizable (Fig. 5 & 6) and in 4 cases chalky, white, brittle plaques 1–10 mm in diameter were found in the thalamic area (Fig. 6). A large haemorrhagic mass containing mineral deposits, approximately 1 cm in diameter and transversed by tortuous blood vessels, was found in the thalamic area of one foetus. Aplasia and hypoplasia of the hippocampus were constant features and, in 2 cases, the hippocampus was only unilaterally developed (Fig. 5). All the foetuses had a medulla oblongata, but in 10 cases this structure consisted only of the 2 parallel pyramidal tracts passing down into the spinal cord. There was no fusion of the tracts and a space about 2–3 mm wide separated them (Fig. 5).

Although no obvious lesions were present in the various peripheral nerves examined, the optic, acoustic and other cranial nerves were obviously hypoplastic (Fig. 7).

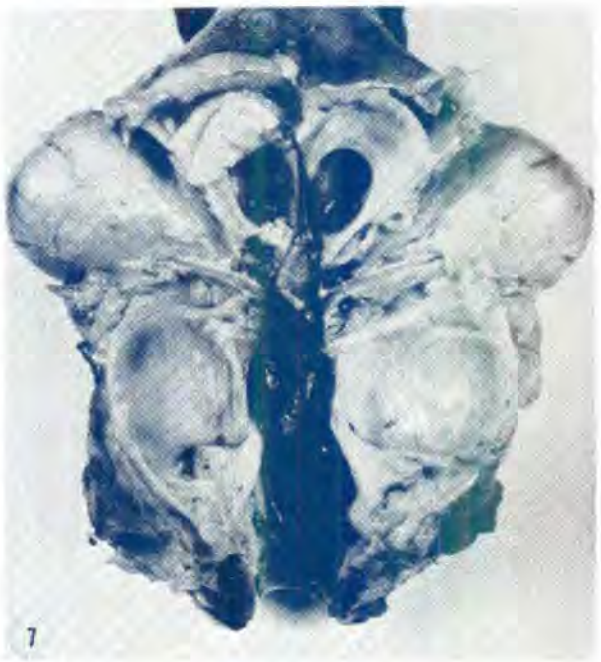


FIG. 7 Hypoplasia of the optic nerves. Only the collapsed cerebral hemispheres and vestiges of the midbrain are present in the cranial cavity

No enlargement of the calvarium was seen and there was no delay in the union of the sutures. In some foetuses the frontal bones were thickened, compressed and flattened. The appearance of the dura mater varied from a thick fibrotic to a thin translucent membrane and, in one foetus, petechial and ecchymotic haemorrhages were present in its inner surface.

The spinal cord was hypoplastic to varying degrees in all the foetuses (Fig. 8). In addition, segmental aplasia was found in one foetus. An oedematous, sometimes gelatinous fluid, surrounded the spinal cord.

