STUDIES ON CANINE, FELINE, AND MURINE HAEMOBARTONELLOSIS

Thesis for the Degree of M. S.
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Aaron M. Leash

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By

Aaron M. Leash

AN ABSTRACT

Submitted to
Michigan State University
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ABSTRACT

STUDIES ON CANINE,
FELINE, AND MURINE
HAEMOBARTONELLOSIS

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Haemobartonella sp. is a group of organisms parasitic on the erythrocytes, and capable of producing a hemolytic anemia in its hosts. In most instances, splenectomy of the host is a prerequisite for the production of anemia. The cat is a notable exception to this rule, since anemia regularly occurs in the non-splenectomized individual. Various studies were conducted on *H. canis* of the dog, *H. felis* of the cat, and *H. muris* of the rat. Test animals were proved negative for these parasites by the examination of Giemsa stained blood films daily for a minimum of 14 days before they were inoculated with infective blood.

Cats, when injected with *H. canis*, do not develop a haemobartonellemia, but their blood becomes infective for dogs.

Dogs, when injected with *H. felis*, develop a haemobartonellemia, and their blood may be infective for dogs and cats.

H. canis is transferred from bitch to puppies, but whether this occurs in utero or through the milk was not

determined.

An attempt to prove the mosquito, Culex pipiens, the vector of H. felis was unsuccessful, as was the attempt to transmit this organism to the laboratory rat, Rattus norvegicus.

A dose of 2 mg/kg body weight of Mapharsen[1] intraperitoneally, was used to successfully treat H. muris infection in rats, and the MLD of this drug for rats was found to be between 22 and 24 mg/kg body weight.

Embryonated chicken eggs, 8-12 days old, could not be infected with H. canis or H. felis.

Filcide[2] was used to successfully treat canine haemobartonellosis on a 3-day schedule at the level of 0.20 ml/lb body weight, intravenously. This drug was unreliable for the treatment of H. felis infection in cats at the level of 0.20 ml/lb body weight, intravenously, for 10 successive days.

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[1]Parke, Davis and Company, Detroit, Michigan, Brand of Oxophenarsine Hydrochloride.

[2]Pitman-Moore Division of Allied Laboratories, Indianapolis, Indiana, Brand of Arsinobenzamide.

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DEDICATION

This thesis is dedicated to my ever patient wife, Fran.

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The author is deeply indebted to Dr. William V. Lumb, his former advisor, now at Colorado State University, who suggested and guided this work. To Dr. Gabel H. Conner, Department of Surgery and Medicine, sincere thanks for "adopting" the author and patiently overseeing the writing of this thesis. The chick embryo work could not have been done without the helpful suggestions of Dr. Esther Smith, Department of Anatomy, and Dr. John P. Newman, Department of Microbiology and Public Health. Dr. Smith was especially generous in affording the author the use of her laboratory and facilities. A special thank you is in order for the former students, now graduate veterinarians, who performed many of the routine procedures for the author, and who cared for the experimental animals: Dr. Theodore Schaub, Dr. James Reaume, and Dr. Ray Jacobs. The efficiency of Miss Susan Richards in the laboratory was extremely helpful and greatly appreciated. Many thanks to the Department of Surgery and Medicine with whose time and facilities most of this study was conducted, and to the departmental secretary, Mrs. Betty Jenkins, for her assistance.

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CHAPTER I

INTRODUCTION

The genus Haemobartonella[1] has been classified most recently as follows:[2] Order Rickettsiales, Family Bartonellaceae.[3]

Haemobartonella is extremely polymorphic. Coccoid forms range from 0.2 to 0.5 microns (u). Rod and violin bow shaped types range from 0.2 to 1.5 u in width by 1 to over 6 u in length. They stain bluish-red with Giemsa and Wright stains.

In severe infections, Haemobartonella parasitize 90 per cent (%) or more of the erythrocytes of the affected animal. Their position is epierythrocytic. They rarely produce disease without prior splenectomy, except in the cat. They are not easily cultured on artificial media. Arthropod transmission has been proved for some species. They are sensitive to organic arsenical therapy.

When splenectomy is performed on a dog which is a latent carrier of Haemobartonella canis, a marked hemolytic

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[1] Tyzzer and Weinman, 1939.

[2] Robert S. Breed, E. G. D. Murray, and Nathan R. Smith, Bergey's Manual of Determinative Bacteriology, 7th Edition, 1957.

[3] Greszcykiewicz, 1939. Revised by Weinman, David, Department of Microbiology, Yale University, September, 1955.

anemia develops. Thus, canine haemobartonellosis may become a problem to the veterinarian who finds it necessary to remove a pathological spleen.

Feline infectious anemia, caused by Haemobartonella felis, is a disease better known by veterinarians. In most cases it presents a clinical picture of a chronic hemolytic anemia. The acute form of the disease is rarely recognized.

The possibility of the dog's being a source of Haemobartonella infection for the cat, and of the cat for the dog, presented an interesting problem. If transmission from one species to the other were possible, several questions could be raised. Are separate species designations, i.e., H. canis and H. felis valid? Might exchange of Haemobartonella between the dog and cat (fighting, blood-sucking arthropods) be a mode of natural transmission of the disease?

In an infectious disease, there exists the possibility of transfer of the etiological agent from mother to offspring. The maternal transmission of H. canis was investigated in this study, and its role in the perpetuation of the disease will be discussed later.

Arthropods have been found to be the vectors of some species of Haemobartonella. Since the mosquito, Culex pipiens, is common in this area, it was investigated as a possible vector of H. felis.

Unsuccessful attempts have been made to transmit H.

canis and H. felis to laboratory rats. In this study, it was intended to infect rats with H. felis. During the preparation of the rats for injection of feline blood by splenectomy and examination of blood smears, H. muris, a fairly common parasite of rats was encountered. This led to treatment of the Haemobartonella-induced anemia in rats, and the determination of the minimum lethal dose (MLD) of the arsenical used as treatment in this species.

Cultivation of Bartonella bacilliformis and Haemobartonella muris in incubating chick embryos has been reported. An attempt was made to cultivate H. canis and H. felis in a similar manner.

Since the veterinarian has few drugs with which to combat canine and feline haemobartonellosis successfully, the efficacy of arsinobenzamide[1] against these organisms was investigated.

Finally, 3 case reports of clinical feline haemobartonellosis are presented. They shed further light on the natural transmission of the disease and treatment of advanced feline infectious anemia.

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[1]Filcide, Pitman-Moore Company, Indianapolis, Indiana.

CHAPTER II

REVIEW OF LITERATURE

A. *Bartonella bacilliformis*, Strong et al (1913)

Biffi (1903) and Gastiaburu (1903) reported red cell granulations in the peripheral blood of a patient suffering from Carrion's disease (Oroya fever). In 1905 and 1909, Barton described the bartonella bodies and stated that they were the etiology of Oroya fever. This was confirmed in 1915 by Strong et al, and the name *Bartonella bacilliformis* was proposed. Noguchi and Battistini (1926) obtained growth of *Bartonella bacilliformis* from citrated blood which had been refrigerated up to 28 days when placed in artificial media. When the cultures were injected into rhesus monkeys, skin lesions and bartonellemia were produced. Pinkerton and Weinman (1937) obtained abundant growth in tissue culture utilizing rat and guinea pig lung, bone marrow, spleen, and tunica vaginalis. Jiminez and Buddingh (1940) inoculated 8 to 12 day chick embryos via the chorio-allantoic (CA) membrane, allantoic sac, amniotic sac, and yolk sac. Growth occurred at 37° centigrade (C), but better results were obtained at 25 to 28° C, although embryo mortality was higher. Organisms appeared in the yolk at 48 to 72 hours. Amniotic fluid remained negative. Better growth on the CA membrane resulted from scarification than when it was left intact.

Peters and Wigand (1955) considered Bartonella bacilliformis to be closely related to the bacteria because of size, form, growth in cultures, binary fission, unipolar flagella, cell walls, and behavior in serological tests. Griesemer (1958) noted that Bartonella is easily cultured on cell-free media, whereas, Haemobartonella is not.

B. Haemobartonella muris, Mayer (1921), Tyzzer and Weinman (1939).

Marked anemia in certain rats following splenectomy has been observed frequently. Wolferth (1917) noted that those rats with splenomegaly developed anemia following splenectomy; those with normal spleens were unaffected. Streuli (1918), Lepehne (1918), and Danoff (1919) noted hemoglobinuria and death within 10 days after splenectomy.

