

EXPERIMENTAL BRUCELLA CANIS INFECTION IN RABBITS

by 1264

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TABLE OF CONTENTS

INTRODUCTION	1
MATERIALS AND METHODS	2
RESULTS	5
DISCUSSION	12
SUMMARY	15
ACKNOWLEDGMENTS	16
REFERENCES	17
APPENDIX	19

INTRODUCTION

Abortions in Beagle dogs due to a previously undescribed bacterium were reported by Carmichael (5), Taul et al. (17) and Moore and Bennett (13). Since these original reports, the organism has been isolated from many breeds and mongrel dogs from each state of the United States (1, 6). These and other reports (7, 8, 9, 11, 12) indicated that the organism was related to the genus Brucella, but was different from previously described species. The name Brucella canis was proposed (8, 12, 13).

In female dogs, B. canis infection has been characterized by early embryonic deaths recognized as conception failures, late term abortions with subsequent vaginal discharges and birth of dead or weak pups which usually succumbed shortly after birth (7). In males, epididymitis, prostatitis and scrotal dermatitis with a loss of libido have been reported (7, 14).

Because of the limited breeding season in canines and their relatively long gestation period, a suitable model was needed to study the pathogenesis of the disease. The hare is naturally susceptible to brucellosis (10, 16, 18) and experimental transmission of B. canis to a few rabbits had been accomplished. The rabbit was considered the logical choice for studying B. canis infections since its gestation period is relatively short and its breeding season long. The purposes of these experiments were to determine if the rabbit

would prove to be a suitable model and to establish the pathogenesis in rabbits.

MATERIALS AND METHODS

Thirty-six multiparous female rabbits (Oryctologus cuniculus) of various breeds were randomly selected and placed in groups of 6 animals each for experiments 1 through 3. Sixteen sexually mature males of varying ages were utilized in experiments 4 and 5. All rabbits were obtained from breeders in the vicinity of Manhattan, Kansas. No history of Brucella canis infections in dogs on the premises was reported from any of the suppliers.

Rabbits were housed in individual cages. Water and a commercial rabbit diet* were fed ad libitum. All rabbits were housed in the experimental area at least one week prior to commencing the experiments. Each female was bred twice at 4-hour intervals. After breeding, each female was observed twice a day for signs of abortion.

The strain of B. canis used in the experiments was isolated from the uterus of a rabbit artificially infected with B. canis strain 56BL. The strain was originally isolated by the Department of Infectious Diseases, Kansas State University. The rabbits in experiment 5 were infected with the same strain but passed once on artificial media.

All infected groups received B. canis grown in brucella broth** at 37 C for 72 hours. The number of viable organisms in the culture was determined by averaged duplicate plate counts. Control groups received sterile brucella broth.

* Rabbit Chow, Ralston Purina Co., St. Louis, Mo.

** Baltimore Biological Laboratory, Baltimore, Md.

Heart blood was collected prior to death and 1 ml inoculated into 10 ml of brucella and tryptose* broths respectively. Blood cultures were incubated at 37 C for 5 days and then transferred to blood agar and the medium described by Nicoletti and Muraschi (15) with 5 per cent sheep red blood cells substituted for bovine serum (modified brucella medium).

The following tissues were collected and placed into individual sterile bags: uterus, placenta, fetuses, ovary, liver, spleen, kidney, lung, mammary gland and popliteal lymph node. Tissues were prepared for culturing by flaming and cutting with sterile scissors. The cut edge was plated directly on blood agar and modified brucella medium. The plates were incubated at 37 C for 14 days. When typical colonies appeared, a Gram stain was made and the organism inoculated onto Simmons' citrate agar, urea agar, MacConkey agar and glucose broth. If no typical colonies appeared, plates were discarded and considered negative. Specific details of experimental procedures for experiments 1, 2 and 3 above are presented in Table 1.