TABLE 2 Main features manifested in the ewe and foetus

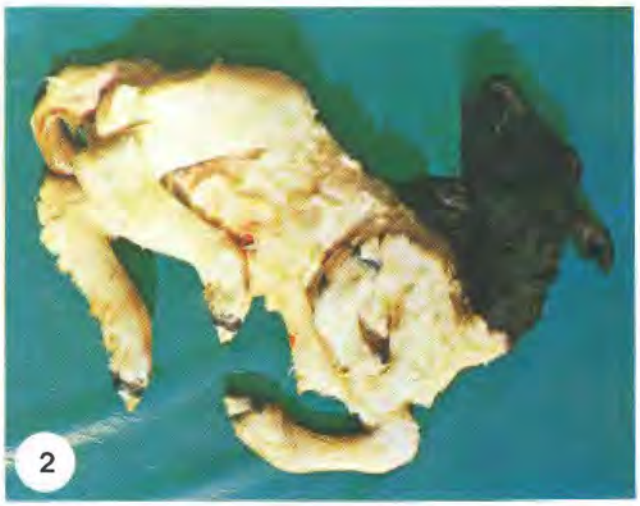
Case No.	Foetus: Arthrogryposis	Hydranencephaly	Brachygnathy inferior	Muscle atrophy	Alive	Dead intrauterine	Aborted	Mass (kg)	Amniotic fluid (litres)	Allantoic fluid (litres)	Ewe: Amniotic membranes: Normal	Amniotic membranes: Oedematous	Allantoic membranes: Normal	Allantoic membranes: Oedematous	Endometrium: Normal	Endometrium: Oedematous	Endometrium: Cystic dilatations
1	+	+	+	+	+				16*		+		+			+	
2	+	+	+	+	+						+		+				
3	+	+	+	+		+										+	+
4	+	+	+	+	+			5,6	18*							+	
5	+	+	+	+	+			3,7	18,4*		+		+			+	+
6	+	+	+	+	+			6,7	16,1	0,6		+	+			+	+
7	+	+	+	+		+		6,1	16,0	0,4		+				+	+
8	+	+	+	+			+						+				
9	+	+	+	+	+			5,8	0,2	1,4	+					+	+
10	+	+	+	+			+										
11						+			1,4			+					
12	+	+	+	+		+										+	+
13	+	+	+	+		+										+	+
14	+	+	+	+		+										+	
15	+	+	+	+	+			3,7	14,0	2,2		+	+			+	+
17	+	+	+	+		+		3,6								+	+
18	+	+	+	+			+	3,8								+	+
19	+	+	+	+			+									+	+
20	+	+	+	+		+		4,0	8,0	0,58	+		+				+
	+	+	+	+		+		4,5	9,0	0,38	+		+				+
21	+	+	+	+	+			4,9	20*		+		+				+
	+	+	+	+	+			5,1			+		+				+
22	+	+	+	+			+	4,8			+		+				+
23	+	+	+	+	+			4,6	12,2	0,8	+		+			+	+

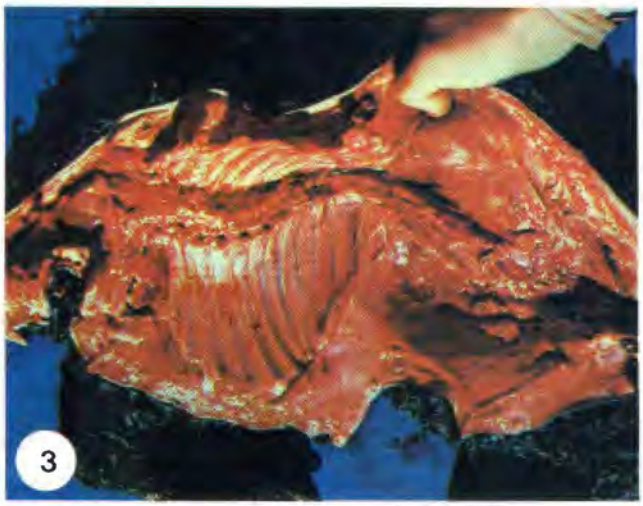
* Total amount of amniotic and allantoic fluid

TABLE 3 Developmental anomalies in the brain and adjacent tissues

Case No.	Calvarium: Normal	Calvarium: Flattened and compressed	Dura mater: Thickened and fibrotic	Dura mater: Thin and translucent	Cerebral hemispheres: Membranous and translucent or absent	Cerebral hemispheres: Mineral plaques	Cerebellum: Aplasia	Cerebellum: Hypoplasia	Basal ganglia	Thalamus	Hippocampus	Choroid plexus	Medulla oblongata	Hypophysis	Spinal cord hypoplasia	Peripheral nerves	Haemorrhages in brain substance	Mineral plaques in brain substance	Fluid in cerebral sacs: Clear	Fluid in cerebral sacs: Blood-stained
1	N		+		+		+					+	+	N	+	N		+	+	
2		+	+		+		+				±	+	+	N	+	N		+	+	
3	N		+		+		+			±		+	+	N	+	N	+	+		+
4	N		+		+			+		+		+	+	N	+	N		+	+	
5	N			+	+		+			+	±	+	+	N	+	N			+	
6	N		+		+	+	+	+				+	+	N	+	N			+	
7	N			+	+	+	+	+		+	+	+	+	N	+	N			+	
9	N			+	+		+	+	+	+	+	+	+	N	+	N			+	
15	N			+	+		+			±	+	+	+	N	+	N			+	
17	N			+	+		+			+	+	+	+	N	+	N			+	
18		+	+		+	+		+	+	+	+	+	+	N	+	N		+	+	
19		+	+		+	+		+	+	+	+	+	+	N	+	N		+	+	
20	N		+		+		+			+		+	+	N	+	N			+	
	N		+		+		+			+		+	+	N	+	N			+	
21	N		+		+	+	+			+		+	+	N	+	N		+	+	
	N		+		+	+	+			+		+	+	N	+	N		+	+	
22	N		+		+		+			+	+	+	+	N	+	N			+	
23	N			+	+	+	+			+	+	+	+	N	+	N			+	

