A STUDY OF THE SYMBIOTIC RELATIONSHIP OF HOG CHOLERA VIRUS
TO EPERYTHROZOON SUIS INFECTION

by

JOHN PHILLIPS GIBSON

B. S., Kansas State University
of Agriculture and Applied Science, 1953

D. V. M., Kansas State University
of Agriculture and Applied Science, 1959

————————————

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Pathology

KANSAS STATE UNIVERSITY
OF AGRICULTURE AND APPLIED SCIENCE

1959

TABLE OF CONTENTS

INTRODUCTION

In the early 1930's a new disease entity of swine became apparent. It was first reported by Kinsley in 1932 (24), and later that same year by Doyle (11), that a condition of swine characterized by anemia and icterus, was being recognized sporadically in midwestern herds. Since that time descriptions of field cases have been published by Kinsley and Ray (23), Dicke (10), Quin (27), Spencer (31), Robb (28), Campbell (5), Splitter (35), Berrier and Gouge (2), and Biberstein et al (3). These reports have all been in agreement as to the symptoms and pathology. Because of the similarity of this disease to bovine anaplasmosis many of these writers called the condition "ictero-anemia" or "anaplasmosis-like disease" of swine. As late as 1956 the disease had been recognized in the following twelve states: California, Georgia, Illinois, Iowa, Kansas, Missouri, Nebraska, North Dakota, South Dakota, Ohio, Wisconsin, and New York (3). The condition has also been reported in South Africa (19) and Britain (20).

In the early reports of the condition by Kinsley (24) and Doyle (11) mention was made of the presence of bodies in, or upon the red blood cells. These bodies were not taken seriously by Spencer (31), as he thought they were Howell-Jolly or Cabot's ring bodies, degeneration products of the erythrocytes, or even artifacts. It was not until 1950 that the etiology of the disease became known. Splitter (35), in his investigation of some field cases of "ictero-anemia" or "anaplasmosis-like disease" noted an unreported blood parasite associated with the condition. Splitter set up experiments to determine if this parasite could be the cause of this condition. Using splenectomized swine he was able to

produce symptoms and pathological changes which were identical to those of field cases of "ictero-anemia." The blood parasite observed by earlier investigators was associated with all experimentally produced cases. Splitter (34) named this blood parasite of swine Eperythrozoon suis.

During the previously mentioned experiments, Splitter (35) also noted another parasite on the red blood cells of splenoctomized swine. This organism appeared to cause little in the way of clinical symptoms or anemia and was considered to be relatively non-pathogenic. Splitter (34) named this organism Eperythrozoon parvum.

Splitter's report (35) was the first evidence presented which incriminated an eperythrozoon as the cause of a disease entity in naturally infected field cases. Since then Crocker and Sutter (9) reported one case of bovine eperythrozoonosis in a Kentucky herd in 1953. The cattle in this herd exhibited anemia or icterus, decreased milk flow, intermittent diarrhea, weakness and poor condition. No abortions were reported, and no deaths occurred although one animal was necropsied. At present, E. suis is the only member of the genus reported to cause frequent loss in production and even death.

Many attempts (3), (15), (17), (28), (35) to transmit acute infections of eperythrozoonosis in normal unsplenoctomized swine have been undertaken. These attempts were unsuccessful, except for one by Splitter (36) in which large doses of heavily infected blood killed two pigs and produced subclinical symptoms in others.

Although "ictero-anemia" has been reported in both hog cholera vaccinated and in unvaccinated swine, there were numerous reports of the condition occurring from two to six weeks following vaccination for hog

cholera (5), (27), (31). Difficulty in transmitting the disease to non-splenectomized swine and field reports of the disease following hog cholera vaccination, were the factors which stimulated the experiments reported in this study.

REVIEW OF LITERATURE

Eperythrozoonosis, "ictero-anemia", or "anaplasmosis-like disease" of swine was recognized by Splitter (36) as an acute febrile disease primarily of young swine in which the predominating symptoms were a severe hemolytic anemia and icterus. The morbidity was usually quite low with only one or two, and rarely over 10 percent, of the animals in a herd being visibly affected. Usually the mortality of affected animals was reported to be high, although some cases did recover. The disease was seasonal in nature being most prevalent during the summer and early fall. Its occurrence was sporadic; however, veterinarians in some areas encountered the disease frequently.

The Etiological Agent

Eperythrozoa were classified and named in Bergey's Manual of Determinative Bacteriology, 7th edition (1), under the order Rickettsiales in the family Bartonellaceae. The order Rickettsiales included small pleomorphic microorganisms which occurred intracellularly or extracellularly, were usually non-filterable and Gram negative, and could be cultivated outside the host, with few exceptions, only in living tissues. These parasitic organisms were almost always intimately associated with the reticulo-endothelial cells or erythrocytes in vertebrates, but were also often found in invertebrates which were thought to act as vectors.

The family Bartonellaceae included those rickettsia found in, or on, the erythrocytes of vertebrates. At present, seven species of the genus have been classified, three in rodents and four in large animals of which two were in swine.

Eperythrozoon suis Splitter (34) was found to be the etiological agent of "ictero-anemia" of swine. It was a large parasite, apparently the largest eperythrozoon known. The usual form observed was a ring structure about 0.8 microns in diameter. However, ring and discoid forms were sometimes seen with a diameter as great as 2.5 microns. Splitter (33) found this microorganism was usually filtered out by filters which retained bacteria, but was completely removed by finer filters. He felt this organism was not the same as the one Foote et al (17) thought to be the cause of virus anemia of swine.

Eperythrozoon parvum Splitter (34) was a very small parasite which readily distinguised it microscopically from E. suis. E. parvum was observed primarily as small coccus forms and occasionally as ring structures. The rings averaged about 0.5 microns in diameter while the coccus forms were somewhat smaller. This parasite exhibited a tendency to accumulate in large numbers upon individual erythrocytes, even when the total number in the blood smear was very low. Splitter (33) found this organism to be capable of passage through filters of fine porosity. It has been demonstrated only in splenectomized swine. Infections resulting from this parasite were innocuous.