In experiment 4 the 5 males used to breed the previously infected females were euthanatized 14 days after breeding to determine if B. canis could be transferred during mating. The testis and head of the epididymis were cultured from all rabbits.

In experiment 5, 7 male rabbits were inoculated intraperitoneally with 5 ml of a 72-hour brucella broth culture containing 6.2×10^7

* DIFCO Laboratories, Detroit, Mich.

Table 1. Experimental procedures for experiments 1, 2 and 3 utilizing female rabbits inoculated with Brucella canis.

Exp. No.	No. animals infected	No. animals (control)	Route of inoculation	No. organisms/ml (amount)	Time of inoculation (Day of gestation)	Time of euthanatization (Day of gestation)
1	6	6	Intra-peritoneal	7.0×10^6 (5 ml)	10	24
2	6	6	Oral	7.0×10^6 (2 ml)	10	24
3	6	6	Intra-peritoneal	4.0×10^7 (5 ml)	-3	24

organisms per ml. Four males were inoculated intraperitoneally with 5 ml of sterile brucella broth incubated at 37 C for 72 hours (controls). All rabbits were euthanatized 14 days later, and tissues were examined bacteriologically as previously described.

RESULTS

The disposition of all the fetuses in experiments 1, 2 and 3 is presented in Fig. 1.

Experiment 1. All blood cultures were negative for B. canis. Results of the cultural examination of tissues from infected rabbits are presented in Table 2. One rabbit in this group was not pregnant. Three rabbits had all but one fetus normal and that was underdeveloped. Purulent material was found in the uterus of two rabbits (Plate I, Fig. 1). One rabbit died 24 hours prior to termination of the experiment. All fetuses were small (less than 5 cm crown-rump). The other rabbit in this group had 3 small and 9 underdeveloped fetuses (Plate I, Fig. 2).

Brucella canis was not isolated from tissues of the control rabbits. Two abnormal fetuses (1 small and 1 resorbing) were found from a total of 60.

Experiment 2. Blood cultures of both the infected and control groups were negative for B. canis. The organism was isolated from the uterus of a non-pregnant rabbit and the spleen of another.

Two rabbits were not pregnant. Three others had all fetuses normal except 2 which were underdeveloped. The other rabbit in the group had 3 normal and 10 resorbing fetuses (Plate I, Fig. 3).

