

On the Etiology of Epizootic or Infectious Equine Abortion.

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IN a previous article Henning, Keppel and Flight (1943) described an outbreak of infectious abortion in mares in the Cape Province. Further evidence in regard to this outbreak is given, another outbreak is described and the results of a number of transmission experiments are recorded.

Since Kilborne (1893) and Smith (1893) incriminated an organism of the hog-cholera group as the causal agent of infectious abortion of equines several workers in different parts of the world have described this disease. Good and Corbett (1913) and Good and Smith (1914) isolated an organism of the "*enteritidis cholerae-suis group*" from the after-birth and foetal organs of aborted foetuses and called it *Bacillus abortivo-equinus*. By intravenous and intraperitoneal inoculations of saline suspensions of cultures of this bacterium they induced pregnant mares to abort in about ten days' time and isolated the organism from the foetal membranes and foetal organs. The disease was also studied in great detail by de Jong (1912), van Heelsbergen (1914), Murray (1919) and others. These authors all found that the majority of abortions occurred from 7-9 months; they also succeeded in producing abortion in pregnant mares by means of intravenous or intraperitoneal inoculation of cultures of paratyphoid organisms which they had previously isolated from the after-birth and foetal organs, but failed to induce premature birth when the cultures were given by the mouth or per vaginam. Later the bacterium associated with equine abortion was called *Salmonella abortus-equi*.

Abortus-equi was generally accepted as the only important etiological agent of infectious abortion in equines until Dimock and Edwards (1936), Dimock (1940), and Dimock, Edwards and Bruner (1942), incriminated a filterable virus as the cause of abortion in some outbreaks of abortion studied by them. But they still regarded *abortus-equi* as a very common causal agent. In agreement with other workers they found that abortion seldom recurs in the same mare a second time, and that a diagnosis of *abortus-equi* abortion can be made by recovering the salmonella from the after-birth and foetal organs, and also by means of agglutination tests. They did not regard the stallion as an important factor in the dissemination of the disease. Like Ostertag (1901) they attributed some forms of infectious abortion in mares to a streptococcus which was thought to enter the body by way of the genital tract and not the mouth.

Dimock, Edwards and Bruner studied several outbreaks of infectious abortion in mares in which cultivable organism could not be incriminated as the cause and in which the serum of affected mares consistently gave negative agglutination tests. They succeeded in inducing abortion in both pregnant guinea-pigs and pregnant mares by means of unfiltered and filtered suspensions of foetal organs. Guinea-pigs that received Seitz filtrates gave birth either to premature or dead foetuses from 14 to 29 days after injection. Of the ten pregnant mares that received filtered foetal material three aborted on the 18th, 23rd and 24th days respectively after the inoculation. The remainder gave birth to normal foals. The aborted foetuses presented pathological changes that were regarded as characteristic of the disease. Another mare placed in an infected stable and fed on infected foetal membranes aborted after 24 days and the foetus showed lesions resembling those of the naturally occurring disease. All

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the foetuses dropped by the experimental mares were found to be bacteria-free. The authors therefore, concluded that a filterable virus should be regarded as the cause of these abortions.

In the outbreaks studied there was an abortion incidence of more than 70 per cent. and more than 90 per cent. of the foetuses were found to be free from cultivable bacteria. The mare apparently suffers no inconvenience and the foetal membranes are not retained. Involution of the genital organs takes place as readily as after a normal parturition and the mare is usually ready to be bred about a week later. The pregnancy that now follows will usually run a normal course and a healthy foetus will be delivered at full time.

The most characteristic pathological changes presented are small multiple, greyish-white areas of degeneration in the liver, accumulation of serous, usually blood-stained, fluids in the peritoneal and pleural cavities, small haemorrhages on the epi- and endocardium, swelling of the spleen and sometimes congestion of the lymph glands of the colon. The amnion may be oedematous. The majority of abortions occur between the 8th and 10th months. In late pregnancies the foals may be born alive but then they usually die within 36 hours. The infection is highly contagious and may spread very rapidly but the stallion is not considered to play a rôle in the dissemination of the disease nor is the recovered mare considered to act as a carrier. The disease does not recur in the same stud during two successive years but may recur after the lapse of 3 to 5 years.

These workers found acidophilic intranuclear inclusion bodies in sections of the liver, lung and epithelium of the respiratory tract of the foetus. The basophilic chromatin of the nucleus is retracted from the centre to lie on the inner margin of the nuclear membrane, leaving a clear space around the inclusion bodies. For diagnostic purposes a histological examination of the liver and lungs is recommended. It is stated that the intranuclear inclusion bodies will be readily found in the epithelium of the bronchioles, bronchi and alveoli, and also in the hepatic cells near the periphery of the necrotic nodules. Propagation of the virus in developing chick embryos was not possible.

Later Hupbauer (1938), Miessner (1938), and Manninger and Csontos (1941) studied a similar condition in Europe. Miessner (1938) and his collaborator, Harms, investigated a highly contagious epizootic of abortion in an exceedingly well-managed stud in Germany during 1936 and had to exclude *abortus-equi* and *streptococci* as causal agents. Infection was thought to occur at pasture where the whole number of mares were running together. The aborting mares apparently suffered no ill effects and the after-births were normally expelled. A number of the foals that were born alive died soon after birth. The lesions found resembled those described by Dimock and Edwards (1936), and a virus was also incriminated as the cause.

Hupbauer (1938) studied epizootic abortion of equines in Yugoslavia where abortions were comparatively common, but it was thought that losses could be prevented by the adoption of proper hygienic measures and inoculations with *abortus-equi* vaccine. In 1937 a particularly severe outbreak occurred about 14 days after the inoculation. The lesions found also resembled those described by Dimock and Edwards. By means of Seitz E.K. filtrates of foetal suspensions Hupbauer was able to induce abortion in both guinea-pigs and mares and concluded that a virus was the cause of these abortions.

The epizootic abortions investigated by Manninger and Csontos (1941), usually occurred between the 6th and 11th months of pregnancy. The lesions resembled those described in the above outbreaks and a virus was also incriminated. Successful transmission experiments were carried out in guinea-pigs and mares by means of both filtered and unfiltered suspensions of foetal organs. Characteristic lesions were revealed in the internal organs of the foals that were born alive as well as in the aborted foetuses.

Agglutination.—The evidence provided in the literature indicates that there is a marked divergence of opinion with regard to the agglutination titre of the sera of normal horses for *abortus-equi*. Thus Good and Corbett (1913), van Heeelsbergen (1914), de Jong (1912), and Verge (1939), regard the titre of a normal horse to vary from 1 : 200 to 1 : 300 as compared to a titre of 1 : 500 and over for an affected equine. Murray (1919), on the other hand, found that the titre of a normal horse seldom exceeded 1 : 40, while Saxer (1938), considered a titre of 1 : 100 as positive. More

recently Stitz and Görkel (1938), in making a serological survey of *abortus-equi* in a military camp, tested 237,687 horses of which 766, including 497 mares, had an agglutination titre of 1 : 800 and over; but they noticed that the titre fluctuated considerably during a short time. Moreover, these authors isolated *abortus-equi* from 128 equines that had died from a variety of diseases, in which this organism could not be associated with the clinical picture or the lesions presented. They also isolated *abortus-equi* from 2 out of 408 samples of faeces, from 8 out of 398 samples of urine, and from 19 out of 67 samples of uterine discharge. Some of the carriers were geldings and some gave a negative agglutination reaction to *abortus-equi*. They, therefore, regarded this organism as a secondary invader of horses, except when found in the genital tract.

In the agglutination carried out by these authors it is not stated whether "H", "O", or mixed "O" and "H" antigens were used.