N = Normal
\+ = Present
± = Partially present





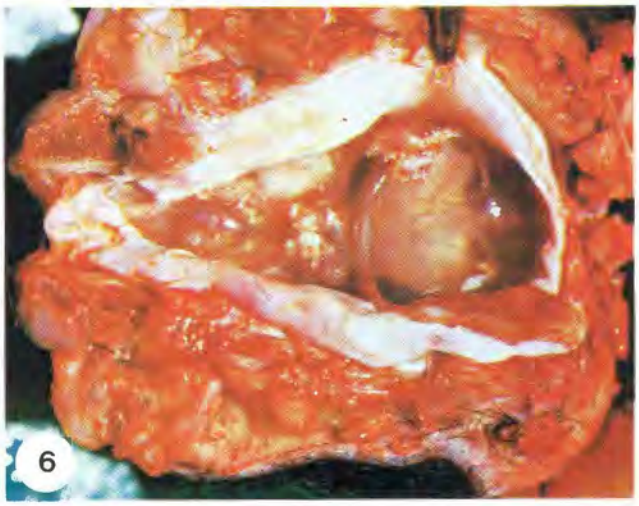


FIG. 1 A Karakul ewe with *hydrops amnii* showing severe abdominal distension

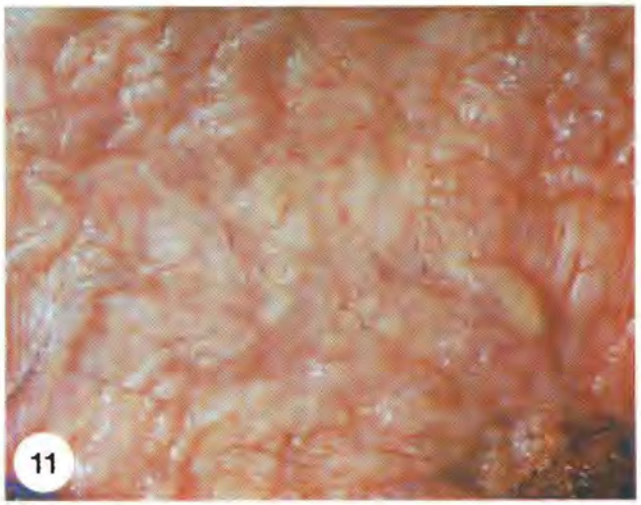
FIG. 2 Arthrogryposis in a foetus

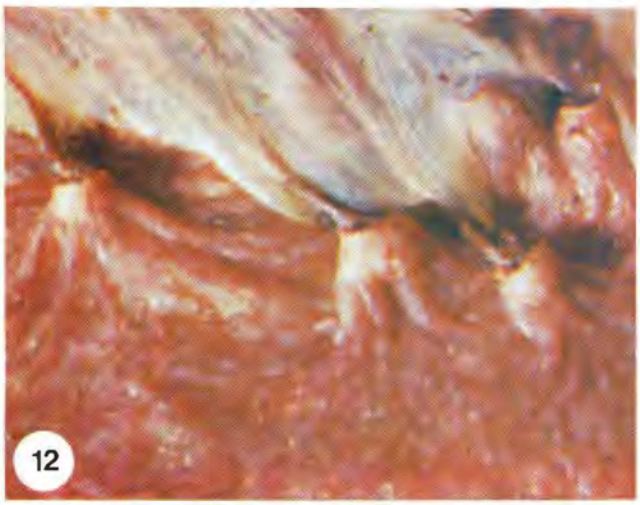
FIG. 3 Foetus showing thoracolumbar lordosis and scoliosis