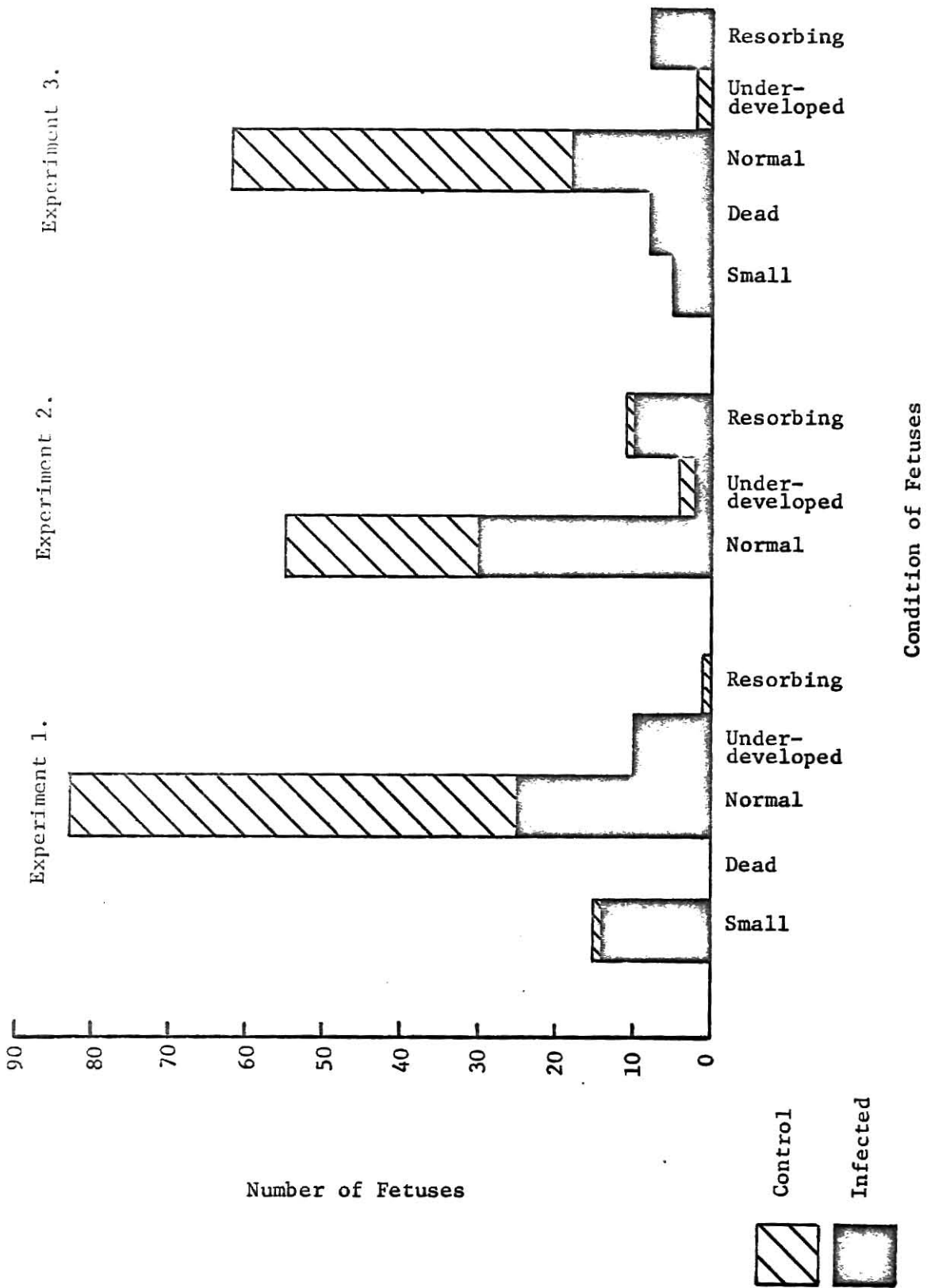


Fig. 1. Disposition of the fetuses of rabbits inoculated with *Brucella canis*.

EXPLANATION OF PLATE I

- Fig. 1. Incised uterus showing normal fetus with a large amount of yellow purulent material in the lumen of the uterus from which Brucella canis was isolated.
- Fig. 2. Underdeveloped fetuses resulting from Brucella canis inoculation on the 10th day of gestation.
- Fig. 3. Resorbing fetuses at gestation day 24 from rabbit infected 3 days prior to mating. Placentas can be clearly seen.
- Figs. 4 and 5. Various stages of arrested development and decomposition on gestation day 24 from a single animal inoculated with Brucella canis 3 days prior to mating. All fetuses were dead at necropsy.
- Figs. 6 and 7. Testes of infected rabbits with unilateral abscesses in the area of the gubernaculum testis. Rabbits were inoculated intraperitoneally and euthanatized 14 days later.

