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WESSELSBRON VIRUS – A VIRUS NOT PREVIOUSLY DESCRIBED, ASSOCIATED WITH ABORTION IN DOMESTIC ANIMALS.

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Outbreaks of abortion among ewes, and heavy mortality among newly born lambs in the Union of South Africa have attracted considerable attention in recent years. Rift Valley fever (R.V.F.) has been found to be responsible for some of these outbreaks (Alexander, 1951) but in many instances the aetiology remains obscure.

During the late summer of 1954/55 an outbreak of disease was reported in a flock of Merino sheep on a farm Magdalena in the Wesselsbron area of the Orange Free State. At the time, this flock comprised about 300 ewes in advanced stages of pregnancy, and about 400 hamels and yearling sheep. Mortality was first observed among newly-born lambs which were dying during the first week of life. It was then observed that many of the ewes were aborting at about full term, and that some of the ewes were dying. No deaths or symptoms of disease were noted in the hamels or yearlings.

About two weeks prior to the outbreak the owner had immunized all the pregnant ewes against R.V.F. with vaccine prepared at Onderstepoort. Since there appeared to be a possibility that the strain of attenuated virus incorporated in this vaccine might be responsible for the abortions an attempt was made to isolate the vaccine virus from material collected during investigations.

From the only lamb that was submitted for examination a virus was isolated which at first appeared to resemble R.V.F. virus but which has since been shown to be distinct. This virus is apparently a hitherto undescribed agent and has been termed " Wesselsbron " virus (WB), strain van Tonder, from the district and name of the owner of the farm from which it was isolated.

In this paper some of the properties of this virus are described.

Methods and Materials.

Strains of Virus.

(*a*) *Wesselsbron.*—A lamb, about eight days of age, which had died on the farm was forwarded to Onderstepoort. It was received two days later in an advanced stage of decomposition. Liver and brain samples were collected and suspended separately in broth-saline. To each suspension were added 500 units of penicillin and 500 micrograms of streptomycin per ml. After light centrifugation the brain suspension was inoculated intracerebrally into each of a family of two-day-old mice while the liver suspension was inoculated intraperitoneally into each of another family.

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Six days later those mice that had been inoculated intracerebrally became sick; they were apathetic, and partially paralysed. Those inoculated intraperitoneally with the liver suspension showed similar symptoms about two days later. Some of the mice from the first family were killed, when moribund, for the collection of infected brain; the remainder died after a period of prostration lasting up to three days. The brains that were harvested were used to start a series of intracerebral passages in infant mice. From time to time infected brains from ascending passage levels were stored under dry-ice refrigeration for future reference.

(*b*) *Rift Valley fever* (Smithburn).—This strain of R.V.F. virus was isolated by Smithburn (1949) in Uganda. When received at Onderstepoort the strain had undergone 82 serial mouse brain passages. Passage in mouse brain was continued to the 102nd passage level. The strain was then taken 56 serial passages in embryonated eggs and a further 12 passages in the brains of infant mice. It is at this passage level that the strain is used for the production of vaccine at Onderstepoort.

(*c*) *Rift Valley fever* (van Wyk).—The van Wyk strain of R.V.F. was isolated from a natural case of R.V.F. in a sheep during the outbreak in the Orange Free State described by Alexander (1951).

Neutralization tests.

For the neutralization tests aliquots of 10% infected brain suspension in 10% horse serum saline were stored in a dry-ice cabinet in sealed glass ampoules. For each test the contents of a phial were thawed rapidly and serial ten fold dilutions were made in serum saline; to 1 ml. of each virus dilution was added 1 ml. of the serum under investigation after inactivation at 56° C. for 30 minutes. After contact for one hour at 37° C. infant mice were injected intraperitoneally with 0·1 ml. of the serum virus mixture. Mice were kept under observation for 12 days. End points were calculated by the method of Reed and Muensch.

Wesselsbron virus was used at the third mouse passage level; R.V.F. (Smithburn) virus was used at 102 mouse, 54 egg, 16 mouse passage level.

For diagnostic purposes 1 c.c. of undiluted inactivated serum was mixed with a dilution of virus emulsion known to contain approximately 10,000 LD_{50} of virus per ml.