In order to determine the agglutination titre of the serum of normal animals for *abortus-equi* several routine samples of serum from equines, cattle, and sheep submitted to Onderstepoort for various purposes were tested serologically. Both "O" and "H" antigens were employed in these tests.

Of 128 samples of horse serum mostly from mares, submitted from different parts of the country, 13 gave "O" agglutination of 1 : 40, 33 of 1 : 20, 41 of 1 : 10 and the remainder (41) failed to react at these dilutions. The "H" agglutination was either lower or very little higher than the "O". But as Henning and Haig (1940) pointed out, for the detection of carriers, "O" agglutination alone can be relied upon, so that not much attention was paid to the "H" agglutination. In addition, 242 samples of bovine serums were tested; of these, three gave an "O" reaction of 1 : 40, 28 of 1 : 20 and the rest (211) had a titre of 1 : 10 or less. From these results it seems reasonable to assume that equine sera with an "O" agglutination titre of 1 : 40 should be regarded as negative. If the reaction is over 1 : 80 the serum is probably positive for *abortus-equi*.

Several agglutination tests were carried out at regular intervals with the sera of the equines involved in the Smal outbreak described by Henning, Keppel, and Flight (1943). The results of these tests are given in Table 1.

The sera of two mares taken some time after abortion were submitted to Onderstepoort for examination. The one sample was haemolysed while the other gave a strongly positive reaction for *abortus-equi*, and a diagnosis of *abortus-equi* infection was made. Arrangements were then made for the examination of sera from all the equines in the stud. The results of the agglutination tests obtained with these sera (Table 1) were not quite clear as the majority of the mares that were known to have aborted from one to three months previously gave negative agglutination reactions while others reacted positively (compare Tables 2, 4 and 6). Sera submitted from two pregnant mares, Nos. 34 and 35, were also negative. Later both these mares aborted and the serological reactions obtained with their sera were regarded as significant.

Mare No. 35 aborted on 26th September, 1942, i.e., one day after her serum had a negative reaction; but when she was bled nearly 3 weeks later (11th October) her serum was positive. Mare No. 34, which aborted about a month later, was still negative on the day of abortion but had become positive within 19 days. The sera of both mares 34 and 35 remained positive

for 2-3 months. *Abortus-equi* was readily recovered from all the foetal organs and the after-births of both these mares; the foetal organs of no other mare were available for examination.

In order to ascertain whether a virus as described by Dimock and Edwards (1936) was present in these *abortus-equi* infected foetal organs, preliminary transmission experiments were carried out with 8 pregnant guinea-pigs and one pregnant mare, using collodion filtrates of foetal organ suspensions as inoculum. The guinea-pigs were inoculated either by the subcutaneous or the intraperitoneal routes, receiving from 2 to 4 c.c. each; the mare was inoculated intravenously with approximately 20 c.c. of the filtrate.

Three of the guinea-pigs that aborted before the 7th day were discarded from the experiment, but the remaining five either aborted or gave birth to weaklings after an incubation period which varied from 9 to 19 days. Nearly all the guinea-pigs that were born alive died after a day or two. The mare delivered a live but very weak foal on the 22nd day; the foal refused to drink and died on the third day. Apart from the presence of abnormal amounts of blood-stained fluids in the pericardial, pleural, and peritoneal cavities no pathological changes were observed. The necrotic nodules described by Dimock and Edwards (1936), could not be seen and a bacteriological examination of the foetal organs was negative. No further material was available and the experiment was interrupted until August, 1944, when a very severe outbreak of infectious equine abortion occurred in the Animal Embarkation Depot at Pinetown, where a number of equines brought from different parts of the country were collected and kept in small camps prior to export to India.

THE PINETOWN OUTBREAK.

Foetal organs preserved in 50 per cent. glycerine were submitted by Capt. B. M. McIntosh to Onderstepoort for examination. In all, organs from eight horse and four donkey foetuses were submitted. Of these, four horse foetuses and all four donkey foetuses yielded *abortus-equi* on culture; from four of the horse foetuses no *abortus-equi* could be obtained. Only horse and donkey foetal organs that were found to be positive for *abortus-equi* were used for transmission experiments. An exception was made in the case of the foetal organs of mare 683 (*vide infra*). Sera obtained from all the affected mares were tested at the time of abortion and again about 2 weeks later, and in most cases again at varying intervals subsequently (Table 2). It will be noticed that, with the exception of mares T.1082 and T.604, the sera of all the mares taken at the time of abortion were negative. The sera of all those whose foetuses yielded *abortus-equi* on culture became positive from 1-2 weeks afterwards, remained positive for some time and then the titre gradually dropped. With the exception of mare T.604 the sera of all the mares whose foetuses were negative for *abortus-equi* remained negative afterwards. The serum of this mare (T. 604), however, was found to be positive on the day of abortion given.

Transmission Experiments.—In the Pinetown outbreak it was found that approximately 90 per cent. of the pregnant horse mares kept at the animal Embarkation Depot aborted. A few gave birth to live foals, but these foals developed joint-ill and had to be destroyed. Large numbers of equines were confined to small camps so that conditions were very favourable for the dissemination of the infection, once it was introduced. A number

TABLE 1

*Outbreak of Equine Abortion H.
Serological Tests with Abortus-Equi, "H"*

No. of Animal.	Date of Abortion.	Date of Bleeding.	O.	H.	Date.	O.	H.	Date.	O.	H.
1.....	11/6/42	25/9/42	20	20	14/11/42	20	20	V2/8/43	40	20
2.....	July/August	25/9/42	160	160	14/11/42	40	80	—	—	—
3.....	July/August	25/9/42	20	20	14/11/42	20	80	V2/8/43	40	40
4.....	6/9/42	25/9/42	80	320	14/11/42	160	160	—	—	—
5.....	June 1942	25/9/42	10	40	14/11/42	10	40	V19/9/43	40	80
6.....	July/August	25/9/42	10	40	14/11/42	20	80	V2/8/43	20	20
7.....	July/August	25/9/42	20	40	14/11/42	40	160	V2/8/43	200*	100
8.....	July/August	25/9/42	20	40	14/11/42	20	40	V2/8/43	40	20
9.....	July/August	25/9/42	20	40	14/11/42	20	40	V2/8/43	40	20
10.....	July/August	25/9/42	20	40	14/11/42	20	80	V2/8/43	40	40
13.....	July/August	25/9/42	320	640	14/11/42	40	320	V2/8/43	80	80
14.....	July/August	25/9/42	10	40	14/11/42	10	40	2/8/43	20	20
15.....	July/August	25/6/42	80	160	14/11/42	80	320	V2/8/43	100	100
16.....	July/August	25/9/42	160	160	14/11/42	80	160	V2/8/43	20	20
20.....	15 August	25/9/42	160	160	14/11/42	80	160	21/12/43	80	0
22.....	July/August	25/9/42	80	160	14/11/42	40	80	2/8/43	80	20
30.....	August	25/9/42	10	80	14/11/42	20	80	V19/4/43	80	80
34.....	27/10/42	25/9/42	10	80	27/10/42	10	40	14/11/42	160	640
35.....	26/9/42	25/9/42	20	80	25/9/42	20	40	11/10/42	320	640
Stallion 44....	Swollen Testis	25/9/42	80	640	14/11/42	320	1280	—	—	—
Stallion 45....	—	25/9/42	20	160	19/11/42	40	160	—	—	—

F = Foaled. V = Inoculated with abortus-equi Vaccine.

* R

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TABLE 1 (cont.)

portion H. J. Smal (1942).
 -Equi, "H" and "O" Antigens.