In 1920, Mayer and Zeiss observed a marked anemia beginning several days after cure of Trypanosoma rhodesiense infection with Bayer 205. They observed bartonelliform bodies on the red blood cells. In 1921, Mayer proposed the name Bartonella muris. In 1925, Lauda suggested the name "infectious anemia of the rat." This followed his production of anemia by injecting blood from anemic splenectomized rats into splenectomized rats which had been normal for 30 days after surgery. Mayer, Borchardt, and Kikuth (1926) repeated Lauda's experiements and observed the bartonelliform bodies. The name Bartonella muris was again proposed.

In 1928, Mayer, Cannon, and McClelland showed the rat louse, Polyplax spinulosus, to be the vector. This was confirmed by Cannon and McClelland in 1926. Eliot (1936) showed that adult lice removed from the host for several hours did not transmit the disease, but nymphs did. He thought that the stronger digestive fluids of the adult destroyed the organisms. Crystal (1959) demonstrated that ingestion of crushed lice from an infected source, or the intraperitoneal injection of their feces did not produce the disease in rats. An infection was produced by crushing and rubbing lice into scarified skin. Lice maintained for 24 hours without access to an infective source did not transmit the disease; whereas, lice fed 4 hours previously on an infected rat did transmit the disease.

Ford and Eliot (1930) postulated that the "virus" reproduces in the spleen, but becomes vegetative on the erythrocytes when the spleen is removed. McCluskie and Niven (1934) produced a bartonellemia in rats by ligation of the splenic blood vessels without removal of the spleen.

Tyzzer and Weinman (1939) subdivided the genus Bartonella into the monotypic genus Bartonella, with the species bacilliformis, and the genus Haemobartonella, type species H. muris.

Peters and Wigand (1955) did not consider Haemobartonella muris to be bacterial because of lack of cellular

structure and resistance to culture. Electron microscopy showed the position of the organism to be epierythrocytic.

Faulkner and Habermann (1957) reported the circulating neutrophils and macrophages to contain Bartonella muris. The disease was produced in splenectomized or non-splenectomized white mice when injected with lymph node suspensions from infected rats.

Infectiveness of the blood was lost after 24 hours at room temperature or 37° C (Ford and Eliot, 1928). Reitani (1929) reported survival of the organisms for 15 days at 4° C in citrated blood. The incubation period of the disease was lengthened after 24 hours at 20° C (Alstead, 1938). Kessler (1942) found no lowering of the death rate when infective defibrinated rat blood was maintained at -70° C for 11 weeks.

Cultivation on artificial media has been attempted many times. Schilling and San Martin (1928) reported colonies of coccoid organisms on blood agar. Inoculation of normal and splenectomized rats produced death. Marmorston-Gottesman and Perla (1932) had growth in 48 hours in semi-solid serum agar. Mayer, Borchardt and Kikuth (1926) found microscopic growth in 5 to 10 days at 30° or 37° C on horse blood glucose agar. Rat inoculations were negative. McCluskie and Niven (1934) were unsuccessful in culturing the organism. Lawkowicz (1938) observed growth on semi-solid rabbit serum agar between the sixth and twelfth days.

Injection into bartonella-free rats produced anemia. Weinman (1944) felt that no report of cultivation was completely satisfactory. He noted that, in pathogenicity tests, information excluding the possibility of survival of organisms from the original inoculum was not offered. Ford and Murray (1959) stated they had offered such proof in cultivating H. muris on Giema's medium. Growth was seen in 72 hours at 28° C. The culture was infective for splenectomized rats carrying Flexner-Jobling tumors and fed a 12% casein diet, and for rats receiving 4 milligrams (mg) of cortisone per kilogram (kg) body weight daily.

Vogelsang and Gallo (1939) infected 9 to 12 day old chick embryos. By the sixth day, all the parasitized eggs were dead, all without parasites living. Splenectomized, arsenic-treated rats showed bartonellae in the blood and died 5 to 8 days after injection with egg material, while control, arsenic-treated, splenectomized rats remained normal.

Mayer, Borchardt, and Kikuth (1927) obtained "sterilization" of the infection with organic arsenicals. This has since been confirmed by many workers. Penicillin, chlortetracycline, and oxytetracycline were reported effective by Griesemer (1958). That oxytetracycline did not alter destruction of the erythrocytes was shown by Rudnick and Hollingsworth (1959). Parasitized erythrocytes had a life span of 10 to 12 days compared to the 25 days of

non-parasitized rat erythrocytes.

C. Haemobartonella canis, Kikuth (1928), Tyzzer and Weinman (1939).

Kikuth (1928) performed a splenectomy on a dog and 6 days later noted bartonella-like structures in blood films. The name Bartonella canis was proposed. Rhoades and Miller (1935) observed bartonella bodies in splenectomized dogs fed a black-tongue producing (niacin deficient) diet and a normal diet. The organisms could not be transmitted to non-splenectomized dogs regardless of diet.

McNaught, Woods, and Scott (1935) depleted the plasma proteins of dogs by bleeding daily and returning the washed erythrocytes. Icterus developed and Bartonella were found "in" the red blood cells. Blood transfusions and treatment with 15 mg of neoarsphenamine per kilogram body weight produced recovery. Infective blood was injected into splenectomized dogs. After bartonellemias developed they were treated with neoarsphenamine.

Knutti and Hawkins (1935) transmitted the organism to 3 out of 4 splenectomized dogs by the intravenous injection of infective blood. An incubation period of 2 to 5 days was noted. No growth was obtained in Noguchi's leptospira medium incubated at 30° C. Fifteen mg of neosalvarsan per kilogram body weight proved curative.

Ray and Idnani (1940) found an incubation period of 19 days for dogs inoculated with H. canis subcutaneously, and 6 to 12 days for dogs receiving intravenous inoculation. Post mortem findings included emaciation, icterus, pale emphysematous lungs, ecchymoses in the heart, hepatomegaly, splenomegaly, and petechiae on the kidneys and intestine.

A spontaneous Haemobartonella infection was found in a splenectomized dog by Carr and Essex (1944). The infection was transmitted to 2 other splenectomized dogs.

Ingle (1946) reported a clinical case of canine haemobartonellosis occurring in a non-splenectomized dog which he treated with blood, iron, and brewer's yeast.

Lumb (1958) described a macrocytic, hypochromic anemia resulting from H. canis infection. A leucopenia of a fluctuating type was also found. Whole citrated blood stored at 6° to 8° C lost its infectivity in 3 days. Treatment was effected by a single dose of 4.5 mg of oxophenarsine per kilogram body weight intravenously. Spontaneous recovery conferred immunity, but a dog could be reinfected if the original infection was terminated by treatment.

Benjamin and Lumb (1959) reported on a case of splenic hemangioma in a dog which was treated by splenectomy. The dog was returned 2 months later because of polydipsia, anemia, and listlessness. Organisms resembling H. canis were seen on examination of blood films. The blood was injected into

splenectomized dogs which then developed bartonellemias. Incubation periods ranged from 15 to 20 days. A non-splenectomized dog was also inoculated and remained normal for 1 year, but was then splenectomized with a resultant bartonellemia.

A case of haemobartonellosis in a non-splenectomized dog was reported by Donovan and Loeb (1960). The disease was transmitted to splenectomized puppies but not to a non-splenectomized puppy by blood inoculation. Transmission did not occur when splenectomized puppies were housed with infected dogs.

Regendanz and Reichenow (1932) reported that ground dog fleas were infective, but not the bite or feces of this insect. The bites of adults or nymphs of Dermacentor reticulatus, Rhipicephalus sanguineus, and Ornithodorous moubata were similarly non-infective. Serial passage of H. canis in the cat was accomplished.

Rhoades and Miller (1935) were unsuccessful in transmitting H. canis to mice, rats, rabbits, guinea pigs, and puppies. Lumb (1958) found no transmission by contact, lice, in utero, and through maternal milk. Ingestion of infective blood produced the disease, and it was postulated that natural transmission may occur through vaginal secretions, dog fights, and unclean medical and surgical procedures.

D. Haemobartonella felis.

An "Eperythrozoon" infection of the cat was first described by Clark (1942) in which practically all of the parasites were ring formed and 0.5 to 1.0 u diameter.