Sera.

The sera used were obtained from sheep which were bled three to four weeks after recovery. Special mention will be made of other sera used.

Experimental Results.

A. *Pathogenicity of Wesselsbron virus for various animals and man.*

(1) *White mice.*

Serial intracerebral passage of the virus in day-old mice was accomplished readily, brains from moribund mice suspended at a dilution of 1 in 100 in horse serum saline being used for these passages.

As stated above those day-old mice which received the original sheep brain inoculum were noticed to be sick on the sixth day after injection. By the third passage the period of incubation had decreased to four days; from that stage it

has remained constant at between three and four days. It is now approaching the sixtieth passage level and has been identified by serum virus neutralization tests from time to time.

Infant mice have been inoculated intraperitoneally with infective brain suspensions at various passage levels. The incubation period in all these cases has been from five to six days and the course somewhat protracted, but the mortality was 100 per cent.

The results of numerous titrations of the infectivity of mouse brain down the passage series have shown the infective titre to vary between 10^8 and 10^9 LD_{50} doses per ml., irrespective of the route of inoculation (intracerebral or intraperitoneal).

Mice of all ages have been found to be susceptible to intracerebral injection of the virus. In the case of adult mice, however, the incubation period was never less than six days. When virus was administered by the intraperitoneal route it was found that mice under the age of 10 days all succumbed; mice over the age of three weeks, on the other hand, were resistant and showed no apparent reaction.

Intranasal instillation of infected brain suspension in day-old mice proved fatal, the incubation period being seven days.

Since the most pronounced manifestation of infection with virus in nature appeared to be the abortions produced in sheep, tests were made to determine its abortifacient properties in white mice. Eighteen pregnant white mice were inoculated intraperitoneally with infective brain material from the third mouse passage level. None aborted and the young were born apparently normally from the fourth to the eighth day after inoculation and survived. No data are available on the effect of infection at an earlier stage of pregnancy.

(2) *Sheep.*

(*a*) *Lambs.*—Nine lambs of different ages were injected intravenously with brain suspension of the third mouse passage.

Of two newly born lambs that were infected, one showed a febrile reaction which commenced after 48 hours. This lamb was dull and fed poorly for about a week and then gradually recovered. The other lamb after a severe febrile reaction died about 72 hours after injection. Post-mortem examination revealed a slight tumor splenis and degenerative changes in the liver. Histopathological examination of this material showed diffuse necrosis of liver cells with marked karyorrhexis; in addition there was infiltration with leucocytes which also showed karyorrhectic changes. These lesions were diffusely distributed throughout the substance of the liver.

From this lamb, virus was recovered by the inoculation of liver and brain suspensions into infant mice.

The other lambs which varied in age from a few weeks to six months showed only febrile reactions. All recovered and all showed the presence of neutralizing antibodies when tested about three weeks after recovery.

(*b*) *Pregnant Ewes.*—In a preliminary trial two ewes in advanced stages of pregnancy received material from the third mouse brain passage. One lambed normally 24 hours later and on the following day commenced a febrile reaction

of short duration (48 hours). It showed no other symptoms and the lamb remained normal. The other ewe showed a similarly short febrile reaction; it aborted 14 days later. Virus was isolated from the liver and brain of this foetus.

In an effort to obtain some data on the possible pathological significance of the Wesselsbron virus, 30 pregnant ewes were given intravenously third mouse brain passage material. All showed febrile reactions which varied considerably in degree, and the incubation periods varied from 24 to 72 hours. Five of the ewes lambed normally within 48 hours of injection and these lambs remained healthy. During the time the ewes were undergoing febrile reactions four weak lambs were born; all died or were killed *in extremis* within 24 hours.

On the morning of the fourth and fifth days after injection of the ewes four apparently full term foetuses were found dead. Attempts at isolation of virus from the brains and livers of these foetuses and from the placentas were unsuccessful.

On the sixth and seventh day another four lambs were born dead. The brains and livers of two of these lambs were collected and inoculated into infant mice. From both lambs virus was obtained which was identified by serum virus neutralization tests as Wesselsbron virus.