Date.	O.	H.	Date.	O.	H.	Date.	O.	H.
V11/8/43	200	12,800	20/8/43	400	50,000	—	—	—
—	—	—	—	—	—	—	—	—
V11/8/43	200	1,200	20/8/43	800	50,000	—	—	—
—	—	—	—	—	—	—	—	—
V29/9/43	6400	50,000	F27/5/43	1600	3,200	—	—	—
V11/8/43	200	1,600	20/8/43	400	12,800	—	—	—
V11/8/43	400	6,400	20/8/43	1600	6,400	—	—	—
V11/8/43	100	6,400	20/8/43	200	12,800	—	—	—
V11/8/43	200	3,200	20/8/43	400	50,000	—	—	—
V11/8/43	100	3,200	20/8/43	1600	6,400	—	—	—
V11/8/43	400	800	20/8/43	800	50,000	—	—	—
F31/8/43	20	40	10/9/43	20	—	—	—	—
V11/8/43	400	50,000	20/8/43	1600	100,000	—	—	—
V11/8/43	200	3,200	20/8/43	1600	50,000	—	—	—
—	—	—	—	—	—	—	—	—
24/9/43	80	40	19/10/43	40	20	—	—	—
V29/4/43	200	6,400	8/5/43	200	50,000	—	—	—
V2/8/43	40	20	V11/8/43	200	3,200	20/8/43	400	500,000
2/3/43	80	320	2/8/43	160	40	—	—	—
—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—

* Reaction on 2.3.43 was O = 100, H = 800.



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TABLE 2.
Agglutination Reactions of Sera Submitted from the Pinetown Outbreak.

No. of Animal.	Date of Abortion.	Foetal Organ Pos. or Neg. for <i>ab. equi.</i>	Date of Bleeding.	Abortus-equi.		Date of Bleeding.	Abortus-equi.		Date of Bleeding.	Abortus-equi.	
				O.	H.		O.	H.		O.	H.
T. 902.....	14/7/44	Pos.	9/8/44	320	640	11/10/44	40	80	—	—	—
T. 604.....	25/8/44	Neg.	25/8/44	320	320	11/10/44	40	20	—	—	—
T. 1899.....	6/9/44	Neg.	25/8/44	20	20	6/9/44	20	0	11/10/44	20	20
T. 1966.....	8/9/44	Pos.	8/9/44	20	20	15/9/44	640	320	11/10/44	160	80
T. 1082.....	11/9/44	Pos.	11/9/44	160	320	11/10/44	1280	2500	—	—	—
T. 828.....	14/9/44	Pos.	15/9/44	40	10	25/9/44	320	10,000	11/10/44	320	640
Donkey V. 1991.....	30/9/44	Pos.	30/9/44	20	40	15/10/44	1280	160	—	—	—
Donkey M. 138.....	3/10/44	Pos.	5/10/44	20	80	15/10/44	80	1,280	—	—	—
Donkey V. 4531.....	11/10/44	Pos.	11/10/44	80	320	20/10/44	320	320	—	—	—
T. 1983.....	11/10/44	Neg.	11/10/44	40	10	20/10/44	20	10	—	—	—
Donkey M. 55.....	11/10/44	Pos.	11/10/44	40	40	20/10/44	80	640	—	—	—
T. 1855.....	11/10/44	Neg.	11/10/44	40	40	20/10/44	40	10	—	—	—

TABLE 3.
*Results of Transmission Experiments with Latapi Minced Infected Foetal
 Organs or Live Cultures of Abortus-Equi Given by the Mouth.*

No. of Animal.	Date of introduction into Experiment.	Nature of Experiment.	Result.	Result of Bact. Examination of Organs or Afterbirth.	Pathological Anatomy.
Horse 176..	17/8/44	Dosed with minced organs of foetus from Pinetown	Aborted on 2/9/44.....	Foetal organs positive for <i>abortus-equi</i>	Sub-epi- and endocardial hæmorrhages. Hyperæmia of intestines.
Horse 21818	12/9/45	Dosed with minced foetal organs from Pinetown	Aborted on 1/10/44.....	Foetal organs positive for <i>abortus-equi</i>	Hydrothorax, hydropericard., petechiæ on surface of lungs and peritoneum. Hyperæmia of intestines.
Horse 682..	27/11/44	Dosed with minced foetal organs from Pinetown	Aborted on 2/1/45.....	Foetal organs positive for <i>abortus-equi</i>	Sub-epi- and endocardial hæmorrhages, hyperæmia of lungs and liver, atelectasis, tumor splenis.
Donkey 691	27/11/44	Dosed with minced foetal organs of 20810	Aborted on 12/12/44...	Foetal organs positive for <i>abortus-equi</i>	Hydrothorax, hydropericard, ascites, sub-epi- and endocardial petechiæ; petechiæ on surface of spleen, tumor splenis, intense hyperæmia of intestine, degeneration of liver and kidneys.
Donkey 687	27/11/44	Dosed with infected joint-oil from foal of mare 22523	Foaled normally on 11/3/45	Afterbirth negative for <i>abortus-equi</i>	—
Donkey 704	30/12/44	Dosed with minced organs of foetus of 683 (<i>abortus-equi</i> free)	Foaled on 10/2/45; foal apparently normal	Afterbirth positive for <i>abortus-equi</i>	—
Donkey 705	30/10/44	Dosed with minced organs of foetus of 683 (<i>abortus-equi</i> free)	Aborted on 20/1/45.....	Foetal organs positive for <i>abortus-equi</i>	Sub-epi- and endocardial hæmorrhages, hydropericard, tumor splenis, hyperæmia of intestines, stasis of liver.
Donkey 728	13/2/45	Dosed with latapi minced organs of 694 and 705	—	—	—
Donkey 693	5/12/44	Dosed with emulsion of 6 mason tube cultures in saline	Foaled on 28/12/44 Foal apparently normal	Afterbirth not examined..	—
Donkey 697	4/12/44	Dosed with emulsion of cultures from 6 mason tubes in saline	Foaled with difficulty on 19/1/45. Foal weak and died few hours later	Foetal organs negative for <i>abortus-equi</i> .	Hyperæmia of liver and lungs.
Donkey 689	4/12/44	Dosed with emulsion of cultures from 6 mason tubes in saline	Foaled 22/3/45.....	Afterbirth negative for <i>abortus-equi</i>	—

of pregnant donkey mares, kept in the same camps as the horses, also picked up the infection, but, although several abortions occurred among the donkeys, the disease did not seem to affect them as seriously as horses, and a greater proportion of donkey foals were born alive. Moreover, the donkey foals that were born alive, remained apparently healthy and developed normally.

Pathological Anatomy.—According to Captain McIntosh the fetuses aborted during the Pinetown outbreak presented the following lesions to a varying degree:—Hydrothorax, hydropericardium, ascites, epicardial and endocardial haemorrhages, petechiae on surface of lungs and spleen, tumor splenis, degeneration of the liver, icterus, and oedema of the placenta.

Notwithstanding the apparently greater resistance of donkey mares to infectious equine abortion so few horse mares were available that a number of pregnant donkey mares had to be employed for some of the transmission experiments.

The material used for the transmission experiments included (1) latapi minced foetal organs, (2) filtrates of latapi minced organs, and (3) live suspensions of 24 hours old cultures of different strains of *abortus-equi*. The foetal organs used were first obtained from aborted fetuses at Pinetown, but later as abortions were experimentally produced at Onderstepoort, the organs of these fetuses were used.

A number of the donkey mares and one horse mare that were admitted into the transmission experiment on the assumption that they were pregnant had to be discharged subsequently as they turned out not to be in foal.

(A) *Transmission by Means of Latapi Minced Abortus-Equi Infected Foetal Organs (Tables 3 and 4).*

Exceptions mares 704 and 705.