Flint and Moss (1953) found "Anaplasma-like" bodies on the erythrocytes of a cat treated for an abscess. The organisms were transmitted to other cats by the intraperitoneal injection of 0.5 milliliters (ml) of blood. Frozen citrated blood remained infective for 2 weeks. Splenectomy was not necessary for the infection to manifest itself. A donor cat which had never shown a positive blood smear was the source of infection for several recipient cats.

Flint, McKelvie, and Douglas (1955) observed that most naturally occurring cases of infectious anemia in cats were in the 1 to 3-year age group. They stated that absence of organisms on blood smear should not be construed as a negative diagnosis if symptoms are present. Treatments suggested were: oxophenarsine, 4 mg every fourth day for 4 doses; chloramphenicol, oxytetracycline, or tetracycline, 100 mg twice daily for 18 to 21 days.

Splitter, Castro, and Kanawyer (1956) found the incubation period after intraperitoneal injection of infective blood into cats to be 3 to 20 days, with an average of 7 7 days. Cultivation attempts on artificial media were

unsuccessful. H. felis was non-filterable. Urine collected during the acute stages of the disease was not infective. Contact transmission could not be effected. Experimental transmission to splenectomized rats, mice, swine, cattle, sheep, and dogs was not accomplished. It was suggested that biting arthropods were the vectors of the disease.

Flint, Roepke, and Jensen (1958) reported on 30 cases in cats, 28 occurring in males. They observed that some factor favors development of the disease shortly after sexual maturity, possibly fighting. The organism may lie dormant until a stress situation occurs. Blood transfusion, oxophenarsine, tetracycline, and chloramphenicol were suggested as treatments.

Schwartzman and Besch (1958) described the bone marrow of a cat with infectious anemia as containing numerous eperythrozoon bodies engulfed by macrophages. The liver was yellow and friable. The spleen was 2 to 3 times normal size. Flint (1959) listed splenomegaly, enlarged lymph nodes, severe anemia, and icterus (in 50% of the cases) as mortem findings.

Flint, Roepke, and Jensen (1959) infected 56 of 76 cats by injection or oral administration of infective blood. Transmission by the intraperitoneal injection of splenectomized dogs, white rats, white mice, and brown deer mice with infective blood was not accomplished. Brown deer mice could

not be infected orally. Contact transmission between cats did not occur. H. felis could be found in the plasma following rupture of the infected erythrocytes. Fleas and mosquitoes were discounted as vectors because of their scarcity in the Salt Lake City area.

CHAPTER III

MATERIALS AND METHODS

A. Experimental Animals

Experimental dogs were mongrels procured from the dog pound. They were vaccinated against canine distemper and infectious canine hepatitis, kenneled individually, and maintained on a diet of prepared dog food, Fromm Meal.[1]

Experimental cats were procured either from farms or private homes. They were vaccinated against feline infectious enteritis. The cats were kenneled either singly, or in twos or threes, depending on available space. A non-infected animal was never housed with an infected one. Their diet consisted of prepared cat food, C/D.[2]

Albino and hooded rats (Rattus norvegicus) were procured from a colony maintained by the Department of Microbiology and Public Health, Michigan State University. The rats were caged in groups, depending on whether they were infected or non-infected. Their diet consisted of Rockland Rat Diet (Complete).[3]

No experimental animal was considered non-infected,

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[1] Federal Foods, Inc., Thiensville, Wisconsin.

[2] Hill Packing Company, Topeka, Kansas.

[3] A. E. Staley Manufacturing Company, Decatur, Illinois.

or used for transmission studies, until a minimum of 14 successive daily Giemsa-stained blood smears were found to be negative for Haemobartonella. This period followed splenectomy in the case of dogs and rats. Cats were not splenectomized.

Blood was used only on days when blood films were positive.

B. Preparation and Examination of Blood Smears

Blood smears were prepared from each animal daily. Dogs were bled by piercing the labial mucosa with a lancet. Cats were bled by piercing the anterior margin of the ear. Rats were bled with a lancet from the tip of the tail. Smears were allowed to air dry. The following staining procedure was then employed:

Methyl alcohol (absolute) ..... 3 minutes

Giesma stain[1] ..... 1 hour

Rinse in tap water

Air dry

The stained smears were examined microscopically under oil immersion using a 100x objective and 10x ocular.

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[1]Preparation of Giemsa's stain:

10 ml Giemsa (Fisher Scientific Company, Fair Lawn, N. J.)
5 ml 1/15 molar NaH_2PO_4
5 ml 1/15 molar Na_2HPO_4
150 ml distilled water

C. Haemobartonellemia Grading System

A grading system was employed to designate the relative number of Haemobartonella on blood smear.

1 plus ..... less than 1 organism per field.

2 plus ..... 1 to 10 organisms per field.

3 plus ..... 11 to 25 organisms per field.

4 plus ..... 26 or more organisms per field.

D. Anti-Haemobartonella Drugs

Filcide[1] is a parenteral solution intended for use in Dirofilaria immitis infections in dogs. Each ml contains:

Sodium p-bis (Carboxymethylmercapto) Arsinobenzamide ..... 1% W/V

(contains arsenic, 1.8 mg)

Water for injection U.S.P.

Benzyl alcohol (Preservative) 0.9%

Mapharsen[2] is distributed in ampoules as a powder and is prepared for use by dissolving in sterile distilled water or saline.[3] It is an effective spirocheticide for treatment

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[1]Pitman-Moore Division of Allied Laboratories, Indianapolis, Indiana, Brand of Arsinobenzamide.

[2]Parke, Davis and Company, Detroit, Michigan, Brand of Oxophenarsine Hydrochloride.

[3]Sterile 0.85% sodium chloride solution used as diluent in this work.

of syphilis. Mapharsen (3-Amino-4-Hydroxyphenyl-arsineoxide Hydrochloride) is supplied as a mixture with sodium carbonate (anhydrous), sucrose, and ascorbic acid which render the solution compatible with the blood. It contains 31% trivalent arsenic.

E. Transmission of Haemobartonella Canis to the Cat

One ml of heparinized blood from a dog (30) positive for H. canis was injected intraperitoneally into each of 4 cats (91, 92, 93, 94; see Chart I). These cats had been proved negative for H. felis by 30 to 42 consecutive days of negative blood smears. On the third, sixth, tenth, and fourteenth days, blood from the cats was drawn into heparinized syringes and pooled. One ml of the pooled blood was injected intraperitoneally into a negative cat, and intravenously into a negative splenectomized dog (19W). This dog (19W), after 4 days, became positive and her blood was inoculated into 2 negative cats (63, 69). When this same bitch (19W) subsequently whelped, possible infection of the puppies was investigated by inoculating their blood into a negative splenectomized dog. All animals remaining negative were challenged with blood of a known positive animal of the same species to prove susceptibility.

F. Transmission of Haemobartonella Felis to the Dog

One ml of heparinized blood from a cat (99) positive for H. felis was injected intravenously into each of 4 splenectomized dogs (5, 12, 13, and 953; see Chart II). These dogs had been proved negative for H. canis by 14 consecutive days of negative blood smears. On the third, tenth, and seventeenth days, blood from the dogs was drawn into heparinized syringes and pooled. One ml of the pooled blood was injected intraperitoneally into a negative cat, and intravenously into a negative splenectomized dog. The dogs developed positive blood smears and their blood was injected intraperitoneally into negative cats. All animals remaining negative were challenged with blood of a known positive animal of the same species to prove susceptibility.

G. Maternal Transmission of Haemobartonella Canis to Puppies

Blood was collected from 4 cats which had been previously inoculated with blood from a Haemobartonella-positive dog (30). One ml of pooled cat blood was injected intravenously into a Haemobartonella-negative, splenectomized, pregnant bitch (19W). The bitch showed blood films positive for Haemobartonella 4 days later. One day after whelping, the puppies were sacrificed and their pooled blood was injected into a Haemobartonella-negative, splenectomized dog (9).

A Haemobartonella-positive bitch (34) had her puppies

delivered by Caesarean section. After 1 day, the puppies were sacrificed and their pooled blood was injected into a Haemobartonella-negative, splenectomized dog (5).

In both cases, the puppies were allowed to nurse.

