CONTRIBUTION TO THE BIOLOGICAL AND SEROLOGICAL STUDY OF *B. ABORTUS EQUI*.

SEIICHI FUJIMURA, TAKEO TOYOSHIMA and TAKEO SUENAGA.

(From the Veterinary Laboratory, Ministry of Agriculture and
Forestry, Nishigahara, Tokyo.)

(Received for publication January 30, 1926.)

It is a severe shock for horse breeders in our country since years that the infectious abortion prevails in mares. In the course of examining a considerable number of aborted colts and fetal membranes in our laboratory, *B. abortus equi* was demonstrated in some cases but in other cases neither such bacilli nor other bacteria were demonstrated.

It is widely believed, however, that abortion and premature birth could be caused by other bacteria or their toxins.

It has been found in Japan, according to the researches already made, that the abortion in mares has most frequently been caused by the infection of *B. abortus equi* which was demonstrated by several investigators.

For this reason our attention has been directed to the comparative study of *B. abortus equi* of Smith and Kilborne and other organisms of *paratyphoid-enteritidis* group.

It has long been known that a certain toxic substance is produced in old culture of organisms belonging to the *paratyphoid-enteritidis* group, but little is known about the toxicity of culture filtrate of *B. abortus equi*. We have demonstrated, however, the presence of toxic substance in a bacteria-free filtrate of 2-4 weeks old broth culture of this organism, and recognized that any considerable variation in the toxic properties of filtrates derived from broth cultures of other organisms of this group.

We have also found that the toxicity of the filtrate varies with the species of animals; guinea-pigs and mice are most susceptible, rabbits much less and pigeons undetermined.

It was found, moreover, that 3-7 days old culture filtrate of this organism was fatal to guinea-pigs, but the filtrate of 3-4 weeks old culture was much stronger in toxicity, while that of

culture of 5 weeks old and upwards exhibited diminished toxicity for both animals. So it may be possible that a filtrate of maximal toxicity can be obtained within the first few weeks of growth.

As regards culture media, it is apparently true that the reaction, whether alkaline or acid, has nothing to do with the toxic properties of the culture or filtrate.

The luxuriant growth is, however, expected in broth medium containing 4 % glycerine or 20 % normal horse serum, and we can always ensure a maximal content of toxic substance in the filtrate of such culture.

It is also noticeable that in the course of cultivation of this organism the increase of degree of PH is not considerable in the culture in which acid remained continuously for about 4 weeks (PH 5.8-6.6).

The filtrate was injected into the ear vein of most susceptible animals, such as rabbits and guinea-pigs, in doses of 0.05-1.0 c.c., after a period varying from 30 to 70 minutes followed a series of manifestations, principally dyspnea, weakness particularly of the hind extremities, faltering, diarrhea and finally death, with or without convulsions, or sometimes gradual recovery.

Now, it is important to decide whether we are dealing with split products of the culture medium produced by the growth of the organism or with the culture medium itself. While control injections of sterile uninoculated medium adjusted to PH 6.2 were not effective, the neutralized filtrate of the culture (PH 7.2) given intravenously in varying doses up to 1.0 c.c. caused the same symptom as mentioned.

Further experiments were made to know whether this substance can favor our idea that it is connected with split product.

Most of the guinea-pigs which received the injection of culture filtrate subcutaneously in less than lethal dose resisted the control injection of the toxic filtrate after 12-16 days.

So we believe that this substance seems to have some antigenic properties.

It has been proved by several investigators that under the influence of *B. abortus equi* some substance is formed in the animal body, the presence of which may be demonstrated by

serodiagnostic method. Thus, for mare, an agglutination value of 1:640-5120 may be considered as positive, in the sense that the animal has been or is infected.

Our knowledge is, however, still far from complete as to whether the mentioned antibody may remain for months and even years in the blood of animal after infection, or after abortion, or even in the state of complete recovery.

We have had no cases to examine the variation of such antibody in the naturally infected mare. But, in two rabbits (A and B) which aborted artificially by the injection of *B. abortus equi* we have obtained some results; namely, every serum showed almost the same decrease in agglutinative titer on the strains of *B. abortus equi* in the course of one year after abortion, as seen in following table:

Table 1.

Weeks after abortion Dilution of serum	1-4	5-12	13-23	24-25	26-38	40-46	47-51
1 : 5120	A, B	A, B	A, B	B A	A, B	B A	A, B
1 : 2560							
1 : 1280							
1 : 640							
1 : 320							
1 : 160							
1 : 80							
1 : 40							
1 : 20							

It must be noted here that the normal rabbit has yielded serum which had no definite agglutinative action above 1:20.

So far as can be gathered from the experiments regarding agglutinative reactions, there are both cultural and serological differences between the bacilli isolated from infectious abortion in mares and other paratyphoid bacilli.

From our repeated serological tests it may be said that the equine strains can distinctly separated from *B. paratyphosus B*, *B. enteritidis* and *B. typhi murium*.

Table 2.

