An Epizootic of Tropical Canine Pancytopenia in Thailand

Principal Investigators: David E. Davidson, Jr., LTC, VC
Garrett S. Dill, Jr., CPT, VC
Markpol Tingpalapong, DVM
Suchai Premabutra, DVM, COL, RTA[1]
Pralong La-or Nguen, DVM, LTC, RTA[1]

Associate Investigators: Miodrag Ristic, Ph.D.[2]
Edward H. Stevenson, LTC, VC[3]

OBJECTIVE: To study the epizootology of Tropical Canine Pancytopenia in a population of military working dogs, and to evaluate the efficacy of currently recommended prophylactic and therapeutic measures in a natural outbreak.

BACKGROUND: Tropical Canine Pancytopenia (TCP) is a tick-transmitted infectious disease of dogs caused by the rickettsia-like organism *Ehrlichia canis*. Infected dogs may be almost asymptomatic, or they may develop a frequently fatal syndrome characterized by fever, anemia, leukopenia, thrombocytopenia, and hemorrhage. The fatal syndrome has been observed most frequently in German Shepherds. Outbreaks of TCP have occurred in many tropical and sub-tropical countries, but until now the disease has not been recognized in Thailand. The present report describes an epizootic of TCP among military working dogs at the War Dog Training Center, Pakchong, Korat Province, Thailand, 185 km northeast of Bangkok.

DESCRIPTION: The War Dog Training Center is a modern facility established by the Thai Armed Forces to breed, train, and issue working dogs to all military services. The population of dogs at the Center averaged about 175 during the period of the study. The Center also supports an additional 125-150 dogs at remote stations. The composition of the dog population at the Center is constantly changing as dogs are issued to and return from remote stations. Most working dogs return to the Center annually for retraining; dogs also return for treatment of serious disease problems, since veterinary care is not generally available except at the Center. Dogs at the Center are maintained in individual pens in several groups of screened or unscreened buildings which are separated by distances as great as several hundred meters. These groups of buildings are designated as the breeding area, the young adult area, the training area, the working dog area, and the hospital area. There is limited direct daily contact between groups, although dogs are periodically moved from one functional area to another as needs of training and utilization dictate. Contact is also possible in the hospital area which services dogs from all areas and from outside the Center. In addition, indirect contact is possible in common exercise, training and working areas. The common brown dog tick (*Rhipicephalus sanguineus*) has been collected from dogs and kennels in all areas. No other species of tick has been found.

TCP was first suspected in March, 1974, among a group of seven German Shepherds working at a military base in Lopburi, 133 km north of Bangkok. Within a two month period, three of these dogs died after episodes of epistaxis. The four surviving dogs, which were in poor condition, were transported to Pakchong and placed in the hospital for observation and treatment. It is believed that these dogs may have initiated the epizootic at Pakchong, although a retrospective analysis of clinical records suggests an increase in the prevalence of febrile episodes among dogs at Pakchong as early as January, 1974. No dogs were imported to the Center from outside Thailand during this time nor during the year prior to the recognition of TCP at Pakchong.

---

1 War Dog Training Center, Pakchong, Thailand.
2 College of Veterinary Medicine, University of Illinois at Champaign-Urbana.
3 Division of Veterinary Medicine, Walter Reed Army Institute of Research, Washington, D.C.

The progress of the epizootic of TCP has been followed by clinical, pathological, and laboratory studies. A clinical record is maintained on each dog, which contains an indication of clinical symptoms observed and treatments administered whenever a dog is brought to the hospital. Complete physical examinations are performed at regular intervals, including surveillance and treatment for heartworm and intestinal parasites. All dogs are regularly vaccinated for canine distemper, canine hepatitis, leptospirosis, and rabies.