FIG. 4 Hydranencephaly. The cerebral hemispheres are reduced to semitranslucent fluid-filled sacs

FIG. 5 Three hydranencephalic brains with cerebellar aplasia or hypoplasia and mineralization of the membranous cerebral hemispheres

FIG. 6 Mineralized plaques 1–10 mm in diameter in the thalamic area of a hydranencephalic foetus





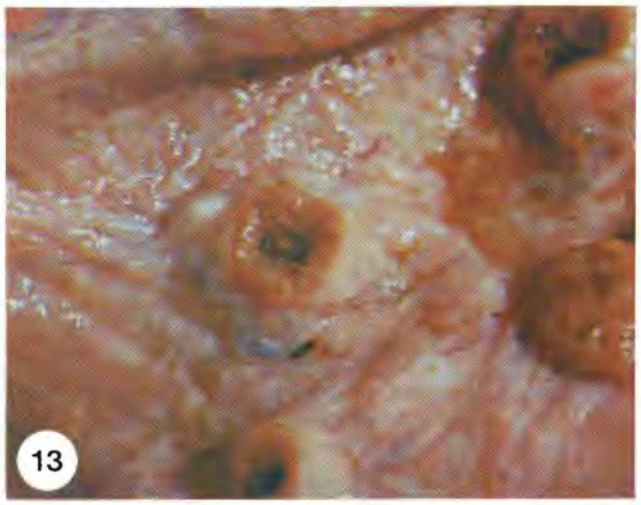


FIG. 8 Hypoplasia of the spinal cord

FIG. 9 Neurogenic muscular atrophy. The muscles have a gelatinous, pinkish-grey to yellow appearance

FIG. 10 Bilateral dilatation of the renal pelvis and calyces

FIG. 11 Raised cystic dilatations in the endometrium containing a clear watery to slightly viscous fluid

FIG. 12 Mineralized foci 1–2 mm in diameter in the endometrium

FIG. 13 Raised caruncles due to oedema of the caruncular stalks. Cysts surrounding the caruncles containing soft, whitish, floccular coagulations

Skeletal musculature

A moderate to marked muscular atrophy was present in all the foetuses that showed arthrogryposis. The foetal carcasses were slender and the bony prominences, e.g. the spinous process of the scapula, sternum and the pelvic girdle, were unduly pronounced. The degree of atrophy varied in the different muscle groups. Affected muscles generally had a range of colour from a grayish-white to cream. (Fig. 9). In foetuses that showed a marked muscular atrophy, the deeper and larger muscles of the back and hindquarters, e.g. M. longissimus dorsi, M. quadriceps femoris, M. gluteus medius, M. semimembranosus and M. semitendinosus had a pinkish-gray to yellow colour and were shiny, gelatinous and moist in appearance. Some of the muscles were so reduced in bulk that identification was difficult. The superficial muscles of the shoulder and thorax, particularly Mm. serratus ventrales, M. latissimus dorsi and the abdominal muscles had a very fatty appearance and a soft consistency. Reduction in size was observed in the tongue muscles which were infiltrated with a yellow, gelatinous fluid, and in the ocular muscles which were grayish-white. Petechiae and ecchymoses were present in the periorbital fat and sclera of a single foetus.

Other organs

There were no distinctive lesions in the internal organs of most of the foetuses, although subepicardial and subpleural petechiae, ecchymoses and haemorrhages in the mucosa of the abomasum were sometimes present. A few foetuses showed distension of the bladder. In some instances the liver had a khaki-brown colour and was compressed into abnormal shapes as a result of the strange postural position of the foetus. Besides the general corneal opacity, two foetuses had haemorrhages in the cornea and in the anterior chamber of the eye. One foetus showed a bilateral dilatation of the pelvis and calyces of the kidneys (Fig. 10). Other changes of interest in this particular case were a moderate ascites and marked subcutaneous and intermuscular oedema.

