First report on *Anaplasma platys* infection in a dog in the Philippines

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Abstract:

Anaplasma platys, previously known as *Ehrlichia platys*, which causes infectious canine cyclic thrombocytopenia, has not been extensively studied in Southeast Asia. Recent reports in the region have been limited to ticks and dogs in Thailand, and to ticks in the Philippines. In this report, DNA fragments of *A. platys* were molecularly detected in a dog in the Philippines. A pitbull puppy exhibited pancytopenia (WBC: 3.5 x10^3/µL, PCV: 11.5%, RBC: 1.2 x10^6/µL, HGB: 3.7 g/dL, platelet count: 24 x10^3/µL), hepatitis, elevated alanine amino transferase (127 u/L), and splenomegaly. Inclusion bodies in the platelets were also seen in the blood smears of this case. *A. platys* sequences obtained in the present study revealed 100% homology with *A. platys* previously detected in a *Rhipicephalus sanguineus* tick in the country. This study documents the first reported case of *A. platys* infection in dogs in the Philippines, and adds knowledge to the current vector-borne disease epidemiology in Southeast Asia.

Case History

Anaplasma platys is a Gram-negative intracellular bacteria belonging to the family of Anaplasmataceae (Dumler et al., 2001), and the etiologic agent of infectious canine cyclic thrombocytopenia (Harvey et al., 1978).

Apart from *Ehrlichia canis*, it is considered to be one of the pathogens of canine ehrlichiosis (Inokuma et al., 2003; Abarca et al., 2007). Co-infection of both species in one dog is also possible, which can produce more severe clinical signs (Gaunt et al., 2010). Although *A. platys* in-fection in dogs has been suspected in the country, it has not been reported yet in the Philippines, except in a *Rhipicephalus sanguineus* tick (JN121381; JQ894779). Nearby countries including Thailand (Suksawat et al., 2001), China (Wen et al., 2003), Korea (Kim et al., 2006), Japan (Inokuma et al., 2003) and Taiwan (Chang et al., 1996) had already reported the detection and characterization of *A. platys* in the respective countries. In this study, a hemi-nested polymerase chain reaction (PCR) based on 16S rRNA was used to detect *A. platys* in a dog in Cebu, Philippines. The clinical details of the case are herein reported.

Clinical Presentation

A 7-week-old male pitbull puppy suspected to have *A. platys* and/or *E. canis* infections was examined in 2011. The case was presented at GPY Veterinare Animale Veterinary Clinic, Cebu City, Philippines. EDTA-anticoagulated peripheral blood was collected from the dog for hematological and serological examinations. Clinical signs were recorded at the time of blood collection, and two thin blood smears stained with Giemsa solution were examined.

Table 1. Results of hematological and serological analyses of the case. (*) Duncan and Prasse, 1986; (**)Boyd, 1984.

Hematologic Parameters	Reference Values	Observation Day			
		1	20	34	41
WBC count (x10^3/µL)	6-17*	5.5	3.5	17.6	14.7
PCV (%)	37-55*	33	11.5	23.3	27.6
Hemoglobin (Hgb) (g/L)	120-780*	110	3.7	62	75
RBC count (x10^6/µL)	5.5-8.5*	3.7	1.2	3.79	4.56
Platelet count (x10^3/µL)	200-900*	200	24	79	227
Differential Count					
Basophils (%)	0-2*	--	--	--	--
Eosinophils (%)	2-10*	--	--	--	--
Monocytes (%)	3-10*	1	4	4.9	3.7
Lymphocytes (%)	12-30*	35	17	17.6	16.9
Segmenters (%)	60-70*	64	79	77.5	79.4
Serum Biochemistry					
Creatinine Kinase (u/L)	0.5-1.6**	--	0.67	0.68	--
ALT (u/L)	8.2-57.0**	--	127	8.5	--

The puppy completed the vaccination scheme of Quantum (Schering Plough, USA). Tick infestation was noted on each scheduled visit.