The classification of these organisms is still under debate. Foote et al (17) stated they should have been classified as viruses while another worker (13) felt they were protozoan in nature. Peters and Wigand (26) suggested Bartonella bacilliformis should be classified with the

bacteria because of its typical characteristics, which were small rod-
shaped form, growth on culture media, propagation by binary fission,
possession of unipolar flagella, a retracted cytoplasm, and bacteria-like
cell wall. On the other hand, they claimed that hemobartonellae and
eperythrozoa possessed no such criteria. These organisms occurred mostly
as coccus and ring-shaped forms. They were nonmotile and did not multi-
ply outside the host's blood. They lacked structural details and cell
walls like those of bacteria. Furthermore, they were so nearly alike in
every respect that it would have been possible to list them under the same
generic name.

Transmission

The first successful transmission of this disease was accomplished
by Splitter (35) following splenectomy of normal swine. Before this time
investigators (28), (15) had attempted to transmit the disease in non-
splenectomized swine without success. Since the discovery that splen-
ectomy reduced resistance and allowed the infection to reach acute pro-
portions much experimental work has been done on the disease. Splitter
(36) found that the urine of acutely infected animals was not infective.
Anthony found that spleen, lymph node, and bone marrow from carrier pigs
were infective when injected into splenectomized swine.[1] Blood and
serum were both infective, but Splitter (33) observed that the presence
or absence of eperythrozoa microscopically in the blood of the donor
animal had an important bearing upon the infectivity of the serum and the
eperythrozoon species recovered. The affinity of these parasites for the

----

1 H. D. Anthony, unpublished data.

erythrocyte (as indicated in microscopic observation) increased the probability of their recovery from whole blood rather than serum alone, particularly when the organisms were rare in the blood.

Thurston (42) showed that E. coccoides was able to withstand very hypotonic solutions, and also that in blood it survived a temperature of 3°C for 11 but not 14 days. The same author showed that E. coccoides could not withstand drying for 24 hours at 3°C. Splitter (33) reported that E. suis could not be maintained in chick embryo by serial passage, but it survived eight days in the yolk sac. Splitter also showed that the parasite would survive for 31 days in frozen blood.

The exact methods of natural transmission have not been demonstrated. It may be said with certainty that transmission does not take place by direct contact between individuals, but probably through the medium of insect vectors such as biting flies or mosquitoes (36). This is further indicated by the seasonal occurrence of the disease although Splitter (36) observed a natural outbreak during February. Splitter (36) was unable to incriminate the hog louse Haematopinus suis as a vector either mechanically or biologically, although Jansen (19) in one case transmitted E. parvum with the hog louse. Eliot (16) succeeded in transmitting E. coccoides using the louse Polypax serrata, though it was probably a mechanical transmission since lice became non-infective if several hours elapsed between feeding on the infected and the susceptible mouse. Jensen (21) observed rapid transmission of E. wenyoni when susceptible calves were exposed to carriers in the presence of large numbers of biting flies.

Mechanical transmission from carrier to susceptible pigs by means of vaccination needles may have occurred since Anthony has found amounts

as small as .01 ml of whole carrier blood could infect splenectomized
pigs.[1] Smaller amounts were not tried. Thurston (42) was able to trans-
mit E. coccoides with 0.2 ml of a one to 100,000 dilution of infected
blood in citrated saline.

The only report of the transmission of an acute infection to normal
non-splenectomized swine has been by Splitter (33). He injected 30 to 80
ml of heavily infected citrated blood intravenously into nine 15 to 30
pound pigs. Two of the pigs developed acute cases of eperythrozoonosis
and died on the sixth and ninth days following injection. Two additional
pigs developed increased temperature only, which was associated with
relatively heavy parasitic invasions of less than twenty-four hours
duration. Mild parasitic attacks occurred in three pigs, and the rest
remained unaffected. In all instances, E. suis was present in the blood
of the inoculated pigs immediately following the injections, though
usually in low numbers.

Berrier and Gouge (2) reported an outbreak of eperythrozoonosis in
90 young pigs. All but 17 died before they were one week of age, and
most died between 24 and 48 hours of age. These writers felt that this
was a case of eperythrozoonosis transmitted to utero from carrier sows to
their pigs.

Pathology

Studies made of field and experimental cases by Splitter (36) es-
tablished that the severity of the disease following injection of E.
suis depended upon the intensity and duration of the resulting parasitic

---

1 H. D. Anthony, unpublished data.

attack. The majority of normal swine were able to suppress the multiplication of E. suis, and consequently the parasite invasion was held to numbers insufficient to cause ill effects. Organisms then disappeared microscopically from the blood and the animal remained a carrier, probably permanently. Pigs in which heavy parasitic infections developed showed increased temperatures when the parasites became numerous. The fever was usually directly related to the number of parasites present, and in many cases reached 107°F. In some cases, the organisms disappeared spontaneously after being very numerous for a day or two. The pigs showed no symptoms other than an elevation of the temperature during the parasitic attack and increased regenerative blood changes following the reduction in numbers and disappearance of the parasites. The anemia that developed was thought to be negligible.

Pigs in which heavy infections persisted developed symptoms of depression and anorexia (as well as fever) on the second or third day that parasites were very numerous. Blood values began to fall very rapidly. A decrease of two million erythrocytes per day was often noted in the blood count. A spontaneous reduction in the number of parasites took place as the anemia developed, and a corresponding decline in the temperature usually occurred. The animal became gaunt, exhibited anemic and usually icteric mucous membranes, dyspnea on forced exercise, and bile stained feces (36). An increase in neutrophiles was observed by some in field cases (11), (32) but in experimental cases this was not true (36). The former was probably due to secondary infections which occurred during the debilitated stage. Anthony noticed some experimental cases which died in lateral recumbency while making paddling motions with their feet. The disease looked similar to cholera and other diseases because

the animals did not have time to develop icterus. Also noted was the fact that animals acutely infected with E. suis usually continued to eat until death approached.[1]

Splitter (36) noted that in splenectomized animals repeated parasitic relapses occurred with each attack repeating the course of the disease as described. The severity of the attack depended upon the number of the organisms and the duration of their presence. In furthur studies, the time from experimental infection to the first appearance of symptoms varied from six to 17 days. In addition, parasites were demonstrated in blood films from two to seven days following intravenous inoculation of infectious blood.