A program to control the epizootic was developed based upon the experiences in Vietnam (1, 2) and upon laboratory studies conducted at the Walter Reed Army Institute of Research in subsequent years (3, 4). Serologic identification of infected dogs was the cornerstone of the control effort (5). The basic elements of the control program were as follows:

1. Identification of infected dogs by serologic testing, clinical signs, and laboratory studies.

2. Treatment of infected or suspect dogs with tetracycline hydrochloride orally 30 mg/lb/day for 14 days. Supportive therapy was utilized as appropriate in severe clinical cases.

3. Prevention of infection or re-infection by continuous daily oral administration of approximately 3 mg/lb/day of tetracycline hydrochloride. (A single 250 mg capsule was opened, and the powder lightly mixed with the pre-weighed food in each dog feeding pan).

4. Elimination of ticks by regular spraying of kennels and dipping of dogs with insecticide.

5. Isolation and treatment of newly introduced dogs.

The laboratory procedures utilized to identify TCP-infected dogs for treatment included serology, hematocrit, total and differential leukocyte counts, and serum protein electrophoresis. The serological method utilized was the indirect immunofluorescent test (5). At each bleeding 10 ml of venous blood was collected. Two to three milliliters were placed in tubes with EDTA for hematologic studies; the remainder was allowed to clot and serum collected for serology and serum protein determinations. For serologic screening, a 1:10 serum dilution was used, and results were reported as "positive" or "negative".

Blood samples were collected from all dogs at Pakchong (except puppies less than six months of age) at three month intervals. During the intervals between quarterly bleedings some additional dogs were bled, including new arrivals missed at the previous bleeding. On occasion, sera were collected at other military bases, but the bleeding of dogs at remote stations could not be comprehensive. Quarterly bleedings were performed on 4 June, 4 September and 18 December, 1974, and on 4 March 1975. In addition, all dogs at Pakchong were bled on 25 July 1974, just before initiation of the tetracycline treatment program. A total of 316 dogs were studied between June 1974 and March 1975. This included 287 German Shepherds, 10 Doberman Pinschers, 9 Labrador Retrievers and 10 Labrador-Shepherd cross-breeds.

Individual dogs were given a complete 14 day therapeutic course of tetracycline for any of the following reasons:

1. Suspicious clinical signs
   a) Unexplained fever
   b) Anemia (hematocrit less than 39)
   c) Leukopenia (WBC less than 6000)
   d) Bleeding (epistaxis, hematuria)
   e) High serum gamma globulin (> 2.5 gm%)
2. Serologically positive for TCP
3. Known or suspected exposure to TCP
4. Admitted to the Center with unknown prior history

In addition, on 26 July, all dogs at the Center older than six months of age (172 dogs) were placed on a 14 day therapeutic course of tetracycline. This was done with the knowledge that 49 per cent of the dogs had been serologically positive on 4 June, and with the suspicion than numerous additional dogs were incubating the disease (31 dogs developed fevers between 4 June and 25 July). At the completion of treatment, all dogs were continued on prophylactic levels of tetracycline daily until 9 September. After a 60 day interruption, tetracycline prophylaxis was again reinstituted on 9 November, and has been maintained until the present time. If at any time a previously treated dog converted serologically, or showed any of the suspicious clinical signs listed above, it was re-treated with tetracycline therapeutically.

PROGRESS: The existence of TCP in Thailand has been demonstrated conclusively for the first time. The evidence for the disease includes the observation of characteristic clinical, hematological and pathological signs among a group of Thai Military Working Dogs, and the confirmation of the disease serologically and by direct observation of morulae of *Ehrlichia canis* in tissue macrophages.