H. Mosquito Transmission of Haemobartonella Felis

An attempt was made to investigate the role of mosquitoes in the natural transmission of H. felis. A mosquito-proof area was constructed in a pre-existing room. The floor of the room was approximately 6 feet by 6 feet and composed of terrazzo; the plaster walls were about 10 feet high. The front wall was constructed of fine mesh wire screening and a door. A 3-foot space was allowed between this door and a second door leading to an outside room. Four cages were placed inside the mosquito-proof room. A Haemobartonella-positive cat was placed in each of 2 cages (36 and 97) and a Haemobartonella-negative cat was placed in each of the remaining 2 cages (25 and 37). The cats were clipped over a majority of their body surfaces to allow the mosquitoes easy access. Pond water was obtained from a wooded area heavily infested with Culex pipiens mosquitoes and placed in the room in an attempt to hatch mosquitoes. Plant material was placed in the room as a shelter for the mosquitoes, and a sugar solution was supplied for supplemental feeding. When no mosquitoes emerged from the pond

water, adult mosquitoes were captured and released in the room. When it was found that the mosquitoes could not be maintained alive for longer than a 3-day period, additional mosquitoes were captured and released into the room at frequent intervals.

I. Transmission of Haemobartonella Felis to the Rat

Three days after being treated with oxophenarsine, rats 51, 52, 53, 54, 55, 56, 57, 58, 59, 62, and 63 were splenectomized.

Giemsa-stained blood films from each rat were examined daily for the presence of Haemobartonella muris. After 13 days of negative findings, rats 51, 53, 55, 57, 59, and 63 were inoculated intraperitoneally with 0.5 ml of blood from a cat (29) infected with H. felis. Rats 52, 54, 56, 58, and 60 served as uninoculated controls. After 48 days, all rats were challenged with blood drawn from a rat originating in a colony from which H. muris-infected rats had previously been obtained. The rat was not exhibiting a haemobartonellemia at the time of bleeding.

J. Treatment of Haemobartonella Muris Infection in Rats With Oxophenarsine

A series of hooded rats (Rattus norvegicus), approximately 6 months old was prepared for transmission studies

of H. felis, by splenectomizing half of them. Giemsa-stained blood films were examined daily for the presence of blood parasites which might be confused with H. felis. Rats 76, 78, 79, 80, 83, and 85 were splenectomized. Rats 82, 84, 86, 87, 88, and 89 served as non-splenectomized controls (see Chart III).

General anesthesia was induced in the rats with pentobarbital sodium.[1] The commercial preparation contains 75 mg of the anesthetic agent per ml (6.5%). This was diluted with sterile 0.85% sodium chloride solution to make a 3% solution. Rats were weighed and injected intraperitoneally with 30 mg/kg body weight of the pentobarbital solution through a 27-gauge hypodermic needle attached to a 0.5 ml tuberculin syringe. Surgical anesthesia was attained in approximately 5 minutes.

Splenectomy was performed on the rats under aseptic conditions, except that the same drapes and instruments were used on each rat, and the operator did not change gloves. The splenic vessels were ligated with size A nylon. Usually only a single ligature was necessary. The abdomen and skin were closed in 2 layers, again using size A nylon. It was subsequently found that the rats chewed out the dermal sutures, causing a dehiscence of the skin incision. All

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[1]Halatal, Jensen-Salsbury Laboratories, Inc., Kansas City, Missouri.

skin closures were then completed with Michel wound clips which were not removed.

Several days after splenectomy, ring-shaped bodies were observed on the erythrocytes of the splenectomized rats. A macrocytic, hypochromic anemia developed, with anisocytosis, poikilocytosis, and polychromasia. The affected rats were treated with 2 mg/kg body weight of oxophenarsine intraperitoneally.

K. Determination of the MLD of Oxophenarsine for Rats

A group of rats (Rattus norvegicus) was selected for use in determining the MLD of oxophenarsine for this species (see Chart IV). Weights ranged from 116 to 524 grams. Oxophenarsine was diluted to 4 mg/ml and was injected intraperitoneally through a 27-gauge needle attached to a tuberculin syringe.

L. Growth of H. Canis and H. Felis in Embryonated Chicken Eggs

A sterile 10-ml syringe was prepared by aspirating a sufficient volume of heparin solution[1] to wet the barrel and ejecting the excess. Ten ml of blood were drawn from a known Haemobartonella-positive dog or cat and transferred to

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[1]Heparin Sodium, 10 mg/ml, Upjohn Company, Kalamazoo, Michigan.

a sterile, rubber-diaphragm, stoppered vial, to be used as inoculum. Sterility of the inoculum was tested by inoculating semi-solid medium.[1] Viable 8 to 12 day-old embryonated chicken eggs were selected. The sites of inoculation were marked and painted with nitromersol tincture[2] or polyvinylpyrrolidine (povidone).[3] These sites were first opened by drilling with an abrasive disc, later by means of an egg punch. Sites inoculated were the chorio-allantoic (CA) membrane, allantoic sac, and yolk sac. Injection was accomplished through a 27-gauge needle. A 0.2-ml dose of inoculum was used. After inoculation the eggs were sealed with paraffin and incubated at a temperature of 37 to 38° C until death of the embryo occurred or 96 to 140 hours had transpired.

Following incubation, the entire contents of the eggs were removed aseptically from their shells directly into a Waring blender and ground until the resulting mixture could be aspirated through a 20-gauge needle. Eggs in which early death of the embryo occurred were refrigerated at approximately 4° C until harvested. Maximum refrigeration time was

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[1]Bacto Brain Heart Infusion plus Bacto Agar 0.15%, Difco Laboratories, Detroit, Michigan.

[2]Metaphen Tincture 1:200, Abbott Laboratories, North Chicago, Illinois.

[3]Betadine 1% available iodine. Talby-Nason Company, Inc., Dover, Delaware.

3 days. Five-tenths to 1 ml of macerated embryos was inoculated intraperitoneally into Haemobartonella-negative animals. Giemsa-stained blood smears were examined daily for Haemobartonella. When they remained negative, the test animals were challenged by inoculation with blood from a Haemobartonella carrier of the same species to prove susceptibility.

M. Haemobartonella Infection in Dogs and Cats Treated with Arsinobenzamide

Nine dogs (9, 13, 953, 19B, 954, 952, 957, 18, and 19W) exhibiting blood films positive for Haemobartonella were selected (see Charts V, VI). Their weights and source of infection were recorded, and they were divided into 2 groups. Arsinobenzamide was administered intravenously at various dosages on 3 and 10-day schedules. A Haemobartonella-positive dog (30) served as the untreated control.

Eight cats (5, 11, 12, 49, 62, 68, 71, and 74) were inoculated intraperitoneally with approximately 1 ml of blood from a cat (99) positive for H. felis (see Chart VII). When a haemobartonellemia occurred, the weight of each cat was recorded and intravenous treatment with arsenobenzamide was commenced. Cats 5, 11, 12, 49, 71, and 74 were treated with various doses on a 10-day schedule. Cats 62 and 68 served as untreated controls.

CHAPTER IV

RESULTS

A. Transmission of Haemobartonella Canis to the Cat

Chart I is an outline of the experimental procedure. Cats did not develop positive blood smears after being injected with H. canis-positive dog blood. The pooled blood of these cats was infective for dogs but not for cats. The blood of puppies from a bitch which whelped subsequent to being infected with the cat blood was infective for a negative, splenectomized dog.

B. Transmission of Haemobartonella Felis to the Dog

Chart II is an outline of the experimental procedure. One of 4 dogs injected with H. felis positive cat blood developed a haemobartonellemia after a 28-day incubation period. The pooled blood of these dogs was infective for a cat on the third post inoculation day and for a dog on the seventeenth post inoculation day. All other attempts to infect dogs and cats with the pooled blood yielded negative results.

C. Maternal Transmission of Haemobartonella Canis to Puppies

A negative, splenectomized dog (9) became positive 5

days after injection of pooled blood from puppies of a positive bitch (19W).

A negative, splenectomized dog (5) became positive 13 days after injection of pooled blood from puppies of a positive bitch, and died 4 days later.

D. Mosquito Transmission of Haemobartonella Felis

Neither susceptible cat (25, 37) exhibited a blood smear positive for H. felis. After a period of 45 days, the negative cats were proved susceptible by challenge with infective blood.

Since the mosquitoes could not be kept alive for an extended period of time, this experiment was abandoned.

E. Transmission of Haemobartonella Felis to the Rat

No positive blood films were demonstrated in any rat, experimental or control. No blood dyscrasias evidenced by anisocytosis, polychromasia, or poikilocytosis were observed.

F. Treatment of Haemobartonella Muris Infection in Rats with Oxophenarsine

The onset, progress, and treatment of haemobartonellosis in rats is recorded in Chart III.