PLATE I

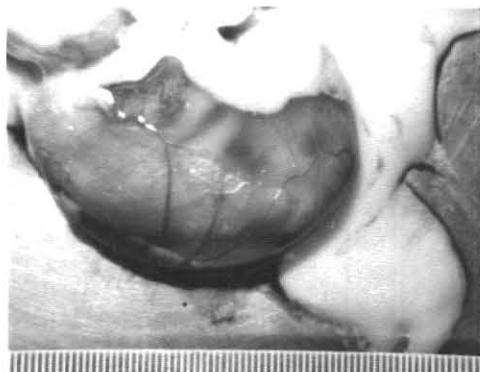


Fig. 1

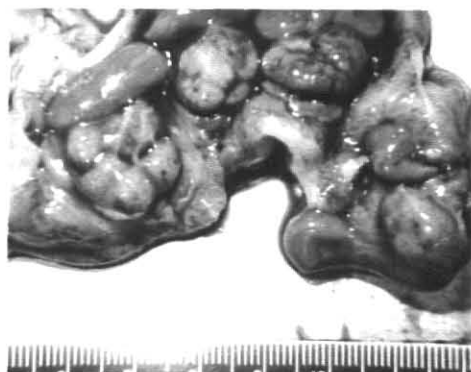


Fig. 2



Fig. 3



Fig. 4



Fig. 5



Fig. 6



Fig. 7

Table 2. Tissues from which Brucella canis was isolated:
rabbits inoculated intraperitoneally on the 10th
day of gestation.

	Animal					
	1	2 [#]	3	4 [*]	5	6
Uterus	+	-	+	-	+	+
Placenta	+	-	-	-	+	+
Fetus	+	-	+	-	+	-
Liver	+	-	-	-	+	-
Spleen	+	+	-	+	+	+
Kidney	-	-	-	-	+	-
Lung	+	-	-	-	+	-
Ovary	-	-	-	-	-	-
Mammary gland	-	+	-	-	-	+
Popliteal lymph node	-	-	-	-	+	-

[#] Gave birth to term fetuses 7 days post inoculation.

^{*} Not pregnant.

Brucella canis was not cultured from any tissue in the control group. One resorbing and two underdeveloped fetuses were found from a total of 23 fetuses.

Experiment 3. Blood cultures of both infected and control groups were negative for B. canis. Results of cultural examination of tissues in the infected group are presented in Table 3. One rabbit was not pregnant and only one had all normal fetuses. Small, dead and resorbing fetuses were found in four rabbits in the infected group. One rabbit had dead fetuses in varying stages of development (Plate I, Figs. 4 and 5). Purulent material was present in the lumen of the uterus of two rabbits.

Brucella canis could not be isolated from any controls. Two underdeveloped fetuses were found from a total of 46.

Experiment 4. Brucella canis was not isolated and gross abnormalities were not noted in rabbits in this group.

Experiment 5. Two rabbits had unilateral small abscesses in the area of the gubernaculum testis (Plate I, Figs. 6 and 7). These were the only gross abnormalities found. Brucella canis was isolated in pure culture from the abscesses. The organism also was isolated from the spleen of all but one rabbit. All blood cultures were negative.

No gross abnormalities were observed in the control group and B. canis could not be isolated from blood or tissues.

Table 3. Tissues from which Brucella canis was isolated:
rabbits inoculated intraperitoneally 3 days prior
to mating.

	Animal					
	1	2	3	4	5	6*
Uterus	+	-	-	-	+	+
Placenta	+	-	-	-	+	-
Fetus	+	-	-	-	+	-
Liver	-	-	-	-	+	-
Spleen	-	+	+	+	+	+
Kidney	-	-	-	-	-	-
Lung	-	-	-	-	-	-
Ovary	-	-	-	-	-	-
Mammary gland	-	-	-	-	-	-
Popliteal lymph node	-	-	-	-	-	-

* Not pregnant.

DISCUSSION

General. Multiparous female rabbits have been described as having up to 60 per cent fetal death with resorption of fetuses (3). Prenatal death of all fetuses is usually complete by post-coitum day 12 (2). Complete resorption of fetuses is accomplished by 8 days after death (4).

In these experiments 22 per cent of the rabbits were not pregnant at necropsy. One female had multiple masses in the uterus which grossly appeared to be neoplastic. The remainder were assumed to have resorbed their fetuses. Further experiments might well utilize virgin females to reduce resorption rate.

Tissues were cultured using both sheep blood agar and the modified brucella medium. In certain instances isolations were made on only one of two media. This occurred in no particular pattern with various tissues, and with either medium. This indicated that for the most complete isolations more than one type of medium should be used.