Kind of serum \ Kind of antigen	<i>B. abortus</i> <i>equi</i>	<i>B. para</i> <i>typhosus</i>	<i>B. typhi</i> <i>murium</i>	<i>B. enteritidis</i> (Gärtner)
<i>B. abortus equi</i> immune serum of rabbit	1 : 10,240	1 : 320	1 : 320	1 : 40
<i>B. paratyphosus B</i> immune serum of rabbit	1 : 1,280	1 : 10,240	1 : 1,280	1 : 160
<i>B. typhi murium</i> immune serum of rabbit	1 ; 2,560	1 : 2,560	1 : 10,240	1 ; 80
<i>B. enteritidis</i> Gärtner immune serum of rabbit	1 : 640	1 : 160	1 : 160	1 : 5,120

In the cultural reactions, the dry, membranous and brittle growth on slanted agar noted by several observers has been manifested on every transfer. However, there have been some strains of this organism in which the cultural distinction was not observed on the agar, and they grew as organisms of the *paratyphoid-enteritidis* group.

By way of this experiment it has been found that their characteristic membranous growth depends upon the hydrogen ion concentration in culture medium.

Taking this matter into consideration, we can say that PH 6.0-8.4 of medium is most suitable for the characteristic growth of this organism, PH 9.0 less advisable and no growth may be observed in PH 9.6 or more. Therefore, we can recommend the slant agar containing 4 % glycerine for the characteristic growth of the organism.

SUMMARY

B. abortus equi can be separated from the various members of the *paratyphoid-enteritidis* group as a special bacillus and is perhaps the only organism causing abortion in mares.

馬流産菌ノ生物學的竝血清學的研究補遺

藤村 誠 一 豊島 武夫 末永 武夫

(農林省獸疫調査所)

本邦ニ於ケル馬ノ流産モ亦泰西ノソレノ如ク其原因多樣ニ亘ルノ觀アルモ今日判然タルモノハ獨リ *B. abortus equi* ニ因ルモノノミナリ

本編ハ Smith 及 Kilborne 兩氏ノ所謂 *B. abortus equi* ト他ノばらちふすB菌屬細菌トヲ生物學的竝血清學的ニ比較研究セル成績ノ概要ナリ

著者等ハ先ヅ本菌ノ肉汁培養無菌濾過液中ニハ他ノばらちふすB菌屬細菌ヨリ寧ロ強烈ニもるもつご及まうす等ニ特異中毒症狀並致死の經過ヲ呈セシムル一種ノ毒性物質ノ溶存セルヲ認メタルモ該毒性ハ極メテ不定ニシテ培養期間、培養基ノ性、培養基ノ種類及培養期間ニ於ケル培養基ノ水素イオン濃度ノ變化等ニ關スル反復試験ノ結果毎常同一程度ノモノヲ得ルコト甚ダ困難ナルヲ知レリ然レトモ大體ニ於テ酸性ぐりせりん(4%)加肉汁ノ2—4週間培養濾過液ハ毒性最モ強ク且培養期間中ニ於ケル水素イオン濃度ノ變化モ亦僅微ニシテ5週間以上培養ヲ繼續スル時ハ著シク毒性衰フルモノ、如シ而シテ本培養濾過液ヲ以テ前處置セルもるもつご及まうすハ10—16日後ニ於ケル致死量2倍ノ濾過液對照注射ニ對シテ殆ント無反應ニ經過シ或ハ對照動物ニ比シ死期ノ延長ヲ見タルモノ多シ故ニ該濾過液中ニハ免疫元的價值ヲ有スル物質ノ溶存スルヲ認容シ得ベシ

次ニ馬流産菌ヲ皮下ニ注射 $\left(\frac{1}{100} - \frac{1}{10}\right)$ 白金耳) シテ流産セシメタル2頭ノ家兎ニ就キ凝集素ノ消長如何ヲ検査セルニ極メテ徐々ニ遞減スルモノニシテ流産當初 1:5120 ノ凝集價ヲ示シタルモノ

滿1箇年ヲ經過セル後尙 1:160 ヲ保持スルヲ認メタリ本菌ニ對スル家兎ノ正常凝集價(最高1:20)ハ殆ント之ヲ認メズ

本菌培養ノ乾燥性皺襞形成ト培養基ノ水素イオン濃度、新舊、乾濕及營養分ノ含有量等トノ關係ニ就テハ明ナラザレトモ發育良好ナル場合ハ形成モ亦旺盛ニシテぐりせりん加寒天ニ於テハ普通寒天培養ニ比シ遙カニ勝レルガ如クぱらちふす B 菌及鼠ちふす菌ニ於テハ全ク其ノ形成ヲ見ズ

又 *B. abortus equi* 及 ぱらちふす B 菌屬細菌ヲ以テ免疫セル家兎血清ヲ用ヒテ行ヘル交叉的凝集反應ノ結果ハ格段ノ差アリテ本菌ハ全クぱらちふす B 菌ト別個ノ菌ナリト信ズルヲ可トス

(自抄)