Four out of 5 rats were successfully treated with 2 mg/kg body weight of oxophenarsine intraperitoneally. Only 1 out of 6 rats did not develop a haemobartonellemia

following splenectomy.

G. Determination of the MLD of Oxophenarsine for Rats

As recorded in Chart IV, rats injected intraperitoneally with up to 22 mg/kg body weight of oxophenarsine lived. The lowest dosage at which death occurred was 24 mg/kg. Rats receiving a sublethal dose exhibited varying degrees of ataxia, salivation, dyspnea, and prostration, from which they eventually recovered. Rats receiving a lethal dose died in a minimum of 4 hours, and a maximum of 8 days. One rat receiving 26 mg/kg lived, as did 1 receiving 30 mg/kg.

H. Growth of Haemobartonella Canis and H. Felis in Embryonated Chicken Eggs

1. Inoculation of the CA Membrane with H. Canis Positive Dog Blood

a. Examination of several eggs 24 hours after inoculation revealed edematous CA membranes with some grayish areas 0.5 cm diameter; livers of the embryos were hemorrhagic. Blood, liver, and CA membrane smears were negative for Haemobartonella.

b. Of 13 eggs inoculated, 5 embryos were dead at 24 hours, and 8 were alive at 96 hours. A suspension was made of the contents of the live eggs, and a susceptible dog inoculated with it.

It remained negative 33 days, was challenged and became positive in 8 days.

c. Six eggs served as uninoculated controls. The CA membranes were dropped and the eggs sealed. Two embryos were dead at 24 hours, 1 at 48 hours, 1 at 72 hours, and 2 were alive at 96 hours. A suspension was made of all the control eggs and a susceptible dog inoculated with it. It remained negative 33 days, was challenged and became positive in 8 days.

d. The blood vessels on the CA membranes of the inoculated eggs appeared cyanotic while those of the controls appeared normal in color.

2. Inoculation of the CA Membrane with H. Felis Positive Cat Blood

a. Of 13 eggs inoculated, 11 embryos were dead at 48 hours, and 2 were alive at 72 hours. A cat inoculated with a suspension made from the contents of all the inoculated eggs remained negative 25 days, was challenged and became positive in 9 days.

b. The CA membranes of 13 embryonated chicken eggs were inoculated with the same embryo suspension

(2a). Three embryos were dead at 24 hours, 5 at 48 hours, 1 at 72 hours, and 4 were alive at 96 hours. A cat inoculated with a suspension made from the contents of all the inoculated eggs remained negative 21 days, was challenged and became positive in 8 days.

c. Five eggs served as uninoculated controls. The CA membranes were dropped and the eggs sealed. One embryo was dead at 48 hours and 4 were alive at 72 hours. A cat inoculated with a suspension made from the control egg contents remained negative 25 days, was challenged and became positive in 2 days.

d. The CA membranes of 3 embryonated chicken eggs were inoculated with the same embryo suspension (2c). All 3 embryos were dead in 24 hours, and bacterial growth was obtained in semi-solid medium. A cat inoculated with a suspension made from the egg contents remained negative 21 days, was challenged and became positive in 9 days.

3. Inoculation of the Allantoic Sac with H. Felis-Positive Cat Blood

a. Of 11 eggs inoculated, 2 embryos were dead at 92 hours, 1 at 116 hours, and 8 were alive at 140

hours. A suspension was made of the contents of the eggs in which embryos were dead at 92 hours, and 2 cats were inoculated. One remained negative 54 days, was challenged and became positive in 12 days. The other remained negative 114 days, was challenged and became positive in 9 days.

b. Of 5 eggs similarly inoculated with 0.1 ml of sterile 0.85% sodium chloride solution, 2 were dead at 24 hours, and 3 were alive at 140 hours.

4. Inoculation of the Yolk Sac with H. Felis-Positive Cat Blood

a. Of 11 eggs inoculated, 3 embryos were dead at 48 hours, 1 at 110 hours, and 7 were alive at 140 hours. A suspension was made from the contents of the eggs in which embryos were dead at 48 hours, and 2 cats were inoculated. The first remained negative 55 days, was challenged and became positive in 12 days. The second remained negative 54 days, was challenged and became positive in 2 days.

b. Of 4 eggs which were similarly inoculated with 0.1 ml of sterile 0.85% sodium chloride solution, 2 embryos were dead at 48 hours, and 2 were

alive at 140 hours. A cat was inoculated with a suspension made from the contents of the eggs in which embryos were dead at 48 hours. It remained negative 115 days, was challenged and became positive in 15 days.

c. Giemsa-stained blood smears taken from the embryos which were dead at 48 hours were negative for Haemobartonella.

I. Haemobartonella Infection in Dogs and Cats Treated with Arsinobenzamide

Charts V, VI, and VII record the treatment of experimentally induced haemobartonellosis.

A dosage of 0.20 ml/lb body weight daily was effective in dogs when used on a 3-day schedule. A dosage of 0.10 ml/lb daily was effective in 1 dog when used on a 3-day schedule, but not effective in a second dog when the same dosage was administered on 10 successive days.

A dosage of 0.20 ml/lb daily was not effective in a cat when used on 10 successive days.

CHART I

TRANSMISSION OF HAEMOBARTONELLA CANIS TO THE CAT

Dog 30 (positive)

Negative cats

Pooled

91..Negative 36 days; challenged and positive in 8 days.

92..Negative 36 days; challenged and positive in 6 days.

93..Negative 36 days; challenged and positive in 2 days.

94..Negative 36 days; challenged and negative for 8 days; rechallenged and positive in 6 days.

3rd day

Negative cat 95...Negative 33 days; challenged and negative in 8 days; rechallenged and positive in 3 days.

Negative dog 19B..Positive in 12 days.

6th day

Negative cat 96...Negative 30 days; challenged: suspicious in 2 days; definitely positive in 18 days.

Negative dog 19W (pregnant)[1,2]..Positive in 4 days.

10th day

Negative cat 67...Negative 49 days; challenged and negative 14 days; rechallenged and remained negative.

Negative dog 18...Positive in 5 days.

14th day

Negative cat 31...Negative 30 days; challenged and suspicious in 3 days; definitely positive in 9 days.

Negative dog 10...Positive in 23 days.

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[1]Evidence of maternal transmission of Haemobartonella infection to the puppies is presented elsewhere in this paper.

[2]Negative cat 69 was inoculated with positive blood from dog 19W. One blood smear positive 63 days post inoculation was attributed to mislabeling of slides. Smears were negative for the next 9 days. The cat was challenged and remained negative 14 days; rechallenged and positive in 3 days.

Negative cat 63 was inoculated with positive blood from dog 19W; remained negative 45 days. The cat was challenged and remained negative 14 days; rechallenged and positive in 9 days.

CHART II

TRANSMISSION OF HAEMOBARTONELLA FELIS TO THE DOG

Cat 59 (positive)

Negative dogs

Pooled

- 5...Negative 65 days; challenged and positive in 12 days; died 4 days later.
- 12..Positive in 28 days and died 38 days later.[1]
- 13..Negative 75 days; challenged and positive in 4 days.
- 953.Negative 75 days; challenged and positive in 4 days.

3rd day

- Negative cat 32...Positive in 11 days and died 13 days later.
- Negative dog 954W.Negative 75 days; challenged and positive in 4 days.

10th day

- Negative cat 11...Negative 39 days; challenged and positive in 5 days.
- Negative dog 9....Negative 53 days; challenged and positive in 5 days.

17th day

- Negative cat 55...Negative 53 days; challenged and positive in 12 days
- Negative dog 952..Positive in 19 days.[2]

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[1]Negative cat 64 was inoculated with blood from dog 12, 22 days after the initial positive blood smear. Cat 64 remained negative.

[2]Negative cat 66 was inoculated with blood from dog 952, 5 days after the initial positive blood smear. Cat 66 remained negative.