By this time six of the ewes had died. Two of these ewes had full term lambs *in utero*. The others had apparently aborted. Post-mortem examinations carried out on these ewes revealed marked liver changes in all cases. The livers were very pale in colour and markedly friable. Macroscopically these livers resembled those seen in cases of pregnancy disease ("domsiekte"). Histopathological examination revealed changes similar to those in the lamb. In addition marked fatty changes were present.

As it was not possible to continue the experiment in isolation under insect-free conditions, and therefore there existed a serious danger of establishing a reservoir of infection in an hitherto clean area, the experiment was discontinued and all the surviving animals were slaughtered.

(3) *Pathogenicity for guinea-pigs and rabbits.*

No apparent reactions were observed in six adult non-pregnant guinea-pigs and two rabbits that received intraperitoneal injections of infected mouse brain material from the fourth serial passage.

It was observed, however, that when pregnant guinea-pigs and rabbits were infected all aborted at about full term or gave birth to young which died within a few days. The abortions or births occurred from two to 14 days after infection. Virus was re-isolated from the rabbit and guinea-pig foetuses.

(4) *Pathogenicity for cattle, horses and pigs.*

Six cattle, two horses and two pigs were given intravenously mouse brain suspension from the fourth serial passage.

The bovines were from one to three years of age. After an incubation period of from 24 to 72 hours, four showed mild febrile reactions of about a day's duration. One of the animals showed signs of increased salivation at this time and was disinclined to move. The other animals showed no clinical reaction.

Two horses that were inoculated with the same suspension of virus showed mild febrile reactions after an incubation period of 48 hours. No other symptoms were observed.

The two pigs were about eight weeks old. Both showed febrile reactions with temperature up to 106° F. which persisted about 48 hours. They showed no other symptoms.

Serum collected from all these animals before inoculation was in each case found to be negative when tested for neutralizing antibodies. On the other hand serum collected three weeks after infection was found to contain antibodies to high titre.

Unfortunately pregnant animals were not available for experimental work.

(5) *Pathogenicity for man.*

During the course of the experiments which were carried out with the Wesselsbron virus, all but one of the laboratory staff who were directly concerned with the work became sick, but not critically ill. The incubation period appeared to vary from three to four days. In most instances it was not possible to determine the exact time of infection. In one case, however, it is known that a worker who had not handled infective material for some days previously had his hands contaminated with fluid from an egg that had been inoculated with mouse brain suspension; four days later this person showed symptoms of infection.

In all cases there was a sudden onset of fever with temperatures of about 102° F. The febrile reaction lasted 10 to 24 hours. Rigors with acute general muscular pain accompanied the fever. In no case was a second rise in temperature observed, but the muscular pains persisted for some time, in one instance for more than a month Hyperaesthesia of the skin, particularly the scalp, was noticed but was not constant. In two, a mild rash appeared on the skin of the abdomen and back.

A summary of the observations made on these cases is given in Table 1.

TABLE 1.

Human Cases of Wesselsbron virus infection.

	CASE.				
	W.	H.	vR.	vP.	Har.
Symptoms shown.....	S.	S.	S.	S.	NS.
Virus isolation.....	+	NT.	NT.	—	NT.
Serum neutralization tests—					
(*a*) Prebleed.....	—	NT.	NT.	—	—
(*b*) Convalescent.....	+	+	+	+	+

S. = Showed symptoms of infection.
NS. = Showed no apparent symptoms.
NT. = Not tested.
\+ = Positive.
— = Negative.

Discussion.—In only one of the human cases to which attention was directed was virus isolated from the blood during the febrile reaction. Two did not report sick until after recovery and one became available for bleeding only towards the end of the febrile reaction, when the attempt at virus isolation was unsuccessful.

Case W., from whose blood a virus was isolated which was identified as Wesselsbron virus by serum virus neutralization tests, is of particular interest. This officer was entrusted with certain aspects of the research work into Rift Valley fever virus in 1951. Soon after initiating a series of mouse passage with a field strain of that virus, even though every operation was carried out with full surgical aseptic precautions (sterile protective clothing, gloves, mask, goggles, etc.) he became infected, was seriously but not critically ill. R.V.F. virus was isolated from the blood during the first phase of the diphasic reaction, and subsequently serum was shown to contain antibodies. This type specific R.V.F. antiserum was available for examination at the time of the Wesselsbron virus infection and was shown to contain no specific neutralizing antibodies to that virus. Today the serum contains antibodies to both viruses.