Diagnostic Testing

At day 1, which was 1 week after the 1st vaccination shot, the dog presented signs of fever (40^{o}C) and lethargy. Complete blood count examination revealed mild leukopenia and anemia (Table 1). The puppy was treated with a non-steroidal anti-inflammatory drug (NSAID) (tolfenamic acid; Vetoquinol, France) and a supplement (Biodyl; Merial, USA). In the morning of day 3 , the puppy was returned to the clinic due to fever (40^{o}C), and was treated with the same NSAID. In the afternoon of the same day, the temperature of the puppy was normal (39.2^{o}C), and was prescribed with ascorbic acid, amoxicillin-clavulanic acid and inosiplex for home treatment. On day 8, the puppy was reportedly recovering as the appetite gradually returned (according to the owner).

On day 20, the puppy was returned to the clinic showing signs of fever (40.4^{o}C), icterus and enlarged abdomen. Radiographic findings showed hepatic and splenic enlargement. Complete blood count examination revealed pancytopenia and elevated alanine transferase or ALT (Table 1, Figure 2). At this time, the puppy was suspected of *E. canis* based on history and complete blood count (CBC) results. It was treated with the same NSAID and a long-acting oxytetracycline (Terramycin LA; Pfizer, USA) at 10 mg/kg dose, and prescribed with metronidazole, liver supplement (Jetepar; Rottapharm B.V. Amsterdam, Swiss Branch, Switzerland), furosemide, lactulose, and doxycycline for 14 days.

On day 34, the puppy was reportedly having an irregular appetite, and was observed to be pale, although the icteric condition had lessened. The abdomen was still enlarged. The skin was also seborrheic, with alopecic areas scattered all throughout the body and a wound at the right rump area (around 5 cm in diameter). CBC results showed improvement from previous condition, but values were still not normal (anemia, thrombocytopenia, mild leukocytosis). The ALT value returned to normal. Giemsa-stained blood smears demonstrated inclusion bodies in the platelets, and was presumed to be caused by *A. platys* (Figure 1). DNA from the whole blood was extracted and stored as previously described (Ybañez et al., 2012). Primer sets fD1/Rp2 and fD1/EHR16SR (Parola et al., 2000) based on the 16S rRNA gene were utilized for the 1st and 2nd round PCR, respectively, using similar methods as previously described (Ybañez et al., 2012). The negative and positive controls used, were double distilled water (DDW) and an *Anaplasma* sp. closely related to *A. phagocytophilum* from sika deer (Ybañez et al., 2012) respectively. Amplification products were visualized, purified and sequenced using previously described methods (Ybañez et al., 2012). Compar-

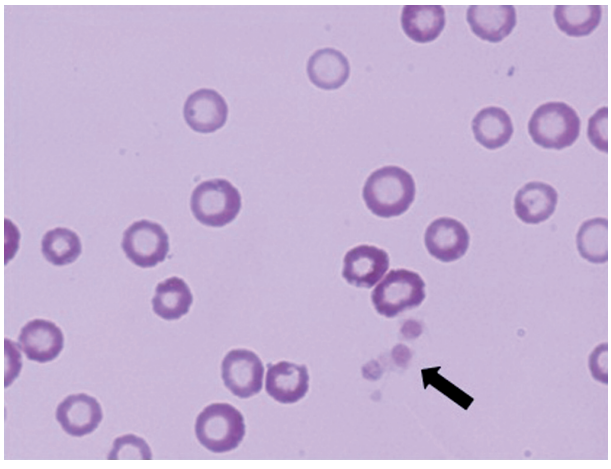

Figure 1. Inclusion bodies (arrows) in platelets were observed in the peripheral blood smear.

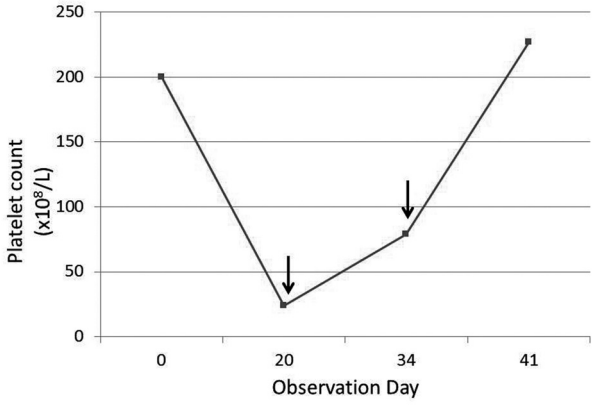

Figure 2. Changes of platelet counts of the case infected with *A. platys*. Recovery from thrombocytopenia was noted after treatment with doxycycline at days 20 and 34 (arrows).