The pathology of E. parvum has confused the picture in experimental cases. Splitter (34) listed the incubation period in splenectomized swine as seven to ten days, but stated that no symptoms of blood damage followed. This may not have been entirely true, as Foote et al (17) mentioned one death possibly caused by E. parvum in a splenectomized pig. The author has seen a hematocrit of 12 in E. parvum infection, although no acute clinical symptoms developed. It was felt that splenectomized swine should be held three weeks to a month before inoculation with E. suis in order to prevent complicating E. suis infections with latent E. parvum infections (35).

Post-Mortem Lesions

The constancy of the lesions was the factor which first allowed the recognition of this disease entity (11), (24). Dicke (10), Spencer (31),

---

1 H. D. Anthony, unpublished data.

and Splitter (35) found the same lesions first reported by Kinsley (24) and Doyle (11). Splitter (42) in his first work with splenectomized swine was able to reproduce these lesions.

Splitter (36) stated that the most noticeable lesion was the generalized icterus of variable intensity which was usually present throughout the body. It was also noticed before necropsy, as a yellowish condition of the mucous membranes and skin, especially of the unhaired portions of the abdomen. For this reason the disease was called "yellow belly" in some localities.[1] The blood appeared thin and watery with yellow plasma or serum, and the erythrocytes sometimes agglutinated spontaneously (36). The gastrointestinal contents were deeply stained with bile, while the heart and kidneys appeared pale, flabby, and yellow (36). A few petechial hemorrhages were noted on the kidneys and were therefore confused with hog cholera.[2] The liver evidenced degeneration and icterus, and the gall bladder contained a thick gelatinous bile (36). The spleen in field cases (11), (24) was markedly enlarged to two or three times normal size, and was very soft and friable. In addition, the body cavities, and especially the pericardial sac, often contained excessive quantities of yellow serous fluid.

Microscopically the spleen showed hyperplasia of the reticuloendothelial cells, hemosiderosis and congestion. The liver damage consisted of central degeneration of the liver lobule due to anoxia (31). Lymphocytic infiltration and hemosiderosis were also present along with some increase in intralobular connective tissue. The heart and kidneys

---

1 M. J. Twiehaus, personal communication, July 10, 1959.
2 Ibid.

exhibited changes of cellular degeneration (36).

Diagnosis

The possibility of eperythrozoonosis should be considered in all cases of anemia in swine. This may also be true of anemias in sheep and cattle although, no field cases have been reported in sheep, and only one in cattle (9). The diagnosis of this disease was overlooked by many veterinarians because the organisms were not demonstrable in the blood stream at all stages of the infection. Additional factors of value in reaching a diagnosis were a marked icterus of all mucous membranes and tissues, the season of the year, the finding of dead pigs in ponds and water holes, the age of the animal, and the usual low herd morbidity.[1] The post-mortem anaplasmosis-like lesions were characteristic, and were considered pathognomonic of the disease (36).

Field diagnosis was difficult in the early stages of the disease prior to the appearance of acute anemia (36). In these early stages, depression and fever of 104.0°F to 107.0°F were the only symptoms. Ordinarily blood slides made at this time showed many organisms on the erythrocytes, because their prevalence usually corresponded to the degree of temperature shown. Splitter (36) however, mentioned cases in which a sudden reduction of parasites occurred during these early symptoms. Observations made by Anthony indicated that the organism was usually rare or absent during the acute anemic phase of the disease.[2]

Splitter (36) stated that blood films should be prepared directly from the living animal without the addition of an anticoagulant as this

_____

1 M. J. Twiehaus, personal communication, July 10, 1959.
2 H. D. Anthony, unpublished data.

may distort the appearance of the organism. Most satisfactory results
were obtained with Giemsa stain. Splitter mentioned that eperythrozoa
were not demonstrated when excessive acid stains were used and the eryth-
rocytes assumed a reddish tinge. Iron reacting inclusions in erythro-
cytes of swine have been observed by Splitter (38) in both acutely and
apparently unaffected swine. These inclusion bodies were confused with
eperythrozoa.

Other methods of diagnosis (36) used were the inoculation of a
susceptible splenectomized pig with blood from the suspected case, or
removal of the spleen from the suspected animal. However, either of
these methods could have resulted in an acute infection if the suspect
was a carrier.

Splitter (32) reported a more positive method of diagnosis in his
study of the complement fixation (CF) test in relation to eperythrozoon-
osis of swine. Animals with carrier infections were generally negative
to the test. Reactions became positive in most infected animals 13 to 30
days after inoculation and continued positive for one to four weeks, and
then became negative. In acute splenectomized cases serum became positive
in an average of 2.5 days after the onset of clinical illness and contin-
ued positive for two or three weeks. Non-specific positive reactions to
the anaplasmosis CF test occurred in the majority of sera from swine
acutely affected with eperythrozoonosis. Splitter felt that this was a
reaction to the erythrocyte stroma.

Treatment and Control

Splitter (37) found that neoarsphenamine produced a specific and
prompt action against E. suis in experimental infections. Single

intravenous doses varying from 15 to 45 mg/kg of body weight were effec-
tive. Erythrocyte destruction was halted almost immediately in most
cases. Spontaneous relapses occurred in nearly all of these experimental
cases, but this probably would not occur in non-splenectomized animals.
An increased resistance of E. suis to repeated treatments was evidenced.
Drugs which were shown to be of no value in these experiments were
sodium cacodylate, antrycide, and piroplasmin.