1. *Horse mare* 176 was dosed on 17th August, 1944, with minced *abortus-equi* infected foetal organs obtained from Pinetown. This mare aborted on 2nd September, 1944, i.e. 16 days later. All the foetal organs were positive for *abortus-equi* infection and the foetus showed the following lesions: Sub-epicardial and sub-endocardial haemorrhages, marked hyperaemia of intestinal mucosa. The serum of the mare was negative at time of abortion, but positive 13 days later.

2. *Horse mare* 21818 was dosed on 12th September, 1944, with *abortus-equi* infected minced organs. It aborted on 1st of October, 1944, i.e. 19 days later. The foetal organs were positive for *abortus-equi* and the foetus presented the following lesions: Hydrothorax, hydropericardium, petechiae on surface of lungs and on parts of peritoneum, and hyperaemia of intestines. The serum of the mare was negative at the time of abortion, but positive 16 days later.

3. *Horse mare* 682 was dosed with minced *abortus-equi* infected organs on 27th November, 1944. It aborted on 2nd January, 1945, i.e., 37 days after infection. All the foetal organs were positive for *abortus-equi* and the foetus presented the following lesions: Sub-epi- and endocardial haemorrhages, hyperaemia of lungs and liver, tumor splenis. The serum of this mare was negative at the time of abortion but positive two weeks later.

4. *Donkey mare* 691 was dosed on 27th November, 1944, with organs of the foal of mare 20810 (see Tables 5 and 6) and aborted on 12th December, 1944. The foetus presented lesions of hydrothorax, hydropericardium, ascites, sub-epi- and endocardial haemorrhages, petechiae on surface of spleen, tumor splenis, severe hyperaemia of intestines, degeneration of liver and kidneys. The foetal organs were positive for *abortus-equi* and the serum of the mare, negative at the time of abortion, was suspicious or slightly positive two weeks later.

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TABLE 4.
Serological Results of the Mares in Table 3 with Abortus-Equi "O" and "H".

No. of Animal.	Date of Abortion or Foaling.	Date of Test.	O.	H.	Date of Test.	O.	H.	Date of Test.	O.	H.	Date of Test.	O.	H.
Horse 176..	Aborted on 2/9/44....	2/9/44	10	10	15/9/44	320	320	23/9/44	80	320	2/11/44	40	20
Horse 21818	Aborted on 1/10/44....	12/9/44	0	10	3/10/44	20	40	17/10/44	640	5,000	19/2/45	40	160
Horse 682..	Aborted on 2/1/45....	27/11/44	10	10	2/1/45	10	40	16/1/45	320	80	8/2/45	640	80
Donkey 691	Aborted on 12/12/44..	27/11/44	40	40	12/2/45	40	40	27/2/45	80	40	8/2/45	40	40
Donkey 704	Foaled on 10/2/45....	30/12/44	0	10	10/2/45	0	40	26/2/45	20	40	10/3/45	10	20
Donkey 705	Aborted on 20/1/45....	30/12/44	0	20	20/1/45	0	20	3/2/45	160	80	8/2/45	320	160
Donkey 728	—	13/2/45	0	10	—	—	—	—	—	—	—	—	—
Donkey 693	Foaled on 28/12/45....	27/11/44	10	40	28/2/44	10	20	11/1/45	320	80	8/2/45	80	40
Donkey 697	Foaled on 19/1/45. Foal died 19/1/45	27/11/44	0	20	19/1/45	0	20	2/2/45	0	20	8/2/45	10	20
Donkey 689	Foaled on 23/3/45....	27/11/44	0	20	23/3/45	640	1,280	26/3/45	160	320	13/4/45	160	10
Donkey 687	Foaled on 11/3/45....	27/11/44	10	40	12/3/45	10	20	26/3/45	20	40	10/4/45	20	40

5. *Donkey mare* 704 was dosed on 30th December, 1944, with the minced organs of the foetus of horse mare 683 which were culturally negative for *abortus-equi*. She foaled on 10th February, 1945, and the foal appeared quite normal, but *abortus-equi* was isolated from the after-birth. The serum of this mare was negative at the time of abortion and remained negative afterwards.

6. *Donkey mare* 705 was dosed on 30th December, 1944, with the minced organs of the foetus of horse mare 683 which were culturally negative for *abortus-equi*. She aborted on 20th January, 1945, and the foetal organs were found to be positive for *abortus-equi*. Her serum was negative at the time of abortion but positive two weeks later. The foetus presented lesions of sub-epi- and endocardial haemorrhages, hydro-pericard, tumor splenis, hyperaemia of the intestine and stasis of liver.

Three donkey mares, viz., Nos. 693, 697 and 689 were each dosed on 4th December, 1944, by means of a stomach tube with the saline emulsion of 24 hours' growth of three strains of *abortus-equi* on 6 Mason tubes (Mason, 1933).

Mare No. 693 gave birth to a live healthy foal on 28th December, 1944. Her serum was negative on the day of abortion but positive 2 weeks later. The after-birth was not examined bacteriologically.

Mare No. 689 gave birth to a live foal on 22nd March, 1945, i.e., after 118 days. Her after-birth was negative for *abortus-equi*, but her serum was strongly positive on the day of abortion.

Mare No. 697 foaled with difficulty on 19th January, 1945, and the foal died a few hours after birth. Apart from hyperaemia of the lungs and liver no abnormal changes were revealed by the internal organs of this foetus. A bacteriological examination of the foetal organs was negative for *abortus-equi*; the serum of the mare was negative at the time of abortion and remained negative afterwards.

Mare 687 was dosed with infected joint fluid from the foal of horse mare 22523 on 27th November, 1944 (foal was suffering from *abortus-equi* infected joint-ill). She gave birth to an apparently healthy foal on 11th March, 1945. Her serum, negative on 12th March, i.e., one day after abortion, remained negative. The after-birth failed to yield *abortus-equi* on culture.

(B) *Transmission by Means of Filtrates (Tables 5 and 6).*

Abortus-equi infected foetal organs were minced in the latapi and approximately 10 gm. of the pulp was suspended in 30 c.c. isotonic saline. The suspension was spun for 3 hours at 3,000 r.p.m. in a Clay-Adams angle centrifuge. The supernatant fluid obtained was passed through an asbestos filter (Elford, 1938). The resultant filtrate was then filtered under two atmospheres of nitrogen, first through collodion membrane with the size of the pores 1,200 milli-micron, and finally through a membrane with the pores 810 milli-micron in size (Elford, 1938, Bauer and Hughes, 1935). The filtrations were carried out by Dr. A. Polson, of the Section of Virus Disease of this laboratory.

The filtrate was tested for sterility by inoculating approximately 2 c.c. either into tubes containing 15 c.c. broth each, or into 30 c.c. chopped meat broth. No growth was apparent during the first four or five days, but after about two weeks incubation a turbidity was noticed in some of the tubes. Sub-inoculation on to MacConkey, with seed material from the various tubes, yielded a growth in some cases but not in others. The growths obtained from the different tubes all yielded different types of colonies, but the colonies in the same culture appeared to be alike. The morphology of the organisms in the different cultures were also different, some being Gram-positive, coccus-like, while others were Gram-positive bacteria. No organisms could be found that could in any way be associated with a salmonella. The growth, obtained in these tubes were, therefore, regarded as due to contamination. Those tubes where no growth could be detected were considered to be bacteriologically sterile.

TABLE 5.
Results of Transmission Experiments with Filtrates of Infected Foetal Organs.