Case Har, too, is of interest. This new appointee was bled for the collection of serum before assuming duty. At no time did she come into contact with virulent or low passage level virus but was entrusted with work involving high passage level virus strains. There is no history of sickness but serum collected as a routine six months later showed high titre specific neutralizing antibodies to Wesselsbron virus.

From these known cases it may be deduced that man is susceptible to WB virus, that the influenza-like reaction is most unpleasant and may be characterized by prolonged convalescence, and that there is a marked antigenic dissimilarity between WB and R.V.F. virus. There appears to be some indication that with continued mouse passage the virus has become attenuated.

B. *Behaviour of Wesselsbron virus in embryonated hens eggs.*

Infective material from the third mouse brain passage was injected into the yolk-sacs of 12 eggs that contained eight day-old embryos. The eggs were reincubated at 37° C.

A few of the eggs died from the second to eighth day after injection but the majority survived until the time of hatching. The embryos of two eggs that died on the fifth day, and also two live embryos, were harvested and used to infect other eggs. This material, in the form of the supernatant fluid of a 10% saline emulsion was inoculated into two families of infant white mice. It was found that virus was present in both samples.

Serial passage of the strain was continued by blind passage of live embryos harvested on the fourth day, the supernatant fluid obtained from these embryos after maceration and light centrifugation being diluted 1/1000 in saline and being injected by the yolk-sac route. Deaths among the inoculated eggs have remained inconstant and irregular. Material from each egg passage was examined for infectivity by mouse inoculation. A sheep inoculated intravenously with infective embryo suspension from the 19th passage developed a febrile reaction after the usual short period of incubation.

During the course of these passages an attempt was made to determine the optimum period of incubation of infected eggs to obtain the highest titre of virus. For this purpose 15 eggs were given, via the yolk-sac, embryo suspension from

the third serial egg passage. Two embryos were harvested daily and the viral activity was titrated by the inoculation of serial ten fold dilutions into infant mice. The results of this experiment are shown in Table 2.

TABLE 2.

Infectivity of embryos harvested at different times after inoculation.

Time after infection.	State of Embryo.	LD_{50}.
2 days	living	5.8
3 days	living	6.5 +
4 days	living	6.5 +
6 days	living	6.5 +
7 days	dead	6.2
8 days	living	5.6
13 days	hatched*	4.5

* Pooled liver and spleen tested.

Result.—From Table 2 it is seen that virus was present in high titre in the embryos from the second day after injection up to and after hatching. The optimum time for harvesting infective material appears to be from the third to sixth or seventh day, when using fertile eggs preincubated eight days.

In another experiment, an attempt was made to determine the effect of the virus concentration of the inoculum on the titre of the infected embryos. Serial ten fold dilutions from 10^{-1} to 10^{-7} of sixth passage embryos were made and each dilution was inoculated into a group of six eggs. On the fourth day after injection two living embryos were taken at random from each group and the virus content was determined by titration in infant mice. Each group of eggs that received dilutions from 10^{-1} to 10^{-6} contained virus and the titre in each case was within the range 5·9 to 6·9. Embryos from eggs that received the 10^{-7} inoculum contained no detectable virus when harvested.

The distribution of virus in infected eggs was determined by harvesting different portions of four eggs that had been infected four days previously with material from the twelfth passage. The viral activity of the various samples was then determined by titration in infant mice. The results of this experiment are shown in Table 3.

TABLE 3.

Distribution of virus in infected eggs.

Portion of egg tested.	LD_{50}.
Allantoic fluid	4·5
Yolk-sac	4·7
Embryos:—	
(*a*) Heads	6·9
(*b*) Bodies	7·0

Result.—From Table 3 it is seen that although virus was present in all parts of the egg examined, the highest concentration apparently was present in the embryos. No significant difference in titre could be demonstrated in the heads and bodies.

C. *Identification of the virus.*

Since Wesselsbron virus showed some similarity to R.V.F. virus a number of tests were made to determine whether or not any relationship exists.