CHART III

TREATMENT OF HAEMOBARTONELLA MURIS INFECTION IN RATS WITH OXOPHENARSINE

Rat No.	Sex	Wt. in Grams	Status	Daily findings on blood smear following splenectomy											
				1	2	3	4	5	6	7	8	9	10	11	12
76	M	178	S	0	0	0	0	0	0	0	0	0	0	0	0
78	F	153	S	0	0	++	++	0^1	0_2	0^1	0^2	0^1	0^1 Died		
79	M	227	S	0	++++	++++	++	++++	++	0^1	0^1	0	3	3	0
80	M	230	S	0	++++	+++	+++	++	+	0	0	0^4	3	3	0
83	M	212	S	+++	+++	++++	++++	++++	0	0	0^5	3	0	0	
85	M	189	S	0	+	+++	++++	+++	0	0^1	0	3	3	0	
82	M	212	NS	0	0	0	0	0	0	0	0	0	0	0	0
84	F	136	NS	0	0	0	0	0	0	0	0	0	0	0	0
86	F	127	NS	0	0	0	0	0	0	0	0	0	0	0	0
87	F	189	NS	0	0	0	0	0	0	0	0	0	0	0	0
88	M	214	NS	0	0	0	0	0	0	0	0	0	0	0	0
89	?	171	NS	0	0	0	0	0	0	0	0	0	0	0	0

S Splenectomized.
NS Non-splenectomized.
0 No Haemobartonella seen on Giemsa-stained blood smear.
1 No erythrocytes seen on slide.
2 Few erythrocytes on slide.
3 Granules seen in plasma.
4 Polychromasia.
5 Polychromasia, anisocytosis, poikilocytosis.
Treated with oxophenarsine, 2 mg/kg body weight intraperitoneally.
\+ Less than 1 organism per field.
++ 1-10 organisms per field.
+++ 11-25 organisms per field.
++++ More than 25 organisms per field.

CHART IV

DETERMINATION OF THE MLD OF OXOPHENARSINE FOR RATS

Ear tag	Sex	Weight in kg	Dose in mg/kg	mg As	mg As/kg	Result
51	M	.448	2	0.28	0.62	Lived
52	M	.449	2	0.28	0.62	Lived
53	M	.479	3	0.48	1.07	Lived
54	M	.465	3	0.43	0.90	Lived
55	M	.524	4	0.65	1.24	Lived
56	M	.434	4	0.54	1.25	Lived
57	M	.390	5	0.60	1.53	Lived
58	M	.311	5	0.48	1.54	Lived
59	F	.296	6	0.55	1.86	Lived
60	F	.286	6	0.53	1.85	Lived
62	M	.310	7	0.67	2.16	Lived
63	F	.298	7	0.65	2.18	Lived
64	M	.128	7	0.28	2.19	Lived
65	M	.116	7	0.25	2.16	Lived
66	F	.113	8	0.30	2.45	Lived
67	M	.129	8	0.32	2.49	Lived
69	M	.116	9	0.31	2.67	Lived
70	F	.126	9	0.35	2.79	Lived
71	M	.141	10	0.44	3.12	Lived
72	M	.152	10	0.47	3.10	Lived
74	F	.138	11	0.47	3.41	Lived
75	M	.128	11	0.44	3.44	Lived

CHART IV (continued)

Ear tag	Sex	Weight in kg	Dose in mg/kg	mg As	mg As/kg	Result
76	M	.154	12	0.57	3.70	Lived
77	M	.132	12	0.49	3.73	Lived
78	F	.155	14	0.67	4.32	Lived
79	M	.155	14	0.67	4.34	Lived
80	M	.123	16	0.61	4.98	Lived
81	M	.179	16	0.89	4.97	Lived
82	F	.160	18	0.89	5.56	Lived
84	M	.161	18	0.90	5.59	Lived
85	M	.138	20	0.86	6.23	Lived
87	M	.157	20	0.97	6.18	Lived
88	M	.145	22	0.99	6.83	Lived
89	F	.160	22	1.09	6.81	Lived
6	M	.176	24	1.31	7.44	Died in 48 hours
31	F	.125	24	0.93	7.44	Died in 24 hours
1	F*	.330	24	2.46	7.45	Died in 5 hours
2	F*	.370	24	2.75	7.43	Died in 4 hours
29	M	.160	26	1.29	8.06	Lived
34	M	.147	26	1.18	8.03	Died in 48 hours
3	F*	.291	26	2.16	7.42	Died in 4 hours
4	F*	.331	26	2.67	8.07	Died in 4 hours
47	F	.128	28	1.11	8.67	Died in 6 hours
43	M	.208	28	1.81	8.70	Died in 6 hours

* Castrated

CHART IV (continued)

Ear tag	Sex	Weight in kg	Dose in mg/kg	mg As	mg As/kg	Result
5	F*	.304	28	1.64	8.68	Died in 5 hours
6	F*	.302	28	2.62	8.68	Died in 6 hours
46	F	.110	30	1.02	9.27	Died in 8 days
39	F	.126	30	1.17	9.29	Lived

* Castrated

CHART V

TREATMENT OF HAEMOBARTONELLA INFECTION IN DOGS WITH ARSINOBENZAMIDE-3-DAY SCHEDULE

Dog number	Source of infection	Incubation period in days	Weight in pounds	ml/lb	mg As/dose	Total mg As	Total mg As/lb	Results
9	Puppy of dog 19W[1]	5	35	0.10	6.3	18.9	0.54	Remained negative during a 52-day period of observation.
13	Dog 30	4	24	0.20	8.66	25.98	1.08	Remained negative during a 52-day period of observation.
953	Dog 30	4	22	0.30	9.48	28.44	1.29	Died 3 days after the last treatment without becoming positive.[2]
19B	Pooled blood of cats inoculated with H. canis	12	27	0.40	19.44	58.34	2.16	Remained negative during a 52-day period of observation.
30	Control. Infected donor	-	-	0	0	0	0	Intermittently positive during a 92-day period of observation.

1. Presented previously as evidence of maternal transmission of Haemobartonella infection to offspring.
2. Gross lesions: Hemorrhagic gastritis and enteritis with gas and fluid, pancreatitis. Reinsch test run on the liver was negative.
   Microscopic lesions: Extensive post mortem changes were present. There was no sign of inflammation in the liver, kidneys, or pancreas.

CHART VI

TREATMENT OF HAEMOBARTONELLA INFECTION IN DOGS WITH ARSINOBENZAMIDE-10-DAY SCHEDULE

Dog number	Source of infection	Incubation period in days	Weight in pounds	ml/lb	mg As/dose	Total mg As	Total mg As/lb	Results
954	Dog 30	4	12	0.025	0.54	5.4	0.45	Positive on the 4th treatment day. 2+ on the 16th post-treatment day. Died 4 days later without becoming positive again.
952	Pooled blood of dogs inoculated with H. felis	19	27	0.05	2.43	24.3	0.9	Remained negative during a 45-day period of observation.
957	Dog 30	6	26	0.10	4.68	46.8	1.8	Remained negative 27 days, then became positive daily for 1 week.
18	Pooled blood of cats inoculated with H. canis	5	17	0.20	6.12	61.2	3.6	Remained negative during a 45-day period of observation.
19W	Pooled blood of cats inoculated with H. canis	4	13	0.30	7.02	70.2	5.4	Remained negative during a 45-day period of observation.
30	Control Infected donor	-	-	0	0	0	0	Intermittently positive during a 92-day period of observation.

CHART VII

TREATMENT OF HAEMOBARTONELLA FELIS INFECTION IN CATS WITH ARSINOBENZAMIDE-10-DAY SCHEDULE

Cat number	Incubation period in days	Weight in pounds	ml/lb	mg As/dose	Total mg As	Total mg As/lb	Results
5	12	5	0.02	0.18	1.8	0.36	2+ on 7th treatment day; 4+ on 11th post-treatment day; intermittently positive until death on 20th post-treatment day.
11	5	5	0.04	0.36	3.6	0.72	3+ on 7th treatment day; intermittently positive until death on 16th post-treatment day.
12	3	7	0.06	0.76	7.6	1.08	3+ on 7th post-treatment day; intermittently positive thereafter.
49	3	8	0.08	1.15	11.5	1.44	Died on 14th post-treatment day without becoming positive.
71	12	5	0.10	0.90	9.0	1.8	3+ on 12th post-treatment day.
74	9	4	0.20	1.44	14.4	3.6	4+ on 13th post-treatment day.
62	3	4	0	0	0	0	Died 5 days after inoculation; 4+ on day of death.
68	2	?	0	0	0	0	Received positive blood from cat 62; died 10 days after inoculation.

1. Gross lesions: Ecchymoses in the spleen. Reinsch test run on the liver was negative. Microscopic lesions: The spleen showed congestion, hemorrhage, necrosis of individual cells, and extramedullary hemopoiesis.

CHAPTER V

THREE CASE HISTORIES OF CLINICAL FELINE HAEMOBARTONELLOSIS PRESENTED AT THE MICHIGAN STATE UNIVERSITY VETERINARY HOSPITAL FOR DIAGNOSIS AND TREATMENT

A. Case 38623

Feline, domestic, male 10 months old, 8 pounds.

History: The cat had been castrated 3 weeks previously; 10 ml Normal Feline Serum[1] was administered at that time; he had been in a fight in which the skin had been broken 2 weeks before being presented at the clinic; lethargy present for 4 days prior to examination; anoretic for the past 24 hours; he had been treated with sulfas.

Physical examination: Temperature 94.7° F; mucous membrane white.

Hemogram:

Hemoglobin 2.1 grams %

Hematocrit 10 vol. % (packed cell volume)

WBC (corrected for normoblasts) 15,800 leukocytes per cubic mm of blood

Differential, percentages

Neutrophils 86

Segmented 18

Non-segmented 62, metamyelocytes 6

Lymphocytes 14

Normoblasts 381/100 WBC

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[1] Fromm Laboratories, Inc., Grafton, Wisconsin.

Polychromasia, anisocytosis

Haemobartonella felis seen on Giemsa-and Wright-stained smears.

Stool Examination: Ascarid and hookworm ova.

Treatment was begun with 35 ml of whole cat blood intravenously. Additional therapy included Normal Feline Serum, 8 ml subcutaneously; Daribiotic,[1] 0.5 ml intramuscularly (discontinued when diagnosis made); Injectoplex[2] and Covifol[3], 1 ml of each intramuscularly. After the Haemobartonella were seen, 2 mg of oxophenarsine (0.5 mg/kg) was administered intravenously.

The following day the temperature was 103.8° F. Treatment was continued with oxophenarsine, vitamin B-complex, and vitamin B-12 injections. The cat was force-fed with Sustagen.[4] The same regimen was followed for a total of 10 days. The temperature fell to normal on the third day and color returned to the mucosae after 5 days. Force feeding was discontinued

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[1]S. E. Massengill Company, Bristol, Tennessee.

[2]Atlas Pharmaceutical Laboratories, Detroit, Michigan. Contains thiamine HCL, nicotinamide, pyridoxine HCL, pantothenic acid, and riboflavine.

[3]Atlas Pharmaceutical Laboratories, Detroit, Michigan. Contains folic acid, vitamin B-12, cobalt gluconate, liver injection.

[4]Mead Johnson and Company, Evansville, Indiana. Dehydrated, powdered, therapeutic food containing all known essential nutrients.

after the fifth day. A hematologic examination was performed on the ninth treatment day.

Hemoglobin 8.5 grams %

Hematocrit 26 vol. % (packed cell volume)

WBC 8,200 leukocytes per cubic mm of blood

Differential, percentages

Neutrophils 59

Segmented 23

Non-segmented 36

Lymphocytes 32

Monocytes 7

Eosinophils 2

Giemsa-stained blood smear negative for Haemobartonella.

Two weeks later a blood smear was negative for Haemobartonella.

The cat remained normal until he succumbed to a streptococcosis 1 year later.

B. Case 38941

Feline, domestic, male, 1 year old, 11 pounds.

History: The cat had been castrated 3 weeks previously; he had been in a fight since that time, with skin being broken; he had been losing strength, appetite, and weight for 10 days, refusing all food but milk.

Physical Examination: Temperature, 105° F; mucous membranes, white.

Hemogram:

Hemoglobin, 5.2 grams %

Hematocrit, 19 vol. % (packed cell volume)

WBC (corrected for normoblasts), 14,300 leukocytes per cubic mm of blood

Differential, percentages

Neutrophils 52

Segmented 21

Non-segmented 31

Lymphocytes 38

Monocytes 7

Eosinophils 3

Polychromasia, anisocytosis

Some neutrophils show slight toxic granulation.

Blue cytoplasmic inclusion bodies in some neutrophils.

Haemobartonella felis seen on Giemsa stain.

Stool Examination: Ascarid ova.

Treatment was begun with 5 mg oxophenarsine intravenously (1 mg/kg). Supportive treatment consisted of Ferro-B[1], 1 tablet, and Tonamine[2], 1 ml orally daily.

The next day the temperature was 103.4° F. The same treatment was continued. Five ml Normal Feline Serum[3] were administered subcutaneously. The cat drank a small amount of Sustagen[4].

The following day the temperature was not recorded. Treatment was altered by the reduction of oxophenarsine to 2.5 mg (0.5 mg/kg).

Temperature was normal the next day and remained so during the 10-day course of treatment. On the tenth day the hematocrit was 32 vol. %. A Giemsa-stained blood smear was negative for Haemobartonella. The cat was discharged, apparently normal.

The animal was returned 1 month later for a hematologic

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[1]Pitman-Moore Division of Allies Laboratories, Indianapolis, Indiana; iron and ammonium citrate, manganese citrate, thiamine HCL, yeast extract.

[2]Strassenburgh Company, Rochester, New York; B-complex appetite stimulant.

[3]Fromm Laboratories, Inc., Grafton, Wisconsin.

[4]Mead Johnson and Company, Evansville, Indiana. Dehydrated, powdered, therapeutic food containing all known essential nutrients.

examination:

Hematocrit, 41 vol. % (packed cell volume)

WBC, 19,250 leukocytes per cubic mm of blood

Differential, percentages

Neutrophils 49

Segmented 14

Non-segmented 35

Lymphocytes 41

Monocytes 4

Eosinophils 6

Giemsa-stained blood smear negative for Haemobartonella

There have been no recurrences of symptoms up to this writing (13 months).

C. Case 40911

Feline, domestic, male, 4 years old, weight 6.5 pounds.

History: Barn cat which was presented because of inactivity, inappetence, and loss of weight.

Physical Examination: Temperature, 101.5° F; mucous membranes, pale; lice present.

Hemogram:

Hematocrit, 14 vol. % packed cell volume

WBC (corrected for normoblasts), 16,280 leukocytes per cubic mm of blood

Differential, percentages

Neutophils 57

Segmented 13

Non-segmented 44

Lymphocytes 35

Monocytes 4

Eosinophils 4

Normoblasts 59/100 WBC

Polychromasia, anisocytosis

Haemobartonella felis seen on Giemsa-stained smears on the fifth day.

Stool Examination: Ascarid, hookworm, and Trichuris ova.

TREATMENT SCHEDULE

Day	Arsinobenzamide	Other	Observations
1		Parabomb-M[1]; Injectoplex[2], 1 ml, intravenously	T. 101.5°; blood smear negative.
2		Parabomb-M; Injectoplex 1 ml; anti-feline distemper serum[3], 5 ml; whole cats' blood, 20 ml, intravenously	T. 102.5°; blood smear negative.
3		Injectoplex, 1 ml	T. 102.4°; active; appetite fair; blood smear negative.
4		Injectoplex, 1 ml	T. 102.2°; appetite poor; blood smear positive.
5	2.6 ml IV (0.4 ml/lb)	Injectoplex, 1 ml	T. 102.0°; anorectic, depressed, mucous membranes pale; blood smear positive.
6	2.6 ml IV	Injectoplex, 1 ml; 5% Dextrose in 1 Quarter Strength Saline[4], 200 ml, subcutaneously	T. 100.9°; listless, appetite poor; blood smear positive.
7		Injectoplex, 1 ml; Dextrose in Saline, 100 ml	T. 101.3°; active; blood smear negative.

[1]Haver-Lockhart Laboratories, Kansas City, Missouri. Ectoparasiticidal spray containing malathion.

[2]Atlas Pharmaceutical Laboratories, Detroit, Michigan. Contains thiamine HCL, nicotinamide, pyridoxine HCL, pantothenic acid, and riboflavine. For intravenous or intramuscular injection.

[3]Fromm Laboratories, Inc., Grafton, Wisconsin. Feline origin, produced from cats exposed to panleucopenia virus and inoculated with feline distemper vaccine.

[4]Abbott Laboratories, North Chicago, Illinois.

[5]Jensen-Salsbery Laboratories, Inc., Kansas City, Missouri.