Experiment 1. In this experiment the infected group had only 51 per cent normal fetuses while controls had 96.7 per cent normal fetuses. The abnormal fetuses in the infected group were small or underdeveloped. Pregnancy diagnosis was missed on one rabbit and it was subsequently inoculated with B. canis during the fourth week of gestation. Inoculation at this stage of pregnancy apparently had no ill effects on the female or the fetuses. All fetuses died, probably from exposure and lack of maternal interest as no nesting materials were available in the cage.

Inoculation of B. canis on gestation day 10 yielded variable results. One rabbit died from unknown causes 24 hours before the termination of the experiment. No signs of illness were observed prior to death. All fetuses were small and this can be attributed to the action of B. canis. Three rabbits had all but one fetus normal and yet the bacterium was isolated from many tissues and fetuses. Apparently when inoculated at the 10th day of gestation, B. canis invades the tissues and appears to arrest fetal development or has no visible effect on the fetuses. Some developmental problems could occur between day 24 and term. The main effect, however, could be the development of infected, weak offspring which die shortly after birth.

Experiment 2. The results of inoculating 1.4×10^7 organisms into the pharynx indicated this was not a good procedure for studying B. canis infections. The bacterium was isolated from only 2 rabbits. The fact that one rabbit had 10 resorbing fetuses can be explained in two ways: (1) this was the result of the action of B. canis; or (2) the presence of live fetuses slowed the resorption rate permitting some to remain after gestation day 21 (4). Since B. canis was not isolated, the second explanation is probably true.

In dogs inoculated orally with 10^9 organisms, 11 of 12 became infected (7). Experiments in rabbits using greater numbers of organisms might produce a higher infection rate similar to that seen in dogs. Inoculation earlier in gestation or prior to mating might produce a higher rate of infection.

Experiment 3. Inoculation 3 days prior to breeding resulted in more fetal abnormalities than did inoculation during gestation. This probably occurred as there was more time to produce embryonic death. While the effects of infecting on gestation day 10 were limited to small and underdeveloped fetuses, infection prior to mating resulted almost exclusively in fetal death and resorption. Why one rabbit had no grossly observable changes in the uterus or fetuses cannot be explained as B. canis was isolated from the spleen.

This experimental group had fewer normal fetuses than any other, infected or control. It is possible that infection prior to breeding caused severe placental pathology which resulted in early fetal death with complete resorption of fetuses by gestation day 24. It is obvious that drastic differences in development occurred within a single individual. Another rabbit may have had complete resorption of all fetuses due to the action of B. canis as the organism was isolated from the uterus.

Effects on the fetus in infected groups and not in control groups indicate that the female rabbit could be a useful model in studying B. canis infections.

Experiments 4 and 5. No gross orchitis or epididymitis was observed. There was evidence that a bacteremia occurred since B. canis was isolated from the spleen. One rabbit had severe atrophy of both testes. Brucella canis was not isolated from this animal. Perhaps a certain sex hormone balance is necessary for infection to occur.

With no transmission by mating and no gross abnormalities of the male sex glands, it appears that the male rabbit is not very susceptible to B. canis infection and is not a good model for studying the disease or its pathogenesis.

SUMMARY

Brucella canis was inoculated into female rabbits intraperitoneally and orally on the 10th day of gestation and intraperitoneally 3 days prior to mating. The uterus and fetuses were the only organs grossly affected. Fetal death, resorption, small size and underdevelopment resulted. B. canis was isolated from all organs cultured at one time or another, except the ovary. In males injected intraperitoneally, small abscesses developed in 2 of 7 rabbits in the area of the gubernaculum testis. No transmission of B. canis resulted from mating males with infected females. The female rabbit appeared to be a suitable model for studying B. canis infections, whereas the male seemed unsuitable.