No. of Animal.	Date of entry into Experiment.	Nature of Experiment.	Result.	Result of Bacteriological Examination of Foetus or Foal.	Pathological Anatomy.
Horse 22523	17/8/44	Inoculated with colloidion filtrate of foetal organ from Pinetown*	Foaled on 7/9/44. Foal apparently normal, but developed joint-ill later	Afterbirth negative for <i>abortus-equi</i>	
Horse 20810	5/9/44	Inoculated with colloidion filtrate of foetal organ from Pinetown	Foaled on 25/10/44. Foal weak and died on 26/10/44	Organs of foal positive for <i>abortus-equi</i>	General icterus, extensive sub-endo- and sub-epicardial petechiae, Oedema of lungs, hyperaemia of stomach and intestines. Retention of meconium.
Horse 20884	15/9/44	Inoculated with colloidion filtrate of foetal organ from Pinetown	Aborted on 17/10/44...	Organs of foetus positive for <i>abortus-equi</i>	Extensive haemorrhages on serous and mucous membranes. Very prominent secondary follicles, haemorrhages in lymph glands
Horse 685..	27/11/44	Inoculated with colloidion filtrate of foetal organ from Pinetown	Foaled on 29/1/45. Foal weak and died on 31/1/45	Organs of foal negative for <i>abortus-equi</i>	Hydropericardium, epicardial petechiae, foetal atelectasis.
Horse 683..	4/12/44	Inoculated with colloidion filtrate of foetal organs from Pinetown	Aborted on 26/12/44...	Organs of foetus negative for <i>abortus-equi</i>	Sub-epi- and sub-endocardial haemorrhages; petechiae on lungs and spleen; hydrothorax and ascites.
Donkey 686	14/12/44	Inoculated with colloidion filtrate of the organs of the foetus of mare 691	Aborted on 17/2/45....	Foetal organs positive for <i>abortus-equi</i>	Hydropericardium, sub-epi- and sub-endocardial haemorrhages; tumor splenis; stasis of liver.
Donkey 692	21/12/44	Inoculated with colloidion filtrate of the organs of the foetus of mare 691	Aborted a 4-5 months old foetus on 20/2/45	Foetal organs positive for <i>abortus-equi</i>	Hydro-thorax, hydro-pericardium, ascites, swelling of the spleen, icterus, oedema of lungs.
Donkey 694	21/12/44	Inoculated with colloidion filtrate of the organs of the foetus of mare 691	Aborted on 25/1/45....	Foetal organs positive for <i>abortus-equi</i>	Hyperaemia of spleen and liver. Degeneration of liver.

* Foal developed joint-ill severely, from which it died later on. *Abortus-equi* isolated from fluid of all affected joints. Agglutination test of mare serum negative.

TABLE 5—(continued).

No. of Animal.	Date of entry into Experiment.	Nature of Experiment.	Result.	Result of Bacteriological Examination of Foetus or Foal.	Pathological Anatomy.
Donkey 731	13/2/45	Inoculated with candle filtrates of organs of foetuses 705 and 694	Aborted a fully grown foal on 22/2/45	Foetal organs and after-birth negative for <i>abortus-equi</i>	Hydro-thorax, hydro-pericard, ascites, oedema of lungs, sub-epi- and endocardial haemorrhages, petechiae on spleen, spleen swollen, marked hyperaemia of intestines, stasis of liver.
Donkey 726	13/3/45	Inoculated with candle filtrates of organs of foetuses 705 and 694	Foaled normally on 2/2/45 foal appears normal	Afterbirth negative.....	—
Donkey 724	13/2/45	Inoculated with candle filtrates of organs of foetuses 705 and 694	Foaled normally on 20/2/45; foal appears normal	Afterbirth negative for <i>abortus-equi</i>	—
Donkey 729	15/2/45	Dosed with 30 c.c. candle filtrate of organs of foetuses 705 and 694	Foaled normally on 12/3/45	Afterbirth negative for <i>abortus-equi</i>	—
Donkey 732	15/2/45	Dosed with 30 c.c. candle filtrate of foetuses 705 and 694	Foaled on 22/3/45.....	Afterbirth positive for <i>abortus-equi</i>	—
Donkey 725	24/2/45	Dosed with 50 c.c. candle filtrate of foetuses 686 and 692	Foaled on 28/3/45.....	Afterbirth negative for <i>abortus-equi</i>	—
Donkey 727	24/2/45	Inoculated with collodion filtrates of organs of foetuses of 686 and 692	Aborted on 30/3/45.....	Afterbirth and foetal organs positive for <i>abortus-equi</i>	Moderate hydropericardium and hydro-thorax sub-endocardial haemorrhages, petechiae on spleen, tumor splenis.
Donkey 733	24/2/45	Inoculated with candle filtrate of organs of foetuses of 686 and 692	Foaled on 9/4/45.....	Afterbirth negative for <i>abortus-equi</i>	—

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Live *abortus-equi* in broth culture or in saline emulsion failed to pass through collodion membranes (pores, 810 milli-micron.)

Filtration was also carried out by means of Berkefeld candles. Approximately 2 c.c. of the filtrate was inoculated into each of six tubes containing chopped meat broth. Although four of the tubes remained sterile after two weeks' cultivation, two tubes showed a turbidity. When the latter were subcultured on MacConkey the one yielded *B.coli* and the other a slow growing Gram-positive coccus, but no organism that resembled a salmonella could be found. These organisms were therefore also regarded as contaminants.

An attempt was made by Mr. D. Haig of the Section of Virus Diseases to cultivate the infective agent present in collodion filtrates on chorio-allantoic membranes, but without any success so far. He found, however, that, whereas chicken embryos were readily killed by means of live *abortus-equi*, nearly all the embryos inoculated with collodion filtrates developed normally.

A histological study of the morbid organs is being carried out by Mr. de Boom of the Section of Pathology and this work will form the subject of a separate paper later on. Meanwhile it can be stated that after a preliminary examination the intra-nuclear inclusion bodies described by Dimock, Edwards and Bruner (1942) have not yet been found.

1. *Horse mare* 22523 was inoculated intravenously on 17th March, 1944, with about 15 c.c. of the filtrate of foetal organs obtained from Pinetown. On 7th September, 1944, she foaled an apparently normal foal. The after-birth was negative for *abortus-equi*, but about 4 weeks later the foal developed joint-ill from which it died. *Abortus-equi* was isolated from the fluid in all the affected joints and tendon sheaths. The serum of the foal showed a strongly positive agglutination reaction, but the mare's serum was negative.

2. *Horse mare* 20810 was inoculated intravenously on 5th September, 1944, with about 15 c.c. of the filtrate of foetal organs obtained from Pinetown. On 23rd October, 1944, i.e., 48 days later, a live foal was dropped which died 24 hours later. The organs of this foal were positive for *abortus-equi* and the foal presented the following lesions: General icterus, sub-epicardial and sub-endocardial haemorrhages, oedema of lungs, hyperaemia of stomach and intestine, hyperaemia of brain and slight hydrocephalus, retention of the meconium. The serum of the mare was negative at the time of parturition and remained negative.

3. *Horse mare* 20884 was inoculated on 15th September, 1944, with the filtrate of foetal organs obtained from Pinetown and aborted on 17th October, 1944, i.e., 33 days later. All the organs of the foetus were positive for *abortus-equi*; the serum of the mare, negative at the time of abortion, was positive 17 days later. The most important lesions presented by the foetus were extensive haemorrhages on the serous membranes, very prominent secondary follicles and haemorrhages in the lymph glands.

4. *Horse mare* 685 was inoculated on 27th November, 1944, with filtrate of foetal organs obtained from Pinetown. It gave birth to a live foal on 29th January, 1945, i.e., 64 days later, but the foal was weak and died two days later, showing lesions of hydropericardium, epicardial petechiae and foetal atelectasis. The organs of the foal were all negative for *abortus-equi*. The serum of the mare was negative on the day of foaling and remained negative for more than a month.