Foetal membranes

The amniotic fluid (S.G. 1,006–1,010) varied from 8,0–18,0 l in volume, was syrupy and greenish-yellow in colour. Floccules of meconium, varying from 0,5–3 cm in length, floated in the amniotic fluid. A brown-green, watery fluid with a SG of 1,017 and measuring 0,4–2,2 l in quantity was present in the allantois. In one ewe the amnion had hardly any fluid in it, though the foetal membranes were still intact. The foetus, which was alive, was covered with a greenish meconium material while the allantois contained 1,4 l of a thick, dark-green fluid. The allantoic membranes were stained green in this case.

There were no obvious signs of inflammation or infection in the placentomes, intercaruncular and intercotyledonary areas. The amniotic and allantoic membranes were oedematous in a few cases and, in one, the cotyledons were particularly small.

(ii) *Ewes*

Uterus

The most striking lesion in nearly all the cases studied was the raised, cystic, tube-like dilatations in the endometrium. These cysts were thin-walled, varying from 1–10 mm in diameter and, in most instances, they contained a clear, watery to slightly viscous fluid (Fig. 11). Some of them also contained soft, whitish, floccular coagulations floating in the fluid (Fig. 12). The cysts were distributed in a focal, disseminated manner in the body and horns of the uterus, in the intercaruncular area and around the caruncles. In one case the cysts were so numerous that the uterine horns had an irregular wavy surface.

The caruncles were raised as a result of oedema of the caruncular stalks (Fig. 12). A pulpy, greenish material covered some of the caruncles but they were otherwise macroscopically normal. Slight to severe oedema caused a thickening of the endometrium and myometrium. Focal chalk-like white deposits, 1–2 mm in diameter, were scattered in the endometrium in 6 of the cases (Fig. 13) and, in one ewe, the broad and ovarian ligaments were markedly oedematous.

Fibrinous adhesions between the uterus and the abdominal wall were present in one case, where mummification of the foetus was taking place. The uterine wall was very friable and had a grayish-brown colour but no putrid odour. There were no signs of separation of the placentomes and the amniotic membrane was infiltrated with a blood-stained oedema.

The other visceral organs showed no significant lesions. Cachexia, fatty degeneration of the liver, ruminal atrophy as a result of pressure from the enlarged uterus, and ascites were sometimes observed, as were signs of trauma of the abdominal muscles, resulting in abdominal and inguinal hernias in some of the ewes.

Experimental cases

A brief summary of the experimental findings are given in Table 4. From these results it is apparent that both the wild-type WBD virus and the attenuated WBD vaccine can cause hydranencephaly and arthrogryposis in the foetus associated with *hydrops amnii*. One ewe infected with the attenuated RVF vaccine showed a prolonged gestation and *hydrops amnii* and contained a foetus with arthrogryposis which had a mineralized micrencephalic brain. Hydranencephaly and arthrogryposis of the foetus without *hydrops amnii* was also seen with the attenuated RVF vaccine in another case.

Other teratogenic defects were observed in foetuses where the ewes were infected I/V with the attenuated WBD virus. This consisted of aplasia of the mandible, atresia of the oesophagus and skin angiomata on the dorsal-sacral region.

Virology

Natural cases

All attempts to isolate RVF or WBD virus from foetal material failed and no conclusions could be drawn from the results obtained from the HI test for RVF and WBD antibodies in the sera of either ewes or foetuses. Some of the ewes not immunized against WBD showed antibodies against this virus, apparently as a result of natural infection, while certain foetuses showed antibodies against either WBD virus or RVF virus.

Experimental cases

Wesselsbron disease

Wesselsbron disease virus was isolated on one occasion only from the uterine tissues of a ewe. Haemagglutination inhibition antibodies against WBD virus were detected in 11 out of 18 ewes and 4 out of 15 foetuses and lambs tested. The antibody titres of the foetuses and lambs were always lower than those of their mothers.