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APPENDIX

The disposition of the fetuses from each individual rabbit of each group in experiments 1, 2 and 3 is presented below:

Experiment 1. Rabbits inoculated intraperitoneally on the 10th day of gestation.

Infected	Control
Rabbit 1 - 7 normal	Rabbit 1 - 10 normal, 1 small
Rabbit 2 - 11 normal	Rabbit 2 - 10 normal, 1 resorbing
Rabbit 3 - 11 small	Rabbit 3 - 13 normal
Rabbit 4 - Not pregnant	Rabbit 4 - 8 normal
Rabbit 5 - 7 normal, 1 under-developed	Rabbit 5 - 6 normal
Rabbit 6 - 3 small, 9 under-developed	Rabbit 6 - 11 normal

Experiment 2. Rabbits inoculated orally on the 10th day of gestation.

Infected	Control
Rabbit 1 - Not pregnant	Rabbit 1 - 7 normal, 1 under-developed
Rabbit 2 - 10 normal, 1 under-developed	Rabbit 2 - 8 normal, 1 under-developed
Rabbit 3 - 11 normal	Rabbit 3 - Not pregnant
Rabbit 4 - Not pregnant	Rabbit 4 - 2 normal
Rabbit 5 - 3 normal, 10 resorbing	Rabbit 5 - 8 normal, 1 resorbing
Rabbit 6 - 6 normal, 1 under-developed	Rabbit 6 - Not pregnant

Experiment 3. Rabbits inoculated intraperitoneally 3 days prior to mating.

Infected	Control
Rabbit 1 - 2 normal, 2 dead and small, 2 resorbing	Rabbit 1 - Not pregnant
Rabbit 2 - 1 resorbing	Rabbit 2 - 13 normal, 2 under-developed
Rabbit 3 - 6 normal, 2 small, 4 resorbing	Rabbit 3 - 12 normal
Rabbit 4 - 10 normal	Rabbit 4 - Not pregnant
Rabbit 5 - 6 dead, 3 small, 1 resorbing	Rabbit 5 - 11 normal
Rabbit 6 - Not pregnant	Rabbit 6 - 8 normal

EXPLANATION OF PLATE II

- Fig. 1A. A normal, 24-day fetus and placenta from a rabbit inoculated intraperitoneally with 5 ml of sterile brucella broth on the 10th day of gestation.
- Fig. 2A. A normal, 24-day fetus from a rabbit inoculated intraperitoneally with B. canis and placenta showing purulent material between fetal and maternal portions.
- Fig. 3A. The incised uterus of a rabbit inoculated intraperitoneally with B. canis 3 days prior to mating and euthanatized on gestation day 24. The purulent material was red-brown in color.
- Fig. 4A. A resorbing fetus from a control rabbit euthanatized on gestation day 24. Fetuses were occasionally found in this condition when normal fetuses were also present in the uterus.
- Fig. 5A. A live, smaller than normal, 24-day fetus from a rabbit inoculated intraperitoneally with B. canis on the 10th day of gestation.
- Fig. 6A. Atrophied testes from a rabbit inoculated intraperitoneally with B. canis and euthanatized 14 days later. Brucella canis was not cultured from any tissue examined.