Splitter (39) recently showed that oxytetracycline and tetracycline
were effective in a single intramuscular dose of at least three mg per
pound of body weight. With this dosage there was marked reduction in the
number of parasites within six hours after therapy. A return to normal
temperature occurred and clinical improvement was evident within 24
hours. As with neoarsphenamine, relapses occurred within three to eight
days in experimental cases. Although oral use of antibiotics was re-
ported to have no therapeutic value (5), recent evidence indicated that
this was not the case.[1]

Experimental evidence indicated that the majority of adult swine in
enzootic areas were carriers, and there was no practical method of de-
tecting these carriers (36). Therefore, the elimination of the disease
by destroying the source of the infection appeared to be impractical.
There has been no work to determine if it would be feasible to eradicate
a carrier infection by the use of antibiotics as has been done with
anaplasmosis (40).

---

[1] H. D. Anthony, unpublished data.

Effects of Splenectomy

The spleen is the largest lymphoid organ in the body. It was described as being a complex structure of reticular material which contained lymphocytes, monocytes, plasma cells, granular leucocytes, megakaryocytes, fixed and wandering histiocytes, and variable amounts of blood (8). Blood flow through the organ was peculiar in that capillaries were lacking in the ordinary sense, and blood passed directly into the splenic pulp. It was then collected by the venous sinuses, which were drained by veins whose union formed the splenic vein (12).

Copenhaver and Johnson (8) stated that the spleen was not essential for life. It was removed without giving evidence of its functions since other organs, particularly the bone marrow, readily took over its functions. The spleen acted as a filtering organ for the blood; the phagocytic cells of the pulp removed foreign particles including bacteria, degenerating leucocytes, and worn out erythrocytes. Digestion of the phagocytized erythrocytes recovered iron and it was stored temporarily in the spleen. The spleen functioned as an organ of blood development during a part of fetal life, and it could resume this function in the adult under certain pathological conditions. The spleen acted as a reservoir for the storage of blood and it increased the volume of circulating blood by contracting. It played an important part in the formation of antibodies and in the production of immunity (8).

Thurston (42) wondered why removal of the spleen allowed eperythrozoa to produce clinical signs of disease. He felt that splenectomy removed the filtering ability of numerous phagocytes; and found the resistance to _E. coccoides_ in splenectomized mice to be the same as in

mice whose spleens were transplanted to another part of the body. Rosenthal and Zohman (30) produced E. coccoides infection in non-splenectomized mice by blocking the reticulo-endothelial cells with injections of India ink.

Effects of Hog Cholera Vaccination

Factors other than the vaccination needle which linked hog cholera to field cases of eperythrozoonosis, were the previously mentioned observations of Quin (27) and Spencer (31) that the disease occurred from two to six weeks following vaccination for hog cholera. Splitter (35) felt that the disease might have been transmitted by hog cholera virus blood. However he found that it was not possible to pass the infection with heavily infected blood, which had been phenolized and held 15 days under the same conditions used to hold hog cholera virus blood. Thurston (42) had similar results with E. coccoides. Biberstein et al (3) in one case, held nine days after splenectomy, were able to transmit E. suis infection with lyophilized blood. If the infection was due to the inoculum, lyophilized vaccines passed through swine on their last passage could have spread eperythrozoonosis.

Many of the effects of hog cholera virus on swine are known (14), and there are probably many which are not known. How these effects might contribute to the establishment of an acute infection of epery-throzoonosis is speculation. Stress has been incriminated as a factor during the hog cholera vaccination reaction. Coles (7) found that leucopenia followed vaccination with antiserum and virulent virus. In many cases the leucopenia was followed by a leucocytosis which resulted in a leucocyte count higher than before vaccination. The drop in

leucocytes was apparent on the third day following treatment and the return to normal took place within three to four days after the initial drop. The severity of the leucopenia was related to the dosage of serum, being more marked in pigs which received lower dosages. Weide and Twiehaus (43) also showed similar results in pigs vaccinated with lapinized vaccines. Thurston (42) felt that stress might have been a factor in causing _E. coccoides_ infection in mice. His experiments showed that daily cortisone injections had no effect upon the course or severity of the disease. Gledhill (18) worked with a virus which caused a mild, rarely fatal, hepatitis in weanling mice. When the virus infection occurred simultaneously with normally harmless _E. coccoides_, a fatal hepatitis was invariably produced.

METHODS AND MATERIALS

The animals used in these experiments were purchased from two farms in the area surrounding Manhattan, Kansas. The pigs in Experiment I were purebred Hampshires six weeks old at the time the experiment was started. The pigs in Experiments II and III were approximately 10 weeks of age at the time of purchase. After they arrived at the Veterinary Research Farm the pigs were sprayed for lice with benzene hexachloride, wormed with cadmium oxide, and identified with metal ear tags.

Splenectomies were performed under intravenous sodium pentobarbital anesthesia. The operative area selected was on the left side immediately posterior to the last rib. An incision, three inches in length, was made through the skin, fascia, muscle, and peritoneum. Hemorrhage in the area was controlled with hemostatic forceps. The splenic vessels were ligated with 1/8 inch umbilical tape, and the spleen removed intact. The

peritoneum and muscle were closed with continuous sutures, while the skin was closed with subcutaneous continuous sutures. It appeared that the wounds healed very rapidly and with a minimum of infection following this type of closure.

Blood samples were taken daily in Experiments I and III and three times weekly in Experiment II. The samples were collected with individual sterile needles and syringes from the anterior vena cava by the method described by Carle and Dewhirst (6). Blood smears were made at the time the samples were taken and the blood for analysis was then placed in tubes containing sodium citrate. The slides were fixed in methyl alcohol, stained with Giemsa stain, and examined microscopically for the presence of eperythrozoon bodies. The citrated blood was studied for packed cell volume (PCV), erythrocyte count and leucocyte count. The PCV was determined by the use of Wintrobe hematocrit tubes. The erythrocyte and leucocyte counts were made using the improved Neubauer hemocytometer. Blood and temperature examinations of the pigs were recorded over a period of approximately one month. Many of the pigs were observed for a period of three months or more.