(i) *Immunity Tests.*

The immunity of four sheep that had recovered from infection with R.V.F. virus and four sheep that had recovered from infection with Wesselsbron virus was challenged by the injection of the heterologous virus.

All showed typical febrile reactions. It was therefore concluded that neither virus afforded any protection to infection with the other.

(ii) *Serum-virus neutralization tests.*

A number of cross neutralization tests were made in which R.V.F. and Wesselsbron virus preparations and immune sera were used. The results of these tests are shown in Table 4.

TABLE 4.

Cross serum-virus neutralization tests between R.V.F. and Wesselsbron virus.

Virus.	Antiserum.	Virus dilution.					
		10^{-2}.	10^{-3}.	10^{-4}.	10^{-5}.	10^{-6}.	10^{-7}.
R.V.F. (Smithburn).....	R.V.F. (Smithburn)...	3/8*	0/9	0/9	—	—	—
R.V.F. (Smithburn).....	R.V.F. (van Wyk).....	2/8	0/8	0/9	—	—	—
R.V.F. (Smithburn).....	Wesselsbron.....	—	9/9	9/9	8/8	8/8	2/9
R.V.F. (Smithburn).....	Neg. Control.....	—	8/8	8/8	9/9	8/8	1/9
Wesselsbron.....	Wesselsbron.....	1/8	0/8	0/9	—	—	—
Wesselsbron.....	R.V.F. (Smithburn)...	—	8/8	7/7	8/8	5/8	1/8
Wesselsbron.....	R.V.F. (van Wyk).....	—	9/9	8/8	8/8	2/9	2/8
Wesselsbron.....	Neg. Control.....	—	8/8	8/8	7/7	3/8	1/8

* 3/8 = Number of mice dead/number of mice inoculated.

Results.—From Table 4 it will be seen that no antigenic relationship between Wesselsbron virus and two strains of R.V.F. virus could be demonstrated by means of serum virus neutralization tests.

(iii) *Complement fixation tests.*

Since the complement fixation test frequently is group reactive in that it may fail to differentiate between antigenically different strains of the same virus, this test was used to determine a possible relationship between WB and R.V.F. viruses.

Antigens were made from mouse brains infected with either Wesselsbron or R.V.F. virus after the method of Casals (1949). Sheep sera inactivated at 56° C. for 30 minutes were used. Complement and cross complement fixation tests were made with these antigens and the results are shown in Table 5.

TABLE 5.

Cross complement fixation tests between Wesselsbron and R.V.F. virus.

Serum.	Serum Dilution.	Antigens.	
		R.V.F.	WB.
R.V.F. Immune	1/2	4*	—
	1/4	4	—
	1/8	3	—
	1/16	—	—
WB. Immune	1/2	—	4
	1/4	—	4
	1/8	—	—
Normal	1/2	—	—
	1/4	—	—

* 4 = Complete fixation of complement.

Result.—From Table 5 it is seen that there is no evidence of cross complement fixation between the two viruses, and their antisera.

(iv) *Gradocol Membrane filtration of Wesselsbron virus.*

A filtration trial showed that the Wesselsbron virus could readily be passed through a gradocol membrane of 172 $\mu\mu$ A.P.D.

Infective brain material was then submitted to Dr. A. Polson of the Wernher and Beit Institute in Cape Town. In a personal communication he stated that he found the particle size of Wesselsbron virus to be of the order of 30 $\mu\mu$.

D. *Distribution of Immune bodies in Southern Africa.*

Serum virus neutralization tests have been made on a number of sera collected from cattle and sheep in different parts of South Africa. It should be mentioned that a number of these cattle sera were forwarded to Onderstepoort to confirm a field diagnosis of brucellosis. Where examination in the contagious abortion laboratories was found to be negative the remainder of the serum was passed to the virus laboratories for tests.

The results of these tests are shown in Table No. 6.

TABLE 6.

Serum neutralization tests on sera from different parts of Southern Africa.