TABLE 4 Summary of experimental findings

Agents used	No. ewes infected	No. of normal lambs born	No. of early unrecorded abortions or resorptions	No. of normal aborted foetuses	No. of aborted foetuses with teratogenic defects	No. of foetuses with hydranencephaly and arthrogryposis associated with *hydrops amnii*	No. of foetuses with hydranencephaly and arthrogryposis unassociated with *hydrops amnii*	No. of foetuses with micrencephaly and mineralization of the brain associated with *hydrops amnii*
Wild-type WBD virus	11	5**	1*** 1* 1**			1* 2**		
Attenuated WBD virus	9	3** 1*		1**	2*	1* 1**		
Attenuated RVF virus	9	3* 1***		1* 1**	1*		1**	1**
Total	29	13	3	3	3	5	1	1

Route of infection * i/v
** s/c
*** direct foetus

Rift Valley fever

All attempts to isolate RVF virus failed, but HI antibodies were detected in 7 out of 9 ewes and 5 out of 6 lambs tested. The titres of these lambs were usually higher than those of their dams.

Discussion

Information obtained from farmers and field veterinarians indicates that the attenuated RVF and WBD vaccines and probably the wild-type WBD virus are associated with this syndrome under field conditions. Serological assays and attempts to isolate viruses from the field cases failed to demonstrate any virus as aetiological agent. The experimental findings reported here prove that both the wild-type and attenuated WBD virus are capable of producing hydranencephaly and arthrogryposis in the foetus associated with *hydrops amnii* in the ewe. However, the complete syndrome as observed under field conditions could not be reproduced with the attenuated RVF virus. One ewe infected with this agent showed *hydrops amnii*; the foetus, however, revealed no hydranencephaly but a severely micrencephalic and mineralized brain associated with arthrogryposis. Another ewe infected with the same agent showed no signs of *hydrops amnii* but contained a foetus with hydranencephaly and arthrogryposis. These findings indicate that the attenuated RVF virus cannot be completely excluded as a cause of the syndrome described above.

The HI test was used to determine the precolostral antiviral titres of the foetuses or lambs in the experimental cases. Of the 15 ewes infected with the wild-type or attenuated WBD virus, only 4 foetuses or lambs tested revealed titres against WBD virus. However, RVF virus antibodies were detected in 5 of the 6 foetuses or lambs obtained from ewes inoculated with the attenuated RVF virus. This may be explained by the fact that RVF virus probably crosses the "placental barrier" more readily or that the foetus becomes immune competent to RVF virus earlier.

The wild-type field virus of RVF, which is considered to be pantropic or hepatotropic, loses its hepatotropism and develops neurotropic properties during intercerebral passages in suckling mice (Smithburn, 1949). The Smithburn strain of RVF virus which had undergone 103 intracerebral passages in suckling mice is used as antigen for the preparation of the attenuated RVF vaccine in tissue culture.

Transplacental transmission of the attenuated RVF virus may take place since the virus was isolated from the foetal brain and afterbirth of a sheep that aborted after inoculation with a neurotropic strain (Kashula, 1953). Weiss (1962) indicated that immunization of pregnant ewes with the neurotropic virus of RVF may result in the death of the foetus with or without abortion. The lamb may be born apparently normal but die within the first few days, or an actively immunized lamb may be born.

A strain of WBD virus which had undergone 145 intracerebral passages in suckling mice was used as antigen for the production of the attenuated WBD. vaccine in tissue culture. There is definite proof that the WBD virus that is hepatotropic also becomes neurotropic during these intracerebral passages in suckling mice (Weiss, unpublished report, 1962). The findings of the present experimental work indicate that both the attenuated and wild-type WBD virus possess neurotropic properties for the developing foetal brain.