In April 1974, Krisda, a one year old, male German Shepherd, was transported from Pakchong to SEATO Laboratory for observation and diagnostic studies. This dog had a history of recurrent fever (102°F–104°F), inappetence, and progressive weight loss. On admission, the hematocrit was 35; leukocyte count 5100 per cmm; fecal specimen negative for ova and parasites. The serum was serologically positive for TCP. During a month-long period of observation, rectal temperature was consistently elevated (103°F–105.8°F), the appetite was poor, and the dog became progressively more debilitated, lost weight, and developed pressure ulcers of the skin of the abdomen and over bony prominences. The hematocrit fell to 17; leukocyte counts ranged from 6,600 to 30,000 per cmm. No chemotherapy was given. The dog was ultimately euthanized. At necropsy a moderate, multifocal bronchopneumonia was observed. Multifocal plasma cell infiltration was prominent in lymph nodes, in portal areas and around central veins of the liver, and interstitially in the kidneys. Lymphocytic and plasmacytic vasculitis, occasionally with minimal perivascular hemorrhage, was observed in the brain. Typical inclusions or "morulae" of *Ehrlichia canis* were observed in macrophages in giemsa-stained impression smears obtained from cut surfaces of lung at necropsy (4).

Between early April and 25 July, many dogs exhibited clinical or hematological abnormalities suggesting TCP. The number of dogs treated with tetracycline because of fever, anemia or epistaxis during these months is indicated in Table 1. During this period of rapid disease transmission, 47 dogs were treated with tetracycline on the basis of clinical signs without knowledge of their serological status (results of 4 June serology were not known until after 25 July). Only two of these dogs were later found to be serologically negative, and thus 97% of these treatments were appropriate. On the other hand, of the 86 dogs serologically positive on 4 June, only 45 (52%) were selected for treatment on the basis of clinical signs or hematology.

After the mass treatment of dogs on 25 July, a dramatic improvement in the general condition of dogs was noted. Thin dogs gained weight, work performance improved, breeding performance improved, and the number of dogs hospitalized was reduced. We continued to initiate tetracycline therapy in dogs developing fever, anemia or epistaxis, as indicated in Table 2. Thirty-eight dogs were treated for symptoms between 26 July 1974 and 31 March 1975. Nineteen of these courses of treatment (68%) were apparently inappropriate, since they were administered to dogs which were serologically negative both before and after treatment.

Compared to the experience in Vietnam, the severity of the clinical disease at Pakchong was mild. Between April 1974 and March 1975, only 19 dogs died at Pakchong. Six of these deaths were from causes other than TCP (accident, heartworm-2, drug toxicity, cystic calculi, heat exhaustion). Three dogs died of unknown causes. In ten dogs the history, clinical findings, laboratory studies, and gross necropsy findings suggested that TCP was the primary or contributing cause of death. Histopathologic confirmation of TCP was possible in only two cases. Only two of the TCP deaths occurred after 26 July. Both of these dogs were returned to Pakchong from remote sites in moribund condition.

Tetracycline therapy had been instituted in only one of the ten dogs which died of TCP. In this case, the dog was exhibiting epistaxis, the hematocrit was 14, and the dog died three days after initiation of therapy. In all other severe cases tetracycline therapy caused remission of symptoms. Nine dogs with epistaxis and 13 dogs with hematocrits of 15—25 have been successfully treated.

Severe symptoms of TCP were observed only in German Shepherds, although 7 of 9 Labradors, 5 of 10 Dobermans, and 1 of 10 Shepherd—Labrador cross—breeds were serologically positive. Two of the serologically positive Labradors had febrile episodes, and two had low hematocrits (28 and 31); they were otherwise asymptomatic. Three Dobermans were febrile, but exhibited no abnormal hematologic signs prior to treatment. The Shepherd—Labrador cross—breed dog was asymptomatic.

The results of serologic studies are presented in Table 3. The progress of the epizootic can be followed both before the institution of control measures on 26 July, and during the period of control.

Initially, on 4 June, 86 serologically positive dogs (49% of those at the Center at that time) were identified. By 25 July, six weeks later, 30 additional dogs had converted to positive (33% of the 90 susceptible), and 17 more dogs entering the Center from remote sites were identified as positive. After the dogs were placed on tetracycline therapy and prophylaxis, the rate at which dogs converted to positive markedly decreased. Between July and October, only 13 converted, and it is likely that the infections producing these conversions were mainly acquired before 25 July. Experimentally infected dogs do not develop detectable titers for 11—28 days (5). The three dogs converting after October spent a portion of their time away from Pakchong, and were not maintained on tetracycline prophylaxis.

All known serologically positive dogs have been treated with tetracycline, and many are now becoming serologically negative. The large number of dogs converting to negative between December 1974 and March 1975 largely reflect the treatment given in June and July. Of the 69 dogs remaining serologically positive, only 16 were bled at the Center in March 1975. The remaining 53 dogs were at remote stations, and although they were serologically positive at their last bleeding, current serology is not available on the majority of them.

Curiously, the prevalence of serologically positive dogs entering the Center from remote sites is decreasing, although tetracycline has not been administered prophylactically except at Pakchong. Apparently the elimination of infected dogs at Pakchong, and improved tick control at remote sites are having a beneficial effect.

Serum protein electrophoresis has been investigated as a diagnostic procedure to supplement serologic and hematologic findings. An elevation of serum globulins, particularly beta and gamma globulins, has been observed in experimentally infected dogs (Huxsoll, personal communication).

Some, but not all, infected dogs at Pakchong have exhibited increased gamma globulins. In most cases this was also reflected in a reduced A/G ratio. Comparison of the mean values for serologically positive and serologically negative dogs revealed no significant differences; however, individual dogs among the infected groups did have gamma globulin values which differed significantly from values observed in serologically negative dogs. Among serologically positive dogs prior to treatment, 30 of 85 (35%) had significantly elevated gamma globulins on 4 June, and 13 of 38 (34%) had elevations on 25 July. Abnormal gamma globulin levels returned to normal after drug treatment more rapidly than the dogs converted to negative serologically. By 25 July, only 6 of 24 treated dogs (25%) had elevated gamma globulins, and by 4 September only 4 of 80 (5%) still had abnormal values.

The ability to identify infected dogs serologically has been indispensible to the control effort. The treatment of dogs using clinical or hematologic criteria has proven unsatisfactory. Many dogs have been treated unnecessarily using symptomatic criteria, and, more importantly, many asymptomatic carriers would have been overlooked.

An important limitation of the serologic screening method is its inability to identify re-infections or failed treatments. Dogs are susceptible to re-infection even though they have serologically demonstrable antibody to *Ehrlichia canis* (3). After treatment, for as long as the dog remains serologically positive, only clinical and hematological observations serve to identify re-appearance of active infection. As of March 1975, 69 treated dogs remained serologically positive. It is entirely possible that some of these dogs are harboring the organism. Hopefully, control of ticks and the continuation of prophylactic tetracycline will prevent transmission of the organism until such time as any carriers among this group can be identified.

Efforts to control the epizootic in Thai military working dogs are continuing. Tetracycline is still being administered prophylactically, and dogs which exhibit suspicious clinical or hematologic signs, or which convert serologically, are being treated with tetracycline. While many dogs have become serologically negative, the efficacy of the combined therapeutic and prophylactic administration of tetracycline cannot be evaluated until the infective status of the remaining 69 serologically positive dogs has been resolved. Efforts to control ticks are being maintained.

SUMMARY: An epizootic of Tropical Canine Pancytopenia (TCP) has been studied in a population of 316 Thai Military Working Dogs. To date, 161 cases have been identified serologically, of which 54 were clinically or hematologically apparent. The prevalence of severe clinical symptomatology was low. Epistaxis was observed in only 9 dogs (2.8%), and only 10 dogs died (3.2%).

A control program including tick control, serologic identification and treatment of carriers, and tetracycline prophylaxis has been instituted. Administration of tetracycline 30 mg/kg/day for 14 days has produced clinical remission of symptoms in all but one of the severely ill dogs. Serologic remission has been observed in all but 69 dogs to date. Early intervention with tetracycline is probably largely responsible for the mildness of the epizootic, but the possibility that the organism was less virulent, or the dogs more resistant than in the Vietnam epizootic, cannot be ruled out.

Clinical, hematological, and serological surveillance is continuing.

REFERENCES:

1. Walker, J.S., Rundquist, J.D., Taylor, R., Wilson, B.L., Andrews, M.R., Barck, J., Hogge, A.L., Huxsoll, D.L., Hildebrandt, P.K., and Nims, R.M.: Clinical and Clinicopathologic Findings in Tropical Canine Pancytopenia. J. Am Vet. Med. Assoc. 157:43-55, 1970.

2. Nims, R.M., Ferguson, J.A., Walker, J.L., Hildebrandt, P.K., Huxsoll, D.L., Reardon, M.J., Varley, J.E., Kolaja, G.J., Watson, W.T., Shroyer, E.L., Elwell, P.A., and Vacura, G.W.: Epizootology of Tropical Canine Pancytopenia in Southeast Asia, J. Am. Vet. Med. Assoc 158:53-68, 1971.

3. Amyx, H.L., Huxsoll, D.L., Zeiler, D.C., and Hildebrandt, P.K.: Therapeutic and Prophylactic Value of Tetracycline in Dogs Infected with the Agent of Tropical Canine Pancytopenia. J. Am. Vet Med. Assoc. 156:1428-1432, 1971.

4. Huxsoll, D.L., Amyx, H.L., Hemelt, I.E., Hildebrandt, P.K., Nims, R.M., and Gochenour, W.S.: Laboratory Studies of Tropical Canine Pancytopenia. Exper. Parasit. 31:53-59, 1972.

5. Ristic, M., Huxsoll, D.L., Weisiger, R.M., Hildebrandt, P.K., and Nyindo, M.B.A.: Serological Diagnosis of Tropical Canine Pancytopenia by Indirect Immunofluorescence. Infection and Immunity 6:226-231, 1972.

Table 1. Major Clinical Signs of TCP Observed at Pakchong, During June – July 1974

Clinical Signs	Number of Dogs Exhibiting Signs	
	Serologically Positive	Serologically Negative
Fever (a)	31	2
Anemia (b)	8	0
Epistaxis (c)	6	0
Total	45	2

(a) Rectal temperature above 103°F. Depression and inappetence were frequently associated with the fever, but diarrhea or vomiting were seldom observed. None of these dogs exhibited anemia, leukopenia or epistaxis.

(b) Hematocrit less than 39. None of these dogs exhibited epistaxis; some were leukopenic, and most were febrile intermittently.

(c) These dogs were also anemic, leukopenic and febrile.

Table 2. Major Clinical Signs of TCP Observed at Pakchong Between August 1974 and March 1975

Clinical Signs (a)	Number of Dogs Exhibiting Signs	
	Serologically Positive	Serologically Negative
Fever	2	0
Anemia	7	19
Epistaxis	0	0
Total	9	19

(a) See footnote for Table 1.

Table 3. Results of Serologic Studies at Pakchong – June 1974 to March 1975

Date	Cumulative Number of Dogs Studied (a)	Serologically Positive Dogs			Serologically Negative Dogs			Cumulative Known Positive Dogs	Cumulative Known Negative Dogs	Percent Positive
		Converted To Positive	Added To Study	Total New Positive	Converted To Negative	Added To Study	Total New Negative			
4 Jun 74	176	—	86	86	—	90	90	86	90	49 %
25 Jul 74	200	30	17	47	8	13	21	120	80	60 %
Sep-Oct 74	242	13	12	25	6	33	39	136	106	56 &
Dec 74–Jan75	294	3	2	5	45	53	98	93	201	32 %
Mar 75	301	0	1	1	23	9	32	69	232	23 %

(a) Dead Dogs Excluded