Area.	Year collected.	Animal.	Result.	Percentage Positive.
				Per cent.
Nyassaland	1955	Sheep	6/40*	15
Wesselsbron, O.F.S.	1955	Sheep	2/4	—
Southern Rhodesia	1955	Bovines	56/116	48
Northern Rhodesia	1955	Bovines	3/11	27
Knysna, Cape Province	1954	Bovines	11/60	18
Knysna, Cape Province	1955	Bovines	40/111	36
Wolmaransstad	1955	Sheep	1/10	—
Johannesburg	1955	Bovines	1/14	—

* 6/40 = 6 positive out of 40 tested.

From Table 6 it appears that antibodies to WB virus are present in animals in widely dispersed areas of Southern Africa. Furthermore it appears that the infection has been present in the Knysna area since at least 1954.

Remarks.—In Table 6 no mention is made of tests on large numbers of sera from many areas in the Union where no positive sera were encountered. A systematic survey is in progress to determine the distribution of this and other virus infections with some degree of accuracy but progress is of necessity slow.

It should be mentioned that all foetal material submitted to Onderstepoort for routine diagnostic purposes is examined for the presence of WB, R.V.F. or other possible viruses. At the time of writing no other strain of WB virus has been isolated. Faulty methods of collecting material, and transport over long distances in the hot summer months without refrigeration is a possible explanation for this failure.

Shortly after the strain of virus, discussed above, was isolated two strains with similar properties were isolated by Smithburn, Kokernot and De Meillon (to be published) in the Ubombo district of Northern Zululand, one from a native undergoing a reaction to natural infection, the other from a suspension of trapped mosquitos. The former, labelled H177, was supplied to us in the form of an emulsion of infective mouse brain on dry ice and has been compared with the van Tonder strain by cross immunity tests in sheep and by serum virus neutralization tests in infant mice. The results indicate that the two strains, if not identical, are so closely related as to be indistinguishable by the limited tests carried out. An exhaustive study was not made because this would have entailed passage of the strain in either sheep or white mice to furnish the requisite experimental material, a procedure which would constitute a risk of contamination of either or both strains in the laboratory. As soon as the identity of the two strains was established to our satisfaction the work was suspended until such time as a more exhaustive study could be carried out under more favourable conditions.

DISCUSSION.

Until recently lamb dysentery and to a lesser extent enterotoxaemia was considered the chief cause of heavy mortality in new-born lambs in South Africa. In 1951 Rift Valley fever virus was identified as the cause of mortality among

sheep and cattle and illness in humans. By causing abortions and the death of new-born lambs, it was responsible for the loss of practically the entire season's lamb crop in the affected area (Alexander, 1951; Mundell and Gear, 1951; Gear, de Meillon, Measroch, Harwin and Davis, 1951). In 1953 a further enzootic occurred in an adjacent area (Gear, de Meillon, le Roux, Kofsky, Rose Innes, Steyn, Oliff and Schulz, 1955). To a considerable extent the disease in sheep and cattle in the latter outbreak was controlled by the application of a method of immunization based upon the use of a modification of Smithburn's mouse neurotropic attenuated strain of virus (to be published). In the late summer of 1954-55, a season characterized by heavy rainfall, reports were received of further abortions amongst sheep. At the time it was feared that immunization of pregnant ewes with the routine Rift Valley fever vaccine might have been responsible for a percentage of the abortions, and the matter was investigated. A virus was isolated, the Wesselsbron virus, which has been shown to be antigenically distinct from Rift Valley fever and to possess other distinguishing characteristics such as non-pathogenicity for adult mice by intraperitoneal inoculation. Even at this stage the significance of this virus infection to the sheep, cattle, horse and pig breeding industries has not been determined with any degree of accuracy.

Essentially Wesselsbron virus may be classified as a pantropic virus with marked neurotropic properties, high pathogenicity for new-born lambs and predilection for embryonic mammalian tissue. Morbidity amongst adult sheep is high but the mortality is low except in the case of pregnant ewes. Pregnant ewes may abort during the primary febrile reaction but in these cases virus could not be isolated from the expelled foetuses. If early abortion did not occur the foetus subsequently became infected *in utero* where its fate might be death with absorption or possible mummification, or retention to normal parturition either as a still-born lamb or as an infected lamb doomed to early death. In these cases no difficulty was experienced in isolating the virus from the dead lambs. It is worthy of note that if a lamb was born before the primary reaction in the ewe, that lamb did not contract infection *post partum*. This applies to the pregnant mouse which shows at most an inapparent infection.