ison of sequences was performed as previously described (Ybañez et al., 2012). A positive band was seen after PCR and visualization. Sequencing the positive amplicons revealed a partial 782 bp-nucleotide, which was found 100% identical to *A. platys*, detected from an *R. sanguineus* tick in the Philippines (JN121378). The sequence was registered at Genbank with the accession number JQ894779. The dog was treated with long-acting oxytetracycline (Terramycin LA; Pfizer, USA) at 20 mg/kg dose, fipronil (Frontline Spot-on®; Merial, USA) and dexamethasone (Cortamethasone; Vetoquinol, France), and was prescribed with azathioprine for 1 week, prednisone for 2 weeks, a liver supplement Livolin Forte (Megalifesciences, Thailand) for 1 month, and doxycycline for 6 weeks.

On day 41, the puppy gained weight, and was more active. Inappetence was not observed anymore by the owner. The skin was less seborrheic, and the wound was recovering. CBC results improved as the platelet and WBC count returned to normal, and the PCV increased. On day 76, the owner was called for a follow-up check-up for the dog, but the owner refused to bring the dog. According to the owner, the appetite was normal, the enlarged abdomen had disappeared, and the skin had fully recovered.

Assessment

The puppy was most likely infected by the *R. sanguineus* ticks coming from the dam and other puppies, which were housed in the same area. *R. sanguineus* is the suspected vector of *A. platys* (Inokuma et al., 2000). The dam and other puppies in the litter reportedly did not show any clinical signs, despite tick infestation. This may imply that the dog was already immunocompromised, and that the administered vaccine may have triggered the clinical expression of the disease.

The platelet counts (Table 1) observed on the days 20 (24 x103/µL) and 34 (79 x103/µL) were indicative of a cyclic thrombocytopenia, which is one of the characteristics of *A. platys* infection in dogs. Anemia, which is also associated with *A. platys* infection (Baker et al., 1988), was also seen in all the observation days. These hematological observations support the diagnosis of *A. platys* infection in the present report.

Although inclusion bodies in the platelets were seen in the present study, blood smear examination is not a reliable method due to the cyclic parasitemia behavior of *A. platys*. Moreover, the organism is usually absent or present in very low numbers in the blood (Bradfield et al., 1996; Harrus et al., 1997). Therefore, PCR can be more accurate than cytology (Otranto et al., 2010). The detection of *A. platys* in the dogs in the Philippines suggests that the pathogen maybe endemic in the area. While *A. platys* infection test kits are not yet widely available in the Philippines, local veterinarians should attempt to examine blood smears, most ideally from buffy coats, whenever possible (Arraga-Alvarado et al., 2003). Canine anaplasmosis should also be made part of the differential diagnoses especially in the cases with histories of tick infestation and cyclic thrombo-

cytopenia. Since concurrent infection of *E. canis* and *A. platys* is possible, it should be considered especially when more severe signs are observed. The duration of treatment, whether 3 weeks or longer, should be evaluated depending on the severity of signs observed, or the presence of inclusion bodies in the blood smears which may suggest the degree of infection.

The present study documents the first reported clinical case of *A. platys* infection in a dog in the Philippines. Further studies are needed to determine the epidemiologic distribution of *A. platys* in the Philippines, as well as in Southeast Asia.

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References

1. Abarca, K., Lopez, J., Perret, C., Guerrero, J., Godoy, P., Veloz, A., Valiente-Echeverria, U., Leon, C., Gutjahr, Azocar, T. (2007) *Anaplasma platys* in dogs. Chile Emerg Infect Dis. 13:1392-1395.
2. Arraga-Alvarado, C., Palmar, M., Parra, O., Salas, P. (2003) *Ehrlichia platys* (*Anaplasma platys*) in dogs from Maracaibo, Venezuela: an ultrastructural study of experimental and natural infections. