Laboratory Studies of Tropical Canine Pancytopenia


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(Submitted for publication 9 June 1971)

Huxsoll, David L., Amyx, Herbert L., Hemelt, Irene E., Hildebrandt, Paul K., Nims, Robert M., and Gochenour, William S., Jr. 1972. Laboratory studies of tropical canine pancytopenia. *Experimental Parasitology* 31, 53-59. Tropical canine pancytopenia (TCP) is a fatal, infectious disease of dogs characterized by hemorrhage, pancytopenia, and severe emaciation. The disease, which has been reported most frequently in the German Shepherd, has been responsible for the death of numerous military dogs in Southeast Asia. TCP has also occurred in Puerto Rico, the Virgin Islands, Florida, and the Mideast. The most striking clinical sign is a sudden onset of epistaxis in an apparently healthy dog. Pathological findings consist of petechial and ecchymotic hemorrhages on serosal and mucosal surfaces of numerous organs. The disease has been successfully transmitted to laboratory dogs. Experimentally infected beagles and mongrels develop clinical signs consistent with the natural disease; however, clinical signs of hemorrhage, including epistaxis, have been experimentally induced only in the German shepherd. *Ehrlichia canis* has been identified as the etiologic agent of TCP.

**Index Descriptors:** *Ehrlichia canis*; Tropical canine pancytopenia, Dogs, military; German shepherd; Epistaxis; Hemorrhage; Plasmacytosis; Anemia; Leukopenia; Thrombocytopenia; *Ehrlichiosis, canine*; Pathology; Mice; Rats; Guinea pig; Hamsters.

Tropical canine pancytopenia (TCP) is a fatal, infectious disease of dogs occurring in tropical and subtropical areas of the world (Wilkins et al. 1967; Huxsoll et al. 1969, 1970a,b; Walker et al. 1970; Seamer and Snape 1970). In Southeast Asia, TCP was first recognized in 1963 in British military dogs in Singapore where it subsequently was responsible for the death of numerous military and privately owned dogs (Wilkins et al. 1967; Spence et al. 1967). The disease was identified in the Republic of Vietnam in 1967 in several Labrador retrievers which had previously been trained as tracker dogs in Malaysia (Walker et al. 1970). To date approximately 200 U. S. military dogs have died of the disease in Southeast Asia. The disease has also occurred in Puerto Rico, the Virgin Islands, Florida, and the Mideast (Huxsoll et al. 1969, 1970a,b; Seamer and Snape 1970). TCP has been reported most frequently in the German shepherd; however, dogs of other breeds have also been affected.

**Clinical and Pathological Findings**

A sudden onset of epistaxis, the most dramatic sign of the disease, is often the first indication that a dog is affected with TCP. Although in some dogs epistaxis may be the only apparent clinical sign, it is often accompanied by one or more of the following signs: anemia; edema of limbs and scrotum; loss of weight; ecchymotic hemorrhages on the abdomen; petechial hemorrhages in the mucosa of the penis, buccal cavity, and conjunctiva; anorexia; dyspnea; fever; corneal opacity; lethargy; lymphad-
enopathy; posterior weakness; melena and hyphema (Wilkins et al. 1967; Huxsoll et al. 1969, 1970a, b; Walker et al. 1970). Death usually occurs within a few days of onset of epistaxis. On the other hand, if hemorrhage is profuse, death may occur within several hours. Some dogs survive the initial hemorrhagic episode. Nevertheless, epistaxis often recurs and the dog eventually succumbs to the disease. Some dogs become chronic bleeders and have intermittent episodes of epistaxis over a period of several months prior to death (Walker et al. 1970). Many of these chronic bleeders become severely emaciated. Bleeding is not limited to epistaxis. In some dogs severe intestinal hemorrhage occurs as evidenced by large quantities of blood in the stool. Coagulation time and prothrombin time are normal; however, bleeding time is prolonged (Wilkins et al. 1967; Huxsoll et al. 1969, 1970a, b; Walker et al. 1970). The erythrocyte sedimentation rate is often elevated (Huxsoll et al. 1969). Electrophoretic examination of sera from affected dogs reveals an increase in the gamma globulin fraction and a depression of the albumin factor (Wilkins et al. 1967; Burghen et al. 1971).

A large number of dogs with TCP do not develop epistaxis or other clinical signs of hemorrhage. However, the hematologic signs in these dogs are similar to those observed in dogs with epistaxis in that severe pancytopenia occurs (Huxsoll et al. 1969; Walker et al. 1970). These dogs, referred to as “pancytopenics” as opposed to bleeders, usually become severely debilitated prior to death.