Animals that died during the experiment were necropsied. The procedure described by Roderick (29) was used, and records were kept of each case. Representative sections were taken from kidney, liver, spleen and other organs of interest, fixed in buffered formalin, and then sectioned. They were stained with hemotoxylin and eosin and also with Giemsa stain.

Blood used for inoculating pigs with E. suis was obtained from acute cases of eperythrozoonosis. These cases were produced by splenectomy of eperythrozoon carrier pigs six days prior to collections of blood for

inoculation.

Experiment I was undertaken to determine if acute cases of eperythrozoon infection could be produced in non-splenectomized pigs, as observed under field conditions. Immunization against hog cholera (rabbit origin vaccine)[1] was used as a stress factor. Twenty-one pigs were divided into three lots with seven pigs in each lot. Two pigs in each of the three lots were splenectomized. Five days following splenectomy all the pigs in each group received an intraperitoneal inoculation of 10 ml of blood from a pig with an experimentally produced acute infection of E. suis. Two days following inoculation with E. suis the pigs were vaccinated for hog cholera in the following manner:

> Lot 1 - Vaccine plus 15 ml anti-hog cholera serum.
>
> Lot 2 - Vaccine only.
>
> Lot 3 - Hog cholera virus plus 30 ml anti-hog cholera serum.

It was anticipated that the leucopenia produced by the vaccine would coincide with the incubation period of E. suis in the inoculated animals. Splitter (34) gave the incubation period of E. suis in splenectomized animals as six to 17 days. It was not known whether this incubation period would be the same in unoperated swine.

Experiment II involved a group of 10 pigs. Seven of the pigs were inoculated intraperitoneally with five ml of E. suis infected blood obtained in the manner already described. Three of the pigs were kept as controls. This experiment was conducted to determine the results of E. suis inoculation in normal swine.

Experiment III was conducted with six pigs. Two pigs were inoculated

---

1 "Rovac" - produced by American Cyanamid, Pearl River, New York.

with virulent commercial hog cholera virus, and four were inoculated with
a field strain of hog cholera virus. All pigs had been inoculated with
E. suis in the manner previously described, at least two weeks or more
before virus inoculation. This experiment was carried out to determine
if E. suis would exert a clinical effect in pigs acutely affected with
hog cholera.

RESULTS

In Experiment I all of the splenectomized animals developed acute
infections of E. suis two days following inoculation. All except pig
No. 35 died from six to nine days following inoculation. The average PCV
of all splenectomized pigs in this experiment dropped a total of 21 per-
cent over a period of seven days or an average of three percent per day
from inoculation time until death. The rate of drop was less during the
early part of the infection and increased rapidly as death approached.
The red cell count dropped similarly but was not determined during the
terminal stages because there was spontaneous agglutination of erythro-
cytes when suspended in diluent. The leucocyte counts of these splenec-
tomized pigs remained fairly constant during the infection except for pig
No. 35 which lived 11 days following inoculation. The leucocyte count of
this pig followed a course similar to the non-splenectomized pigs in the
experiment. All splenectomized pigs were treated with neoarsphenamine
in the manner described by Splitter (37). All died within one or two
days following treatment except No. 35, which lived five days longer.
The PCV of this pig remained below 11 percent from the time of treatment
until death. Temperatures during the acute stage of the infection ranged
from 105° to 107°F. In pig No. 35 the temperature was abnormal the last

four days of life.

The results of the average daily blood determinations on the non-splenectomized pigs in this experiment can be seen in figure 1, Plates I, II, and III. The lowest leucocyte counts in all lots appeared at six to seven days following hog-cholera vaccination. The counts began dropping about three to five days following vaccination and were back to their original level in seven to nine days. The leucopenia was present from about the sixth to the tenth day following E. suis inoculation. Lot No. 3 which received serum and virulent virus was the only group in which leucocyte counts reached higher proportions than they had before vaccination. Lot No. 2 showed the lowest average white cell count.

Erythrocyte packed cell volume and erythrocyte count showed a steady decline in the vaccinated pigs throughout the experiment. The PCV in all lots dropped from 40 to 33 percent. There was a reduction in erythrocytes from approximately 7.6 million/cu mm to 6 million/cu mm. Microscopic examination of Giemsa stained blood slides revealed a few E. suis at least once in two of the five unoperated pigs in Lot 1, three of the five in Lot 2, and four of the five in Lot 3. The time of appearance of the organisms seemed to be of no special significance. Daily temperatures were recorded, but showed no deviation from the normal.

Two of the pigs which had not exhibited E. suis in the blood during the experiment were later splenectomized. Both pigs became acutely infected with E. suis as evidenced by the large number of organisms which were demonstrated on the erythrocytes in stained blood smears.

In Experiment II, the blood studies revealed little difference between the control animals and those inoculated with E. suis as shown in figure 2 on Plates I, II, and III. Erythrocyte counts and PCVs in both

EXPLANATION OF PLATE I

Fig. 1.   Averages of erythrocyte counts of the non-
          splenectomized animals in Experiment I.

          A.   Time of inoculation with E. suis.
          B.   Time of vaccination against hog cholera.

Fig. 2.   Averages of erythrocyte counts of animals
          in Experiment II from the time of inocu-
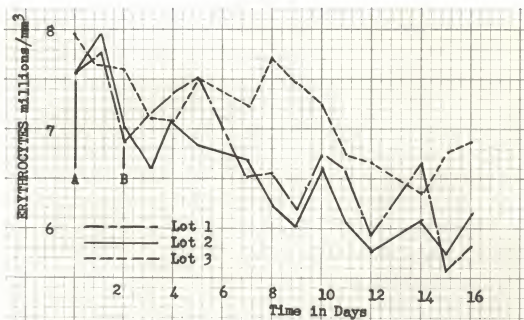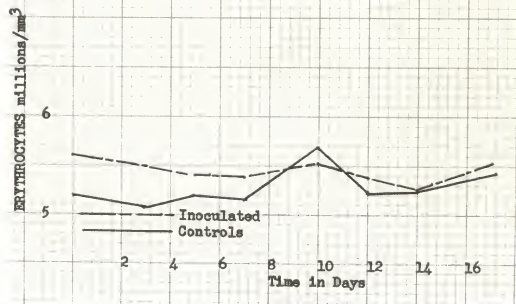   lation of experimental animals with E. suis.

PLATE I

FIG. 1

FIG. 2

EXPLANATION OF PLATE II

Fig. 1.   Averages of erythrocyte packed cell
volumes of the non-splenectomized
animals in Experiment I.

A.   Time of inoculation with E. suis.
B.   Time of vaccination against hog cholera.

Fig. 2.   Averages of erythrocyte packed cell
volumes of animals in Experiment II from
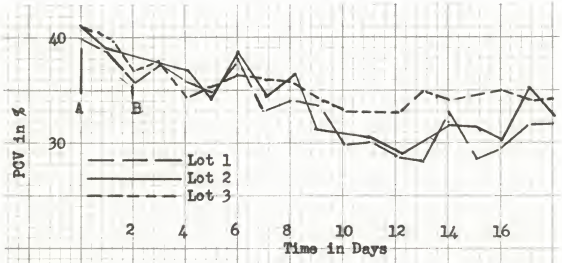the time of inoculation of experimental
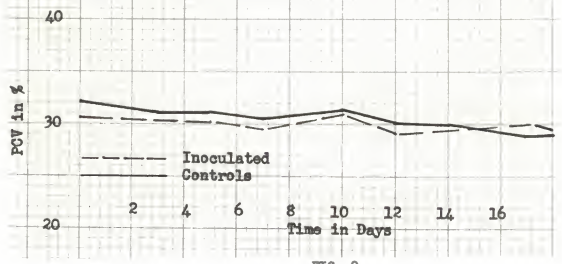animals with E. suis.

PLATE II

FIG. 1

FIG. 2

EXPLANATION OF PLATE III

Fig. 1. Averages of leucocyte counts of the non-
         splenectomized animals in Experiment I.

         A. Time of inoculation with E. suis.
         B. Time of vaccination against hog cholera.

Fig. 2. Averages of leucocyte counts of animals in
         Experiment II from the time of inoculation
         of experimental animals with E. suis.
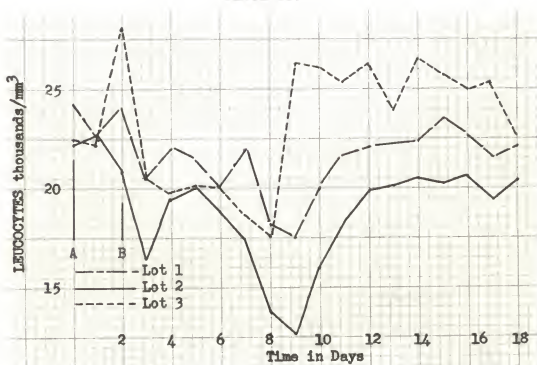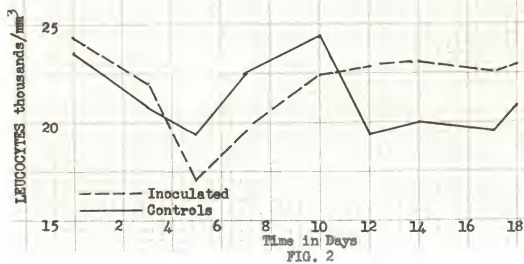
PLATE III

FIG. 1

FIG. 2

groups followed almost identical courses. The white cell counts, al-
though erratic, followed a somewhat similar course. Following the blood
studies, three of the inoculated pigs were splenectomized and all became
acutely affected with E. suis.

In Experiment III all pigs died without showing any evidence of E.
suis infection on microscopic examination. The two pigs that received
the virulent hog cholera virus died five days following inoculation.
The leucocyte counts of these two pigs began dropping on the second day
following inoculation and continued to drop until death, at which time
both were below 6,500 cells per cubic mm. There was an average decrease
of five percent in PCV from the time of inoculation to death.

In the group of four pigs that received the field strain of hog
cholera virus, the disease process extended over a much longer period
before death ensued. Three of the animals were dead by the fourteenth
day and the other pig lived 15 days following inoculation. Animals in
this group exhibited a definite leucopenia beginning on the fourth day
following virus inoculation. In two of the pigs this leucopenia continued
until the eighth day when the white cell count increased. This increase
probably resulted from the secondary infection found at necropsy. In
the other two pigs the leucopenia was present until death. Erythrocyte
PCV of the four animals was approximately 32 percent at the time of
inoculation. Five days later a gradual drop in PCV began. This drop
continued until death, at which time the PCV averaged 20 percent.
Nucleated erythrocytes were observed frequently during this period.
The temperatures of all pigs rose rapidly on the second day following
inoculation and stayed between 105° and 107°F until near death.

DISCUSSION

The splenectomized pigs in Experiment I showed signs of acute eperythrozoonosis in PCV, erythrocyte count, temperature, microscopic blood cell examination, and necropsy lesions. Leucocyte counts of all pigs except No. 35, remained the same throughout the experiment. This is in contradiction to a field case reported by Spencer (31), but agrees with the report by Robb (28) that the leucocyte count in acute cases of eperythrozoonosis was not usually affected. This increase in leucocytes might have been due to a bacterial infection which occurred simultaneously with the eperythrozoon infection.

In the normal pigs of all three lots it was noted that there was a definite drop in erythrocyte numbers and PCVs. Weide and Twiehaus (43) reported an increase in erythrocytes following vaccination of young pigs for cholera. Although no acute infections became apparent, it is possible that the vaccination reaction was a factor which might have allowed the eperythrozoon to produce a sub-clinical anemia. White cell counts of all pigs followed very closely the pattern given by previous workers (7), (43) and probably were not affected by the E. suis inoculation.