PLATE II

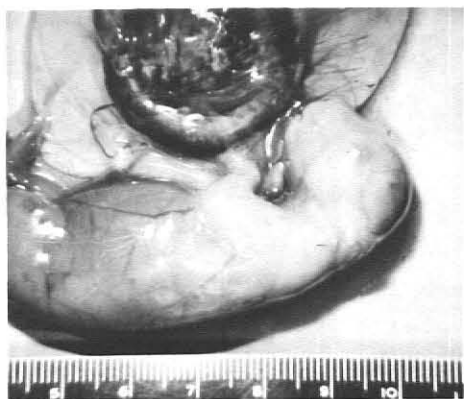


Fig. 1



Fig. 2

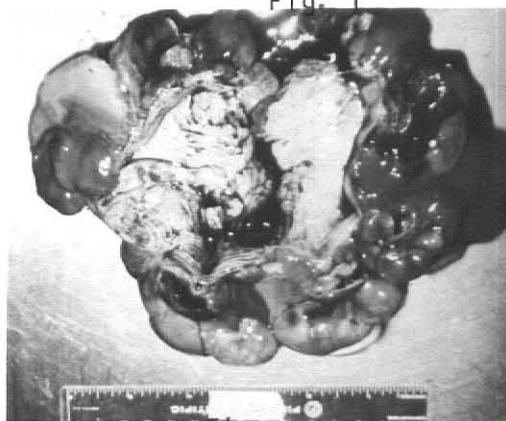


Fig. 3



Fig. 4

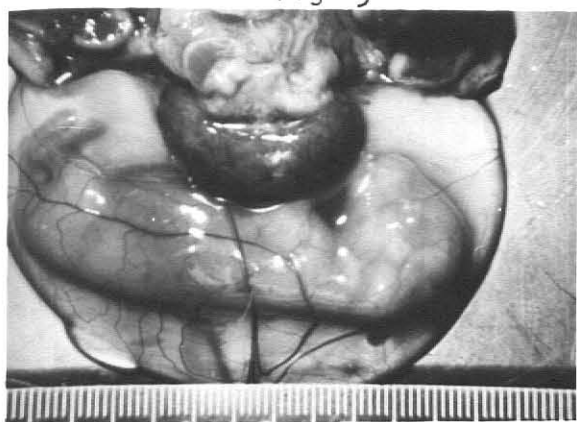


Fig. 5

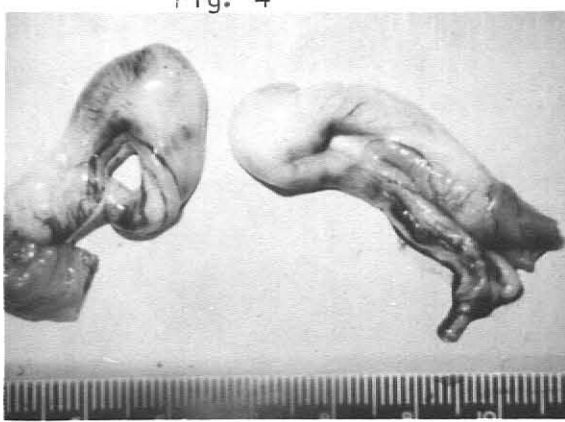


Fig. 6

EXPERIMENTAL BRUCELLA CANIS INFECTION IN RABBITS

by

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B. S., Pennsylvania State University, 1958
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AN ABSTRACT OF A MASTER'S THESIS

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1970

Brucella canis infection was established in rabbits to determine the suitability of this species for studying the pathogenesis of the infection. Thirty-six multiparous females and sixteen sexually mature males were used. Three groups of females were each given a 72-hour broth culture: (1) intraperitoneally on the tenth day of gestation; (2) orally on the tenth day of gestation; (3) intraperitoneally prior to breeding. All were euthanatized on the twenty-fourth day of gestation.

Brucella canis was isolated erratically from those exposed orally. Intraperitoneal exposure permitted recovery of the organism from the uterus, placenta, fetuses, spleen, liver, kidney, lung, popliteal lymph node and mammary gland. A purulent placentitis was the predominant gross abnormality. Intraperitoneal tenth day exposure produced 49 per cent small and underdeveloped fetuses while infection prior to breeding resulted in 42.8 per cent dead and resorbing fetuses and 5.7 per cent small fetuses. Oral infection produced 28.6 per cent underdeveloped and resorbing fetuses.

Males injected intraperitoneally showed no gross evidence of orchitis or epididymitis. Unilateral abscesses containing B. canis were found in two animals. The spleen was the only organ from which B. canis was recovered. Venereal transmission could not be demonstrated in males. Results indicate that the female rabbit could be a valuable model in studying B. canis infections.