There has been much speculation about the possible pathogenesis of hydranencephaly and porencephaly. Osburn *et al.*, (1971), working with modified bluetongue virus in sheep, suggested that defects in embryogenesis, e.g. the arrest of cell migration from the ventricular and subventricular zone (subependymal plate, germinal zone or matrix) may play a rôle. Prenatal infections with wild-type bluetongue virus (Griner, McCrory, Foster & Meyer, 1964; Schmidt & Panciera, 1973) may be responsible for a similar defect in embryogenesis in sheep and in cattle (Richards, Crenshaw & Bushnell, 1971; Jochim, Luedke & Chow, 1974; Barnard & Pienaar, 1976). The related Akabane virus has also been incriminated as a cause of hydranencephaly and arthrogryposis in calves (Omori, Inaba, Kurogi, Miura, Nobuto, Ohashi & Matsumoto, 1974; Inaba, Kurogi & Omori, 1975)

and in sheep and goats (Inaba *et al.*, 1975). Recently Parsonson, Della-Porta & Snowdon (1975) experimentally reproduced congenital hydranencephaly and arthrogryposis in lambs with Akabane virus.

Arthrogryposis is a well-known syndrome in domestic animals and can be manifested alone or accompanied by hydranencephaly. There are reports where arthrogryposis and hydranencephaly occurred together in calves (Blood, 1956; Whittem, 1957) and in lambs (Hartley & Kater, 1962) with unknown aetiology. Omori *et al.* (1974) incriminated Akabane virus as the cause of arthrogryposis and hydranencephaly. In sheep the syndrome was experimentally reproduced with this virus (Parsonson *et al.*, 1975). In the cases reported here these 2 entities were almost always associated. The pathogenesis of arthrogryposis may be due to the neurogenic muscular atrophy resulting from the hypoplastic spinal cord and malformed brain and subsequent partial loss of the trophic effect of nervous stimulation on the skeletal muscle.

The normal volumes of amniotic and allantoic fluids at 150 days of gestation in sheep are 3,0 l and 1,14 l, respectively (Malan & Curson, 1937). The volume, colour and viscosity of the amniotic fluid differs at various stages of gestation (Arthur, 1965). In the cases studied, the composition of the fluids was normal but the amniotic fluid was greatly increased in volume. Arthur (1959) suggested that the volumes of amniotic and allantoic fluids are primarily controlled by the swallowing of amniotic fluid by the foetus and excretion of allantoic fluid via the kidneys. If there is a defect in the foetus or in the foetal CNS, the impaired swallowing process may result in hydramnios, e.g. in hydrocephalus and achondroplasia in Dexter cattle (Crew, 1924), in hereditary lethal muscle constructure in sheep (Roberts, 1929), in Guernsey cattle with a small defected foetus (Roberts, 1971) and in foetal anencephaly in man (Cassidy & Cailliteau, 1967). It appears that the *hydrops amnii* in this study can be ascribed to the hydranencephaly which probably impaired the swallowing reflex. In addition the fixation of the jaws and muscles of deglutition will further interfere with the swallowing process. An increased permeability of the uterine wall and vessels to the foetal fluids as reflected by the cystic and oedematous endometrium may also play a rôle.

Most of the affected ewes had a prolonged gestation period. The pathogenesis of this is not clear but the large amount of amniotic fluid, abnormally large and malformed foetuses may result in uterine inertia and dystocia.

In the cases reported here, *hydrops amnii* became clinically apparent at 3½–4 months of gestation, and the abdominal distension gradually increased up to the end of pregnancy. Weiss (unpublished observations, 1962) observed *hydrops amnii* in sheep vaccinated with the attenuated WBD vaccine, but, as far as can be ascertained, *hydrops amnii* has never been associated with any other virus infection in veterinary literature. It is of interest to note that although hydranencephaly can be caused by both attenuated and wild-type bluetongue virus, *hydrops amnii* has never been reported to accompany it, neither has *hydrops amnii* been reported to be associated with the hydranencephaly and arthrogryposis syndrome caused by Akabane virus.

As WBD is a zoonosis, the outcome of further research into the effect of wild-type or attenuated WBD virus on pregnant animals may have a bearing on the human foetus.

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