TREATMENT SCHEDULE (continued)

Day	Arsinobenzamide	Other	Observations
8	1.3 ml IV (0.2 ml/lb)	Dextrose in Saline, 100 ml; Injectoplex, 1 ml	T. 103.0°; appetite fair; blood smear negative.
9	1.3 ml IV	Dextrose in Saline, 100 ml; Injectoplex, 1 ml	T. 102.0°; active, appetite good; blood smear negative.
10		Dextrose in Saline, 100 ml; Injectoplex, 1 ml	T. 102.0°; improving; blood smear negative.
11		Dextrose in Saline, 100 ml; Injectoplex, 1 ml	T. 101.1°; mucous membranes pink; blood smear negative.
12	1.3 ml IV	Dextrose in Saline, 100 ml; Injectoplex, 1 ml	T. 100.8°; appetite good, very active; blood smear negative.
13	0.6 ml IV (0.1 ml/lb)	Dextrose in Saline, 100 ml; anti-feline distemper serum, 4 ml	T. 100.8°; condition good; blood smear negative.
14	0.6 ml IV	Injectoplex, 1 ml	T. 100.8°; condition good; blood smear negative.
15	0.6 ml IV	Injectoplex, 1 ml	T. 101.2°; condition good; blood smear negative.

Hemogram 4 days after termination of arsinobenzamide therapy:

Hemoglobin 7.5 grams %

Hematocrit 23 vol. % (packed cell volume)

WBC, 17,150 leukocytes per cubic mm of blood

Differential, percentages

Neutrophils 63

Segmented 24

Non-segmented 39

Lymphocytes 32

Monocytes 4

Eosinophils 1

The cat continued to eat and the condition gradually improved. A slough occurred along the femoral vein where some of the arsinobenzamide had been extravasated. The cat was wormed, and when the sloughed area was well granulated, the cat was discharged.

Two months later the owner reported that the cat was back to its normal activity and had gained weight.

Hemogram 2 months post treatment:

Hemoglobin, 8.5 grams%

Hematocrit, 27.5 vol. % (packed cell volume)

WBC, 12,400 leukocytes per cubic mm of blood

Differential, percentages

Neutrophils 67

Segmented 45

Non-segmented 22

Lymphocytes 30

Monocytes 1

Eosinophils 2

Giemsa-stained blood smear negative for Haemobartonella.

One year later the owner reported that the cat was apparently normal.

CHAPTER VI

DISCUSSION

Haemobartonella muris and *H. canis* have been known for many years. There is adequate justification for their inclusion in the genus *Haemobartonella*. Such is not the case with *H. felis*. This organism is not included in the 7th Edition of Bergey's Manual. The application of the name *Haemobartonella felis* to this organism is apparently the result of a letter from Dr. David Weinman, a recognized authority on the *Bartonellaceae*, published by Flint and Moss (1953). Dr. Weinman felt that the feline parasites were either *Haemobartonella* or *Eperythrozoon*, on the basis of morphology. He also believed it unusual for these organisms to produce disease without splenectomy. Whether the feline organism will remain in the genus *Haemobartonella* remains to be seen.

In any transmission study, it is first necessary to prove the test animals negative for the organism which is to be transmitted, so that the experimental results can be properly evaluated. When Regendanz and Reichenow (1932) reported that *H. canis* had been transmitted to a cat, they were unaware of the existence of *H. felis*, and consequently their experimental results might be questioned. In this study,

great care was taken to prove the test animals negative for Haemobartonella before transmission was attempted.

The incubation period of haemobartonellosis is that time between the introduction of infectious material, or the splenectomy in the case of a latent infection, and the appearance of Haemobartonella in the peripheral blood. Based on published incubation periods, the minimum pre-inoculation observation period was set at 14 days. Most animals designated Haemobartonella-negative were observed for a longer time.

Kikuth (1932) reported the post-splenectomy incubation period of H. canis in dogs to be 6 days after surgery. Knutti and Hawkins (1935) found the incubation period of H. canis to be 2 to 5 days. Ray and Idnani (1940) reported the incubation period to be 19 days in subcutaneously inoculated dogs, and 6 to 12 days in intravenously inoculated dogs. Benjamin and Lumb (1959) made observations on 4 dogs with incubation periods of 15 to 20 days.

Splitter, Castro, and Kanawyer (1956) reported the incubation period of H. felis in cats to be 3 to 20 days, and averaging 7 days.

Flint, Roepke, and Jensen (1959) reported an average incubation period of 15.7 days, and ranging from 9 to 34 days after intraperitoneal inoculation. After intravenous inoculation, the incubation period averaged 16.4 days,

ranging from 8 to 23 days. Their results with splenectomized cats were approximately the same.

In this study, the average incubation periods should be regarded in groups according to the source and route of administration of infective blood.

1. Canine to canine, intravenously (11 dogs): range 4 to 28 days, average 9 days.

2. Canine to feline, intraperitoneally, and back to canine intravenously (4 dogs): range 4 to 23 days, average 11 days.

3. Feline to feline intraperitoneally (18 cats): range 2 to 15 days, average 7 days.

4. Feline to canine intravenously, and back to feline intraperitoneally (1 cat): 11 days.

While cats did not develop positive blood smears, H. canis remained viable in this foreign host, and the cat blood was infective for dogs. When blood from these dogs was inoculated into cats, haemobartonellemia did not occur in the cats. H. felis, on the other hand, produced a haemobartonellemia in a dog, and pooled blood of dogs, inoculated with H. felis was infective for a dog and a cat. This indicates that the cat, while not showing a haemobartonellemia, may be a carrier for H. canis. Dogs develop a

haemobartonellemia when injected with H. felis. It is conceivable, therefore, that cross infection occurs in nature, due to exchange of blood in fighting, or perhaps due to parasitic arthropods. H. canis and H. felis appear to be separate entities.

Maternal transmission of H. canis to puppies was demonstrated in 2 instances. In both cases, the puppies were allowed to nurse. It, therefore, remains to be determined whether the infection of the puppies occurred in utero, or whether it was passed through the bitchs' milk. Maternal transmission may be an important factor in the perpetuation of the disease.

The attempt at mosquito transmission of H. felis was inconclusive.

The inability to produce an infection in splenectomized rats with H. felis confirms the reports of other workers. Oxophenarsine proved to be effective in the therapeutics of H. muris infection in rats, and should be considered for use if it is ever necessary to splenectomize rats for experimental use. The MLD of this drug for rats was found to be between 22 and 24 mg/kg body weight. This coincides with the published MLD for rats of greater than 23 mg/kg body weight (Spector, 1956).

Inoculation of incubating chick embryos via the chorioallantoic membrane, allantoic sac, and yolk sac, with H. canis and H. felis did not yield viable organisms. Gross

examination of the embryos and their membranes suggested that infection had occurred but that the organism did not survive. It was impossible to infect dogs or cats with harvested egg material. Further work in this area should include the intravenous route of embryo inoculation as well as variation of the incubation temperature.

Arsinobenzamide is commonly used as a treatment for canine heartworm infection. A 3-day treatment with this drug at 0.20 ml per pound of body weight is suggested as therapy for canine haemobartonellosis. The same dosage, on a 10-day schedule, is unreliable in the cat, although it may be effective in certain instances (see case reports).

Oxophenarsine has been recommended for treatment of H. felis infection. Two case reports are presented in which it was used successfully.

CHAPTER VII

CONCLUSIONS

1. Cats may be carriers of Haemobartonella canis, and thus potential sources of infection for dogs.

2. Dogs can be infected with Haemobartonella felis, showing a haemobartonellemia. The dog blood is then infective for dogs and cats.

3. Maternal transfer of Haemobartonella canis to puppies occurs either in utero, through the milk, or possibly through contamination at birth. This may play an important part in the natural transmission of the disease.

4. Mosquitoes were not proved to be vectors of Haemobartonella felis.

5. Rats could not be infected with Haemobartonella felis.

6. Haemobartonella muris infection in rats, which becomes manifest after splenectomy, can be successfully treated with oxophenarsine, 2 mg/kg body weight intraperitoneally.

7. The MLD of oxophenarsine for laboratory rats is between 22 and 24 mg/kg body weight.

8. Embryonated chicken eggs, 8 to 12 days old, could not be

infected with Haemobartonella canis or H. felis.

9. Arsinobenzamide can be used successfully to treat canine haemobartonellosis on a 3-day schedule at the level of 0.20 ml/lb body weight.

10. The use of arsinobenzamide at a level of 0.20 ml/lb body weight for 10 days was unsuccessful in the treatment of feline haemobartonellosis. Results in 1 clinical case, however, suggest that it may be effective in certain instances.

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