A review of clinical records of several hundred dogs affected with TCP has provided evidence that the clinical course of the disease is prolonged (Walker et al. 1970). Dogs with TCP usually have a history of a febrile episode occurring 2 or more months prior to the onset of epistaxis. The duration of the febrile episode may vary from a few days to several weeks. The fever may be accompanied by anorexia, severe weight loss, decreased stamina, and edema of the limbs and scrotum. In a few instances mild epistaxis may be evident during the febrile period. In all dogs red and white blood cell counts are lowered.

The febrile episode is usually followed by a period of apparent recovery referred to as the “subclinical phase” (Walker et al. 1970). Dogs will usually regain a normal physical appearance even though, anemia, leukopenia, or thrombocytopenia often persist. Therefore, prior to the onset of epistaxis, the only sign of the disease in many dogs is an altered hemogram. In those dogs without clinical signs of hemorrhage, death may be attributable to extensive internal hemorrhage or secondary infections which are associated with severe anemia and leukopenia.

Pathological findings have been consistent in all forms of the disease (Hildebrandt et al. 1970a). Macroscopic findings include a generalized lymphadenopathy, petechial and ecchymotic hemorrhages on serosal and mucosal surfaces of most organs and in subcutaneous tissue, brownish motting of the lungs, and a moderate enlargement of the spleen in some dogs. The most striking histologic finding is a perivascular plasma cell infiltrate in numerous organs, especially the lungs, meninges, kidney, and spleen. The bone marrow is hypoplastic.

Epizootiological studies have provided evidence that ticks are vectors of the disease (Nims et al. 1971). Outbreaks of TCP have usually been associated with severe tick infestations, and in those kennels where rigid tick control measures were enforced, the disease disappeared or the incidence was markedly reduced.

**Etiology and Experimental Disease**

In our laboratory TCP has been successfully transmitted to laboratory dogs. Beagles and mongrels develop signs of disease within 10–15 days after intravenous or intraperitoneal inoculation of fresh whole blood from an affected dog. Early signs of
disease are fever, serous nasal and ocular discharge, anorexia, depression, lowered thrombocyte count, elevation of the erythrocyte sedimentation rate, and lowered red and white blood cell counts. The duration and severity of these signs are extremely variable; however, in many experimentally infected beagles, signs of disease persist for several weeks. Most dogs develop anemia, leukopenia, and thrombocytopenia. Intracytoplasmic inclusions of *Ehrlichia canis* can be demonstrated in monocytes in capillary blood smears prepared during the early stages of the disease (Huxsoll et al. 1969). These inclusions are more readily found in mononuclear cells in impression smears prepared from lung tissue (Fig. 1). In many instances inclusions of *Ehrlichia canis* can also be demonstrated in impression smears of spleen, liver, and kidney tissue. The inclusions can be demonstrated with any Romanovsky-type polychrome stain. They occur as single or multiple morula-like bodies in the cytoplasm and appear to be aggregates or colonies of elementary bodies. These inclusions have been demonstrated in capillary blood smears or tissue impressions of all experimentally infected dogs.

Most experimentally infected beagles and mongrels recover from the disease but remain infected. In a few instances, relapses characterized by a reappearance of earlier signs, have occurred. Epistaxis has never been observed in experimentally infected beagles or mongrels.

German shepherd dogs inoculated with fresh whole blood from an affected dog develop disease indistinguishable from the natural disease. Onset of disease in the German shepherd is similar to that in the beagle although the disease is generally more severe (Fig. 2). A few experimentally infected German shepherds have not recovered from the early stages of the disease. Most, however, survive the early stages and may regain a normal physical appearance. Abnormal hematologic signs may partially disappear. Nevertheless, relapses frequently occur and, as in the beagle, are characterized by a reappearance of earlier signs. Epistaxis and other forms of hemorrhage

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**Fig. 1.** Impression smear prepared from lung tissue of a dog experimentally infected with tropical canine pancytopenia. The monocyte contains an intracytoplasmic inclusion of *Ehrlichia canis*. May–Grunwald–Giemsa stain. × 2000.
have occurred in experimentally infected German shepherds as early as 10 days and up to 120 days postinoculation. During the early stages of the disease a blood-tinged, seropurulent nasal discharge is evident in most experimentally infected German shepherds. This sign, however, is distinct from the profuse epistaxis which usually occurs later in the course of the disease. Hemorrhage is associated with severe thrombocytopenia. Other signs observed in experimentally infected German shepherds during the course of the disease include ulceration of the nasal mucosa, corneal opacity, hyphema, petechial and ecchymotic hemorrhages in the mucosa of the penis and buccal cavity, posterior weakness, dyspnea, and edema of the limbs.

We have recovered *Ehrlichia canis* from 11 dogs with signs of tropical canine pancytopenia in Southeast Asia, Puerto Rico, the Virgin Islands, and Florida. Four of the 11 dogs had concurrent infections with *Babesia canis*. However, the production of experimental TCP in laboratory dogs is not dependent on dual infections. Experimental disease, indistinguishable from the natural disease, is produced with *Ehrlichia* alone.

Pathological findings in experimentally infected dogs are compatible with those in the natural disease (Hildebrandt et al. 1970b). Macroscopic changes include generalized lymphadenopathy and petechial and ecchymotic hemorrhages on serosal and mucosal surfaces of major organs. The bone marrow is hypoplastic and perivascular cuffing with plasma cells is evident in most organs.

Dogs that recover from the disease remain infected. Therefore, healthy dogs may serve as a source of infection. After inoculation, dogs have remained infected during an observation period of 1 year.

Laboratory mice, rats, guinea pigs, and hamsters inoculated with the agent of TCP show no evidence of infection. Attempts to propagate the agent in embryonating eggs and many different cell cultures have been unsuccessful. There is no evidence of human infections.

**TREATMENT**

Effective means of treating all stages of the disease have not been developed. Preliminary studies have provided evidence that tetracycline may be effective during the early stages of the disease and at low dosages may be an effective prophylactic. Walker and co-workers (1970) reported that antibiotics, hemostatic drugs, and massive blood transfusions have little beneficial effect on animals in the terminal stages of the disease.
TROPICAL CANINE PANCYTOPENIA

DISCUSSION

In 1935 Donatien and Lestoquard identified rickettsial-like organisms in monocytes of tick-infested dogs in Algeria and proposed the name, *Rickettsia canis*, for the newly described agent. The organism is now classified in the Order *Rickettsiales* under the name *Ehrlichia* (Philip 1957; Ewing 1969). Infections with *Ehrlichia canis* have been reported in Africa, the Middle East, and the Orient (Ewing 1969). In 1957 Bool and Sutmöller reported the first case of *Ehrlichia* infection in dogs in the Western hemisphere. In 1963 Ewing described a mixed infection of *Ehrlichia canis* and *Babesia canis* in dogs in Oklahoma. It is apparent from these reports that other hemotropic parasites, particularly *Babesia*, frequently complicate *Ehrlichia* infections and account for the severe disease which has been reported. Uncomplicated ehrlichiosis has been reported to be a relatively mild disease (Ewing 1969). In contrast tropical canine pancytopenia is a severe manifestation of uncomplicated *Ehrlichia canis* infections in certain breeds of dogs, particularly the German shepherd (Huxsoll et al. 1969, 1970a,b; Walker 1970; and Seamer and Snape 1970). The identification of *Ehrlichia canis* is based solely on its morphological characteristics in infected cells. The agent of TCP is indistinguishable from *Ehrlichia canis*, originally described by Donatien and Lestoquard in 1935. The conclusion that *Ehrlichia canis* is the etiologic agent of tropical canine pancytopenia is based on the consistent recovery of *Ehrlichia* from dogs affected with TCP from diverse geographical locations and the production of disease, indistinguishable from the natural disease, in laboratory dogs experimentally infected with *Ehrlichia*.

In 1938 Shirlaw described a disease known as Lahore canine fever which was responsible for the death of many dogs in India and Pakistan. The clinical signs of Lahore canine fever closely resemble those of TCP, including nasal and intestinal hemorrhage. Shirlaw concluded that *Babesia gibsoni* was the etiologic agent of this disease, although *Babesia* organisms could not always be demonstrated. Recently Seneviratna (1965) suggested that *Ehrlichia canis* was the probable cause of Lahore canine fever.

Tropical canine pancytopenia is apparently a previously unrecognized manifestation of infection with *Ehrlichia canis*. However, the precise relationship of the agent of TCP with previously described strains of *Ehrlichia canis* has not been resolved.

REFERENCES


HUXSOLL ET AL.


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FIFE: Have you any clues as to what might be the route of infection?

HUXSOLL: Transmission by the tick, Rhipicephalus sanguineus has been confirmed in our laboratory.

D’ANTONIO: Is the anemia here due to bleeding, or is it primarily due to the thrombocytopenia, or is it secondary to some primary event in the bone marrow? Is there bone marrow depression?

HUXSOLL: The bone marrow is hypoplastic and we are sure that the bleeding is associated with thrombocytopenia. There may be other factors. In many cases the thrombocyte counts in these dogs are extremely low. In fact, a decrease in thrombocyte counts occurs very early in the course of the disease. We see dogs with thrombocyte counts of 10-15 thousand showing no signs of clinical hemorrhage. In these instances it is hard to understand the absence of hemorrhagic signs. Many dogs will start to recover and then relapse. At this time the thrombocyte count decreases for the second time and the dogs often bleed. At this point it is often difficult to find any thrombocytes. We have looked for changes in the vascular system that could contribute to the bleeding but have found no significant changes.

FIFE: Have you any idea why the thrombocytes go out so fast?

HUXSOLL: Probably because the thrombocytes are very short-lived, and I’m sure that the bone marrow is attacked. Those components which have a short life appear to go first.

FIFE: Will the serum clump platelets?

HUXSOLL: Yes, we do see some agglutination of platelets, white cells, and even red cells. Anemia is severe, the packed cell volume drops precipitously in just a day or so in some cases.

NUSSENSZWEIG: What is the effect of the enormous numbers of plasma cells which you showed in the picture? Do you find an increase in levels of globulins in those animals?

HUXSOLL: Yes, we see an increase in the beta and gamma globulins and a decrease in the albumin.

NUSSENSZWEIG: Does the agent which you showed inside monocytes occur only in monocytes or does it occur in other cells and other organs?

HUXSOLL: It seems to be limited primarily to monocytes or mononuclear cells. In the peripheral blood, we occasionally see a lymphocyte and very rarely even a neutrophil infected. I am very interested in knowing whether hypersensitivity occurs or whether autoimmunity might be involved in the pathogenesis of this disease. The disease is very similar to Aleutian mink disease.
FERRIS: Are there reports of human infection?

HUXSOLL: As far as we know, there is no evidence of human infection.

COX: Rickettsia commonly infect the endothelium. Have you seen any signs of this? The possibility of endothelial infection is important in view of the massive changes in vascular permeability. Have you looked for such things as antibody to cardiolipin or RH factor, and other things of this nature? I think these might serve as rather significant kinds of indicators of autoimmune disease.

HUXSOLL: To answer your first question on the role of the endothelium, we feel this where the organism may be growing. We have some sections now of experimentally infected dogs where we can see endothelial cells bulging into the lumen and they contain the inclusion. An interesting thing, in all natural cases that have been examined in our pathology department I think there is only 1 case out of 100 where we have found an inclusion in histologic sections which were stained with hematoxylin and eosin. In experimentally infected dogs, killed 10 days post inoculation, when early disease signs were apparent, we could readily find inclusions in histologic sections. We have not studied the other factors.

COX: It's unfortunate that so much of our histologic studies are done on autopsy or postmortem materials. I think these things have to be done at timed intervals through acute infection.

HUXSOLL: I think that in many cases this is true.

RISTIC: Is depression of the bone marrow involved in the anemia?

HUXSOLL: Depression of the bone marrow involved. If the bone marrow is attacked and cell production is halted, a decrease in red blood cell and white blood cell counts would become evident in a short time. Sludging of cells in the vascular bed would also account for some of the anemia, and we have some histological sections that suggest this might be occurring. We also see agglutination of red cells. There is tremendous elevation in the erythrocyte sedimentation rate.

RISTIC: What is the involvement of the spleen in this disease?

HUXSOLL: It has been suggested that splenectomized dogs develop more severe disease than do nonsplenectomized dogs. We haven't seen any significant difference in our studies. In some of the older studies on *Ehrlichia canis*, concurrent infection with *Babesia* may have interfered. When dogs with dual infections were splenectomized they may have developed severe babesiosis and subsequently died.

RISTIC: You call the disease "canine pancytopenia." Now ehrlichiosis has been known for many years as ehrlichiosis. Why not call the disease ehrlichiosis?

HUXSOLL: When the disease was first described in Southeast Asia it was known as idiopathic hemorrhagic syndrome, canine hemorrhagic fever, and tropical canine pancytopenia. The name tropical canine pancytopenia was originated by the British. I agree with you that the name ehrlichiosis should be associated with the disease.