In cattle the significance and importance of this virus is quite obscure. During recent years there has been instituted an intensive campaign for the control of brucellosis by the application of universal calf-hood immunization with freeze dried strain 19 vaccine. In 1955 approximately half a million doses of this vaccine were distributed and used. Critical examination of the records at Onderstepoort showed that there was a significant increase in the number of serum samples submitted to confirm a diagnosis of suspected contagious abortion that were in fact negative to the agglutination test applied. As a routine measure these sera were then forwarded to the section of Virus Diseases for qualitative examination by *in vitro* serum virus neutralization in infant mice for the presence of antibodies against Rift Valley fever and Wesselsbron viruses. Even at this early stage of the survey it is apparent that infection with these viruses is far more common than had been anticipated and that they may play a major role in producing abortion particularly among breeding females introduced into the affected areas. Statistically significant data have not been accumulated but it *is* apparent that the two infections are encountered in the same herds and that Wesselsbron virus infection tends to predominate. In keeping with the world-wide drive for increased agricultural production, a drive which has materially altered general agricultural practice and animal breeding policies, this problem merits attention with a view to introducing a systematic method of protective immunization. The development of an attenuated virus by serial passage in infant

mice and propagation in chicken embryos is under way but conclusive results are not yet available. Reference must be made to the fact that in considering the cases of known human infection Har., who handled only high level passage virus, developed high titre serum antibodies with no history of illness. Similarly it is of interest to report that case vP whose prebleed for the test for immunity against Wesselsbron virus was negative (Table 1) and was shown at the time to be negative against R.V.F. has developed high titre antibodies to this virus. In the interim he handled only the attenuated strain of R.V.F. virus used for routine serum neutralization tests, and there is no history of any illness.

The significance of this virus in the pig and horse breeding industries is a matter of mere speculation but it must be borne in mind that the Thoroughbred mare is carrying her foal during the late summer months when the incidence of infection is at its highest.

The marked predilection of the virus for the foetus of the common laboratory animals, the rabbit and the guinea-pig, in both of which it produces only an inapparent infection, as well as the multiplication of the virus in the chick embryo with no constant lethal effect is of interest.

Finally it should be noted that the observation that the adult mouse is resistant to intraperitoneal injection of virulent virus furnishes a rapid method of differentiating roughly between WB and R.V.F. viruses.

It is apparent that the Wesselsbron virus was active in widely separated areas in the Union in 1955. It has been established that the virus is mosquito transmitted (Smithburn *et al.* 1956)., that infection may be contracted by handling infective material and in the case of infant mice by aspiration following intranasal instillation. No opinion can be expressed as to when the virus was introduced into the Union nor the mode of introduction. Evidence has been accumulated that Wesselsbron virus was present in the Knysna area of the Cape Province in 1954 and that in 1955 it was widespread in certain regions of Southern Rhodesia.

SUMMARY.

1. There has been isolated from a lamb a pantropic virus with neurotropic properties and a well marked affinity for embryonic tissue.

2. In the field the vectors are mosquitoes which have yet to be identified accurately.

3. In sheep infection with the virus causes a febrile reaction after a short incubation period of about one to four days, the mortality rate not being high. Pregnant ewes may abort during the febrile reaction in which case virus could not be isolated from the foetuses. Subsequently the virus invades the foetus causing death usually with abortion. The mortality amongst foetuses carried to full term and new-born lambs is very high (practically 100%). New born lambs suckling reacting ewes did not become infected by contact with the reacting ewe.

4. In addition to sheep, cattle, horses, pigs, mice, rabbits, guinea-pigs and man are susceptible.

5. Infant and adult mice are equally susceptible to intracerebral inoculation, the mortality being 100%. Infant mice are fully susceptible to intraperitoneal infection but in adult mice the reaction is at most inapparent.

6. The virus multiplies in the developing chick embryo among which the percentage mortality is low.

7. Infection was present in widely separated parts of the Union in 1955.

8. The virus named Wesselsbron virus, strain van Tonder, is antigenically distinct from Rift Valley fever virus.

9. The significance of this virus to the animal industry is discussed.

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