The results of Experiment II indicated that there was little or no effect on erythrocyte count, leucocyte count, and PCV in normal pigs due to E. suis inoculation. There are no reports of the effects on these blood values following attempted transmission of the disease in normal pigs, except one case in which Splitter (33) used large doses of inoculum. The animals in Experiment II apparently became carriers of the disease as they exhibited acute infections following splenectomy.

In Experiment III there was a definite drop in PCV during the acute

phases of hog cholera. Kernkamp (22) in his study of 198 cases of exper-
iemtal hog cholera stated that no significant change in the number of
erythrocytes occurred in this disease. Lewis and Shope (25) cited other
authors who indicated there was a slow decrease in the number of red
cells during the disease. The results of this experiment indicated that
E. suis may be the cause of sub-clinical anemia occurring during hog
cholera.

SUMMARY

Attempts to produce acute eperythrozoonosis in non-splenectomized
swine were unsuccessful. There was evidence that Eperythrozoon suis
caused sub-clinical anemia following hog cholera vaccination and during
acute cases of hog cholera. This could account for the reports of mild
anemias during hog cholera infections. These studies further indicated
that there was no increase in the leucocyte count during acute infections
of eperythrozoonosis. From these experiments it would seem that some
factor or factors in addition to hog cholera virus or vaccine are neces-
sary to produce acute cases of eperythrozoonosis in normal swine.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Bergey's Manual of Determinative Bacteriology. Seventh edition.
       Baltimore: Williams and Wilkins, 1957.

(2) Berrier, H. H. Jr., and R. E. Gouge.
       Eperythrozoonosis transmitted in utero from carrier sows to their
       pigs. J. Am. Vet. Med. Assn., 1954, 124:98.

(3) Biberstein, E. L., et al.
       Eperythrozoonosis of swine in New York State. Cornell Vet.,
       1956, 46:288.

(4) Bower, S. J.
       Eperythrozoonosis in swine. Allied Vet., Nov., 1958, 21 p.

(5) Campbell, R. L.
       Icteric condition found in hogs in northern Missouri. North Am.
       Vet., 1945, 26:347.

(6) Carle, B. N., and W. H. Dewhirst, Jr.
       A method for bleeding swine. J. Am. Vet. Med. Assn., 1942,
       101:495.

(7) Coles, C. G.
       Leucocyte counts on the blood of normal, cholera-infected, and
       recently immunized pigs. J. Am. Vet. Med. Assn., 1932, 81:392.

(8) Copenhaver, W. M., and D. D. Johnson.
       Bailey's textbook of histology. Fourteenth edition. Baltimore:
       Williams and Wilkins, 1958.

(9) Crocker, K. W., and M. D. Sutter.
       Bovine eperythrozoonosis. Vet. Med., 1954, 49:305.

(10) Dicke, W. E.
        Anaplasmosis-like disease of swine. Vet. Med., 1934, 29:288.

(11) Doyle, L. P.
        A rickettsia-like or anaplasmosis-like disease in swine. J. Am.
        Vet. Med. Assn., 1932, 81:668.

(12) Dukes, H. H.
        The physiology of domestic animals. Seventh edition. Ithaca:
        Comstock, 1955.

(13) Dunlap, J. S.
        Protozoa. Diseases of swine. Edited by H. W. Dunne. Ames:
        I.S.C. Press, 1958. 452 p.

(14) Dunne, H. W.
        Hog cholera. Diseases of swine. Edited by H. W. Dunne. Ames:
        I.S.C. Press, 1958. 111 p.

(15) Dykstra, R. R., et al.
      Studies in anaplasmosis II 1938-1948. Kans. Ag. Exp. Sta. Bul.,
      1948, 66:19.

(16) Eliot, C. P.
      The insect vector for the natural transmission of Eperythrozoon
      coccoides. Science, October, 1936, 84:397.

(17) Foote, L. E., W. E. Brock, and B. Gallaher.
      Ictero-anemia, epery-osis, or anaplasmosis-like disease of swine
      proved to be caused by a filterable virus. North Am. Vet., 1951,
      32:17.

(18) Gledhill, A. W.
      Quantative aspects of the enhancing action of eperythrozoa on the
      pathogenicity of mouse hepatitis virus. J. Gen. Microbiol., 1956,
      15:292.

(19) Jansen, B. C.
      The occurrence of Eperythrozoon parvum Splitter, 1950 in South
      African swine, Onderstepoort Jr. Vet. Res., 1952, 25:5.

(20) Jennings, A. R., and J. Seemer.
      A new blood parasite in British pigs. Nature, London, 1956,
      178:153.

(21) Jensen, R.
      Eperythrozoonosis in cattle and sheep of Louisiana, Preliminary
      report. Louisiana Ag. Exp. Sta. Bul., 336, 1943.

(22) Kernkamp, H. C. H.
      The blood picture in hog cholera. J. Am. Vet. Med. Assn., 1939,
      95:525.

(23) Kinsley, A. T., and J. D. Ray.
      Anaplasmosis-like disease in swine. J. Am. Vet. Med. Assn., 1934,
      37:391.

(24) Kinsley, A. T.
      Protozoan-like body in the blood of swine. Vet. Med., 1932,
      27:196.

(25) Lewis, P. A., and R. E. Shope.
      The study of the cells of the blood as an aid to the diagnosis
      of hog cholera. J. Am. Vet. Med. Assn., 1929, 74:145.

(26) Peters, D., and R. Wigand.
      Bartonellaceae. Bact. Rev., 1955, 19:150.

(27) Quin, A. H.
      A heard condition of swine characterized by icterus and anemia.
      J. Am. Vet. Med. Assn., 1938, 46:327.

(28) Robb, A. D.
      Ictero-anemia in growing swine. Vet. Med., 1943, 38:271.

(29) Roderick, L. M.
      Necropsy procedures for swine. Veterinary necropsy procedures.
      Edited by T. C. Jones and C. A. Gleiser. Philadelphia: Lippin-
      cott, 1954.

(30) Rosenthal, L., and B. L. Zohman.
      Bartonella anemia in non-splenectomized rats. Arch. Path., 1931,
      12:405.

(31) Spencer, R.
      Anaplasmosis-like disease of swine. Vet. Med., 1940, 35:294.

(32) Splitter, E. J.
      The complement-fixation test in diagnosis of eperythrozoonosis
      in swine. J. Am. Vet. Med. Assn., 1958, 132:47.

(33) _____.
      Eperythrozoonosis in swine – filtration studies. Am. Jr. Vet.
      Res., 1952, 13:290.

(34) _____.
      Eperythrozoon suis n. sp. and Eperythrozoon parvum n. sp. Two
      new blood parasites of swine. Science, 1950, 111:513.

(35) _____.
      Eperythrozoon suis, the etiological agent of ictero-anemia or
      anaplasmosis-like disease of swine. Am. Jr. Vet. Res., 1950,
      11:324.

(36) _____.
      Ictero-anemia of swine. Prod. U. S. Lvst. San. Assn., 54th Ann.
      Meeting, 1950. 279 p.

(37) _____.
      Neoarsphenamine in acute eperythrozoonosis of swine. J. Am. Vet.
      Med. Assn., 1950, 117:371.

(38) _____.
      Observations on an erythrocytic inclusion in swine. Am. Jr.
      Vet. Res., 1953, 14:575.

(39) Splitter, E. J., and E. R. Castro.
      Antibiotic therapy in acute eperythrozoonosis of swine. J. Am.
      Vet. Med. Assn., 1957, 131:293.

(40) Splitter, E. J., and J. G. Miller.
      The apparent eradication of the anaplasmosis carrier state with
      antibiotics. Vet. Med., 1953, 48:486.

(41) Splitter, E. J., and R. L. Williamson.
       Eperythrozoonosis in swine, a preliminary report. J. Am. Vet.
       Med. Assn., 1950, 116:360.

(42) Thurston, J. P.
       Observations on the course of _Eperythrozoon coccoides_ infections
       in mice, and the sensitivity of the parasite to external agents.
       Parasitology, 1955, 45:144.

(43) Weide, M. D., and M. J. Twiehaus.
       Hematological studies of normal, ascarid-infected, and hog cholera-
       vaccinated swine. Am. Jr. Vet. Res., 1956, 20:562.

A STUDY OF THE SYMBIOTIC RELATIONSHIP OF HOG CHOLERA VIRUS
TO EPERYTHROZOON SUIS INFECTION

by

JOHN PHILLIPS GIBSON

B. S., Kansas State University
of Agriculture and Applied Science, 1953

D. V. M., Kansas State University
of Agriculture and Applied Science, 1959

————————————

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Pathology

KANSAS STATE UNIVERSITY
OF AGRICULTURE AND APPLIED SCIENCE

1959

This study was undertaken in an attempt to produce acute cases of eperythrozoonosis in normal non-splenectomized swine. This disease entity was recognized in 1932 as a sporadic condition in midwestern swine herds. Icterus and anemia were the main features of the disease and it was therefore called "ictero-anemia" or "anaplasmosis-like disease" of swine. Early investigators were unable to reproduce the condition. Splitter in 1950 demonstrated that by removing the spleen, an inoculated pig developed an acute case of the disease. The condition was noted in both hog cholera vaccinated and unvaccinated swine. Some investigators reported that it occurred frequently following vaccination for hog cholera. In this study both virulent and attenuated hog cholera virus were used in an attempt to produce the condition in non-splenectomized eperythrozoon inoculated swine.

The pigs used in these three experiments were obtained from farms in the area surrounding Manhattan, Kansas.

Experiment I consisted of 21 pigs divided into three groups of seven each. Two pigs in each group were splenectomized. Two days following inoculation with blood heavily infected with Eperythrozoon suis the pigs were vaccinated for hog cholera. Group 1 received attenuated vaccine and serum, Group 2 attenuated vaccine only, and Group 3 received virulent virus and serum.

Experiment II was a study of the effects of E. suis on normal pigs. It consisted of 10 pigs, seven inoculated with E. suis and three were kept as controls.

Experiment III consisted of six pigs; two were inoculated with virulent commercial hog cholera virus, and four with a field strain of hog cholera virus. Blood samples were taken at regular intervals during

the experiment; leucocyte counts, packed cell volume (PCV) determinations, and erythrocyte counts were made routinely on these samples. Blood smears were also made and examined microscopically for the presence of E. suis. Temperatures of all animals were recorded during the experiments.

Blood studies in Experiment I indicated that the leucocyte count was unchanged during acute cases of E. suis infection in splenectomized pigs. Leucocyte counts in non-splenectomized pigs showed typical hog cholera vaccination leucopenia. This started on the third day, reached a low on the sixth day, and was back to normal by the ninth day after vaccination. Erythrocyte counts and PCVs indicated that a definite sub-clinical anemia was produced in these animals.

Experiments II showed that there was little or no effect upon the blood counts of the normal animal when inoculated with E. suis organisms. Erythrocyte counts and PCVs of normal and inoculated animals were almost identical. Leucocyte counts were variable, but there was no indication of significant change.

In Experiment III, an anemia was produced in all acute cases of hog cholera. A leucopenia also occurred.

From the comparison of these studies with the work of other investigators it was felt that sub-clinical anemia in some herds following hog cholera vaccination might have been due to unobserved infection with E. suis. One investigator with nearly 200 experimental cases stated that there was no change in the erythrocyte count in acute hog cholera. Other investigators have reported that a mild anemia is often present. This study showed that anemia occurred when both infections were present concurrently.

No case of acute eperythrozoon infection was produced in normal

swine following hog cholera vaccination or during an acute case of hog cholera. In a few instances, however, the organism was observed on few erythrocytes in the blood at least once during the experiments.