5. *Horse mare* 683 was inoculated on 4th December, 1944, with filtrate of foetal organs obtained from Pinetown. It aborted on 26th December, 1944, i.e., 16 days later, and the foetus presented the following lesions: Sub-epi- and sub-endocardial haemorrhages, petechiae on the surface of the lungs and spleen, slight hydrothorax and ascites. The foetal organs were negative bacteriologically and the serum of the mare was negative at the time of abortion, remaining negative for at least two months.

6. *Donkey mare* 686 was inoculated on 14th December, 1944, with the filtrate of foetal organs of donkey mare 691. She aborted on 17th February, 1945, i.e., 65 days afterwards. The foetal organs were positive for *abortus-equi* and presented lesions of

TABLE 6.
Serological Reactions of the Sera of Mares in Table 5 with Abortus-Equi "O" and "H".

No. of Animal.	Date of Abortion or Foaling.	Date of Test.	O.	H.	Date of Test.	O.	H.	Date of Test.	O.	H.	Date of Test.	O.	H.
Horse 22523	Foaled on 7/9/44.....	2/9/44	40	640	15/9/44	40	160	30/9/44	20	80	3/11/44	20	40
Horse 20810	Foaled on 23/10/44. Foal died 24/10/44	4/9/44	20	10	25/10/44	20	10	30/10/44	20	20	3/11/44	20	10
Horse 20884	Aborted on 17/10/44.....	12/9/44	0	10	17/10/44	40	40	3/11/44	160	320	19/2/45	20	20
Horse 685..	Foaled on 29/1/45. Foal died 31/1/45	27/11/44	20	10	29/1/45	10	20	8/2/45	40	20	26/2/45	40	20
Horse 683..	Aborted on 26/1/45.....	27/11/44	0	20	27/12/44	0	20	15/1/45	20	20	8/2/45	20	20
Donkey 686	Aborted on 17/2/45.....	27/11/44	40	80	19/2/45	40	160	8/3/45	160	1280	26/3/45	80	640
Donkey 692	Aborted on 20/2/45.....	27/11/44	10	40	20/2/45	40	80	10/3/45	40	1280	26/3/45	1280	320
Donkey 694	Aborted on 23/1/45.....	27/11/44	10	40	23/1/45	40	160	8/2/45	320	160	15/3/45	640	80
Donkey 731	Aborted on 22/2/45.....	13/2/45	40	20	22/2/45	40	20	5/3/45	40	20	16/3/45	40	10
Donkey 726	Foaled on 2/3/45.....	13/2/45	20	20	2/3/45	40	20	20/3/45	160	80	26/3/45	320	160
Donkey 724	Foaled on 20/2/45.....	13/2/45	20	10	20/2/45	40	20	8/3/45	20	40	26/3/45	80	80
Donkey 729	Foaled on 12/3/45.....	13/2/45	20	40	13/3/45	0	10	26/3/45	160	80	3/4/45	320	80
Donkey 732	Foaled on 22/3/45.....	13/2/45	10	0	22/3/45	20	80	3/4/45	20	0	13/4/45	80	20
Donkey 725	Foaled on 28/3/45.....	13/2/45	80	40	28/3/45	160	320	3/4/45	80	40	21/4/45	320	160
Donkey 727	Aborted on 30/3/45.....	13/2/45	20	40	31/3/45	20	80	10/4/45	40	2560	21/4/45	160	2560
Donkey 733	Foaled on 9/4/45.....	13/2/45	40	10	10/4/45	20	320	21/4/45	10	320	—	—	—

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hydropericardium, sub-epicardial and sub-endocardial haemorrhages, swelling of spleen and stasis of the liver. The serum of the mare, negative at the time of abortion, was positive 19 days later.

7. *Donkey mare* 692 was inoculated on 21st December, 1944, with the filtrate of the foetal organs of donkey mare 691. She aborted a 4-5 months old foetus on 20th February, 1945, i.e., after 61 days. The foetal organs were culturally positive for *abortus-equi* and the foetus presented lesions of hydrothorax, hydropericardium, ascites, swelling of the spleen, icterus, degeneration of the liver and oedema of the lungs. The serum of the mare, negative at the time of abortion, was positive 19 days later.

8. *Donkey mare* 694 was inoculated on 21st December, 1944, with the filtrate of the foetal organs of donkey mare 691. She aborted on 23rd January, 1945, i.e., after 33 days. The foetal organs were positive for *abortus-equi* and the foetus presented lesions of hyperaemia of the liver and spleen and degeneration of the kidneys. The serum of the mare was suspicious or slightly positive on the day of abortion but became strongly positive 2 weeks later.

9. *Donkey mare* 731 was inoculated on 13th February, 1945, with candled filtrates of the foetuses of donkey mares 705 and 694. She aborted a fully-developed foal on 22nd February, 1945, i.e., 9 days afterwards. The foetal organs were culturally negative for *abortus-equi* and the foetus presented lesions of hydrothorax, ascites, hydropericardium, sub-epicardial and sub-endocardial haemorrhages, petechiae on surface of spleen, swelling of spleen, oedema of lungs, stasis of liver and intense hyperaemia of the intestines. The serum of the mare was negative at the time of abortion, and remained negative.

10. *Donkey mare* 726 was inoculated on 13th February, 1945, with candled filtrates of foetal organs of 705 and 694. It gave birth to an apparently normal foal on 2nd March, 1945. Its after-birth was negative for *abortus-equi*. Its serum, negative on the day of parturition, was positive 18 days later.

11. *Donkey mare* 729 was dosed with candled filtrates of the foetal organs of donkey mares 705 and 694 on 15th February, 1945. She foaled an apparently normal and healthy foal on 12th March, 1945, and the after-birth was negative for *abortus-equi*. Her serum was negative on the day of parturition, but positive 2 weeks later.

12. *Donkey mare* 732 was dosed on 15th February, 1945, with candled filtrates of foetuses of donkeys 694 and 705. She delivered an apparently normal foal on 22nd March, 1945. The after-birth was positive for *abortus-equi*. The serum, negative at the time of abortion, was still negative 2 weeks later.

13. *Donkey mare* 725 was dosed on 24th of February, 1945, with approximately 50 c.c. of candled filtrates of the organs of the foetuses of donkeys 686 and 692. Its after-birth was culturally negative for *abortus-equi*, but its serum was positive at the time of parturition.

14. *Donkey mare* 727 was inoculated on 24th of February, 1945, with about 20 c.c. of collodion membrane filtrates of the organs of foetuses of donkeys 686 and 692. It aborted on the 30th of March. The organs of the foetus showed lesions of endocardial haemorrhages, petechiae on surface of spleen, tumor splenis, moderate hydropericardium and hydrothorax. The foetal organs and after-birth readily yielded *abortus-equi* on culture. The serum of the mare was negative at the time of abortion, but positive two weeks later.

All the equines used in the experiment gave negative tests for dourine. Five horse mares, Nos. 22523, 20810, 80884, 176, and 21818, were selected at random from a group of twelve pregnant mares that had been running for a number of years on Kaalplaas, a farm adjoining Onderstepoort. The five mares used in the experiment were transferred to Onderstepoort, while the remaining seven remained at Kaalplaas. The latter all gave birth to apparently normal and healthy foals, that have remained healthy for more than twelve months.

The other equines used in the experiment were obtained from an area where infectious equine abortion is not known to occur.

It was found that the mares in which abortion was produced experimentally did not suffer in any way as the result of the premature birth; the after-birth was expelled immediately after parturition and involution of the uterus took place as in a normal birth. The majority of the experimentally produced abortions occurred from 7-10 months of pregnancy, but one foetus was dropped when it was barely 5 months old while another was fully developed at the time of delivery. The incubation period also varied considerably; in one case a foetus was expelled 9 days after the mare was inoculated with filtrate, whereas in three other cases the abortion took place after the lapse of over 60 days (61, 64 and 65 days respectively). As a rule the incubation period varied from 15 to 33 days; it appeared to be shorter in the mares given minced organs by the mouth than in those inoculated with filtrate—probably because the former received far more infective material.

The virulence of the infecting agent appeared to decrease during the end of an epizootic. In the Smal outbreak only two live foals were produced by 29 mares during a period of 10 weeks whereas three of the last five mares that carried their foals till the end of the outbreak delivered live foals, only two abortions occurring. At Onderstepoort nearly a 100 per cent. abortions were produced during the first part of the experiment, while the majority of donkey mares subsequently infected by means of either filtrates or unfiltered material gave birth to live foals.

In the Smal outbreak the disease ran its course during one season (1942) with an abortion incidence of over 85 per cent. and then completely disappeared. During the subsequent breeding seasons (1943 and 1944) not a single abortion was reported.

At present it cannot be stated whether the disappearance of the disease should be attributed to the immunity resulting from the infection or to the attenuation of the virus during the course of the outbreak.

SUMMARY OF RESULTS.

1. (See Tables 3 and 4.) Three horse mares, Nos. 176, 21818 and 682, and two donkey mares, Nos. 691 and 728, were dosed with minced foetal organs infected with *abortus-equi*. All three the horse mares aborted after an incubation period varying from 16 to 37 days; one donkey mare (691) aborted after 15 days, the other (728) turned out not to be pregnant.

The foetuses of all three horse mares and of the one donkey mare were infected with *S. abortus-equi*.

The sera of the three horse mares and one donkey mare, No. 691, were negative when abortion occurred but became positive within two weeks.

Two donkey mares, Nos. 704 and 705, were dosed with the minced foetal organs of horse mare 683 which were culturally free from *abortus-equi* infection. The one, No. 705, aborted after 21 days, while the other one, No. 704, gave birth to a live foal after 42 days. The foetal organs of 705 and the after-birth of 704 were infected with *S. abortus-equi*. The serum of 705 was negative at the time of abortion and positive 14 days later, but the serum of 704 was negative at the time of parturition and remained negative.

Three donkey mares, Nos. 693, 697 and 689, were dosed with large amounts of live *abortus-equi* culture. The one, No. 693, gave birth to an apparently normal foal after 23 days, while the foal of another mare, No. 697, died soon after birth, apparently as a result of dystokia. No. 689 gave birth to a healthy foal after 118 days. The organs of the foal of No. 697 were culturally negative for *abortus-equi* and the mare's serum was also negative. The serum of 693, negative at the time of parturition, was positive about two weeks later, while the serum of 689 was positive at the time of parturition.

The lesions presented by the foetal organs of horse mares 176, 21818 and 682 and of donkey mares 691 and 705 were typical of those found in the naturally occurring disease but no definite lesions could be found in the foal of donkey mare 697, which was dosed with live culture.

One donkey mare, No. 687, was dosed with the infected synovial fluids of the foal of mare 22523 which was suffering from joint-evil. It foaled apparently normally after 102 days. Its after-birth was culturally negative for *abortus-equi* and its serum was also negative.

2. (See Tables 5 and 6.) Five horse mares, Nos. 22523, 20810, 20884, 685 and 683, and six donkey mares, Nos. 686, 692, 694, 688, 727 and 730, were inoculated intravenously with collodion filtrates of *abortus-equi* infected foetal organs.

Horse mares, Nos. 20884 and 683, and donkey mares 686, 692, 727 and foal of 22523 developed *abortus-equi* infected joint-ill from which it died. The foal of 20810 lived for 24 hours and the foal of 685 for about 48 hours; on post mortem both presented lesions which are typical of infectious abortion, the organs of the former being culturally positive for *abortus-equi* but those of the latter negative. The sera of all three mares were negative at the time of parturition and remained negative thereafter.

Horse mares, Nos. 20884 and 683, and donkey mares 686, 692, 727 and 694 aborted and all their foetuses presented lesions which resemble those found in the naturally occurring disease. The organs of the foetuses of horse mare 20884 and donkey mares 686, 727, 692 and 694 were culturally positive for *abortus-equi*, but the organs of the foetus of 683 were negative. The sera of all six mares were negative at the time of abortion and became positive in the case of 20884, 686, 692, 694, and 727, but the serum of 683 remained negative.

Donkey mares 731, 726, 724 and 733 were inoculated intravenously with candle filtrates of *abortus-equi* infected foetal organs, while Nos. 729, 732 and 725 were dosed with similar filtrates. No. 724 gave birth to a live foal on the 7th day while No. 731 aborted after 9 days; Nos. 726, 732, 725 and 729 also produced live foals. The lesions presented by the foetus of 731 were typical of infectious abortion but the foetal organs were all negative for *abortus-equi*. *Abortus-equi* was recovered from the after-birth of No. 732 (and also from 704), but not from Nos. 724, 729, 731, 725 and 726. The sera of all these mares were negative at the time of parturition or abortion and remained negative, except in the case of 726, whose serum was positive 18 days after parturition.

The preliminary results obtained with eight guinea-pigs and one mare in 1942 when foetal material from the Smal outbreak was used, are not given here.

DISCUSSION.

In the evidence presented in the review of the literature it is apparent that the etiology of infectious (epizootic) equine abortion requires elucidation. Most workers agree that a bacterium, *S. abortus-equi*, is the most important etiological agent, but during recent years a number of investigators have incriminated a virus as the cause of some outbreaks of abortion. Thus Dimock and Edwards (1936), Miessner (1938) and Hupbauer (1938), although recognising the importance of *abortus-equi* as the cause of the majority of epizootics, were unable to find this organism in the foetal organs examined by them during some outbreaks and incriminated a virus.

On the other hand several other investigators like Kilborne (1893), Smith (1893), Good and Corbett (1913), Van Heelsbergen (1914), Schofield (1914), MacFadyean and Stockman (1917), Murray (1919), Verge (1939), and Saxer (1938) have isolated *abortus-equi* from the majority of aborted foetuses examined during the outbreaks investigated by them. During the transmission experiments carried out by some of these workers it was possible to produce abortion only when live cultures of the organism were inoculated intravenously or intraperitoneally and, as the abortion occurred after a comparatively short incubation period, it was probably the result of an *abortus-equi* septicaemia. Good and Corbett (1913) admit that the experimental mare may be off colour for a few days as a result of the inoculation. Premature birth could *not* be produced when the cultures were given by the mouth or per vaginam.

Van Heelsbergen (1914), Hupbauer (1938), and others, although recommending the immunization of pregnant mares with killed cultures of *abortus-equi*, admit that this form of immunization will not protect against exposure to infection. Notwithstanding this, dead cultures have been found to produce a very high agglutination titre in inoculated animals (Table 1).

The fact that infected mares do not abort more than once, and usually give birth to healthy foals during succeeding pregnancies, is an indication that they have had an immunity conferred upon them as a result of the previous natural infection. Because this infection may be associated with invasion of the tissues by *abortus-equi* it is no proof that this organism is the cause of the abortion, nor does it follow that *abortus-equi* has conferred the resistance.

The disease reported by Dimock and Edwards (1936), Miessner (1938), and Hupbauer (1938), has so many features in common with the one described by Good and Corbett (1913), van Heelsbergen (1914), Murray (1919), and others, that they cannot be differentiated on clinical and pathological grounds. They both showed a number of common features, viz., the infection is extremely contagious, the abortion incidence is very high (up to 90 per cent.), the disease appears suddenly during one season and usually does not recur in the same stud during two consecutive years, the affected mare apparently suffers no ill-effect and usually takes the stallion as readily as an unaffected mare.

On the other hand, it should be pointed out that we have not yet succeeded in demonstrating the presence of the "inclusion bodies" described by Dimock, Edwards and Bruner (1942).

In the experiments reported here abortion was readily provoked in both horse and donkey mares by means of filtered and unfiltered suspensions of infected foetal organs, but it was not possible to induce abortion when

large doses of live *abortus-equi* cultures were administered by the mouth. All the aborted fetuses presented the same lesions, whether the mare had received filtered or unfiltered organ suspensions.

The question arises, therefore, what rôle *abortus-equi* plays in the etiology of infectious equine abortion. Of the five horse mares that were inoculated with filtrate two aborted and three dropped live foals. Of the latter, two were so weak that they died within 48 hours and presented lesions which are typical of the disease; *abortus-equi* was recovered from the one, but not from the other foal; the third foal developed joint-ill from which it died. When the *abortus-equi*-free, unfiltered organs of the foetus of horse mare 683 were given to two donkey mares (704 and 705), the one (705) aborted, while the other (704) gave birth to a live foal. But, notwithstanding the absence of *abortus-equi* in the foetal organs dosed, this organism was recovered in large numbers from the organs of the aborted foetus of 705 and in smaller numbers from the after-birth of 704. *Abortus-equi* was also recovered from the after-birth of another mare (732) that had delivered a live foal.

In spite of their apparently greater resistance to infectious equine abortion five of the donkey mares inoculated with filtrate aborted; in the case of four, collodion membrane filtrates were used and in the other one Berkefeld candle filtrates. The foetal organs of all five presented typical lesions of equine abortion. *Abortus-equi* was recovered from the organs of the first four foetuses but not from the last.

When the serological results (Tables 1, 2, 4 and 6) are studied it will be noticed that, if the organs of an aborted foetus have been invaded by *abortus-equi*, the serum of the mare, negative at the time of abortion, invariably becomes positive in about two weeks. If the foetal organs are free from *abortus-equi* the agglutination reaction is likely to remain negative.

When a live foal has been born and it dies within 48 hours the serum of the mare generally remains negative whether the foetal organs have been invaded or not. (See agglutination reactions of mares 20810 and 685.)

Several workers have published information which shows that *abortus-equi* may occur in the body of equines as a secondary invader. Thus Stitz and Görkel (1938) isolated this organism from the faeces, urine and uterine discharges of horses; they also obtained it from a variety of diseases in which it could not be associated with the clinical picture or the lesions. Geldings as well as mares were found to be carriers. Sometimes *abortus-equi* invades the tissues and may be associated with suppurations and abscessation—Martinaglia (1929), Good and Corbett (1913), Good and Smith (1914), Schofield (1914), MacFadyean and Stockman (1917), Miessner and Berge (1917), Watanabe (1937), Oguni and Koihutsu (1938).

In the experiments reported here *abortus-equi* sometimes was found to invade the tissues of a foetus when the mare had aborted after receiving bacteria-free filtrates, whereas in other cases the organism could not be recovered from the aborted foetus when the mare was inoculated with a similar filtrate. Sometimes *abortus-equi* was obtained from the after-birth of a mare which has delivered a live foal (704 and 732). The fact that the serum of a mare is usually negative at the time of abortion but positive 2 weeks later, provided the foetus is invaded, is an indication that the tissues of the mare have not been entered long before the abortion occurred.

It would appear, therefore, that the rôle played by *abortus-equi* in relation to infectious equine abortion is analogous to that of *S. cholerae-suis* in relation to hog-cholera. For many years after the discovery by Salmon and Smith (1885) of *S. cholerae-suis* in the morbid tissues of pigs that had died from swine fever this organism was universally regarded as the cause of the disease. Later, however, de Schweinitz and Dorset (1903), and Dorset, Bolton and McBryde (1904), showed beyond doubt that a filterable virus is the real cause and that *S. cholerae-suis* serves merely as a secondary invader, entering the tissues only after the virus has lowered the resistance of the body.

On the other hand, it may be possible that, as with *Haemophilus influenzae suis* and swine influenza virus (Bang, 1943), there is a synergistic action between *S. abortus-equi* and the filterable infecting agent of equine abortion.

The results reported here clearly indicate (1) that the infective agent of infectious equine abortion is present in the morbid tissues of an aborted foetus and that it is filterable through collodion membrane (pores, 810 millimicra) and Berkefeld candles; (2) that *abortus-equi* may or may not be present in the infected foetal tissues and (3) that live cultures of *abortus-equi*, when given alone, cannot produce abortion, whereas bacteria-free filtrates of infected organs generally cause premature birth.

The fact that *abortus-equi* is present in the morbid organs of the majority of the aborted fetuses studied, though not in all, cannot be accepted as proof that it is the cause of infectious equine abortion. Like *S. cholerae-suis* it should be regarded as a secondary invader, which frequently enters the tissues of the foetus and the foetal membranes after their resistance has been lowered by the filterable infective agent which is the primary cause.

The association of a salmonella as a secondary infective agent with the primary cause (a virus) of two epizootic diseases, viz. swine-fever and infectious equine abortion, suggests the advisability of further investigating the etiological significance of other salmonellas found in epizootic diseases like calf paratyphoid and fowl typhoid.

SUMMARY AND CONCLUSIONS.

1. Two outbreaks of infectious equine abortions are reported; both with a very high abortion incidence.
2. In the majority of the abortions studied the foetal organs were found to be extensively invaded by *S. abortus-equi* but in others this organism could not be recovered from the foetus or after-birth.
3. Both donkey and horse mares were found to be susceptible, donkeys being on the whole more resistant than horses.
4. Abortion was successfully produced in both horse and donkey mares by means of (a) the oral administration of minced *abortus-equi* infected foetal organs (4 cases), (b) intravenous inoculation of collodion membrane or Berkefeld candle filtrates of these organs (9 cases, including 2 foals that died within 48 hours of birth) and (c) the oral administration of minced foetal organs that were culturally free from *abortus-equi* (one case).
5. The twelve aborted fetuses and the two foals that died within 48 hours of birth all presented lesions typical of infectious equine abortion.

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6. *Abortus-equi* was recovered from the organs of ten of the aborted foetuses and one of the dead foals, but not from two aborted foetuses and one dead foal. It was also isolated from the after-birth of two mares that had given birth to live foals (Nos. 704 and 732).

7. All the mares gave negative agglutination reactions at the time of abortion or parturition, but in those cases where *abortus-equi* was recovered from the foetus—not the foal—the reaction became positive in about 2 weeks. When the organs of the foetus were free from *abortus-equi* or when a live foal was born, whether its organs contained *abortus-equi* or not, the agglutination reaction of the mare generally remained negative.

8. The aborting mares did not suffer any ill effects as the results of the abortions and the after-births were expelled normally.

9. It is concluded that the primary cause of infectious equine abortion is an infecting agent that will pass either through collodion membrane, with the size of the pores 810 milli-micra, or through Berkefeld candles.

10. It was not possible to produce abortion in three pregnant donkey mares dosed with large amounts of live *abortus-equi* culture.

11. The significance of *S. abortus-equi* in equine abortion is comparable with that of *S. cholerae* in swine-fever. Like *cholerae-suis*, *abortus-equi* is regarded as a saprophyte which frequently occurs in the body of the horse without causing any obvious disturbance, invading the tissues of the body only when conditions become favourable. When the resistance of the foetus, foetal membranes and uterus has been lowered by the primary cause of infectious abortion, viz., the filterable infecting agent, this organism enters and causes a secondary infection. Alternatively *abortus-equi* plays the role of a synergist as *H. influenzae suis*.

12. The advisability of further investigating the etiological significance of salmonella encountered in epizootic diseases like calf paratyphoid and fowl typhoid is suggested.

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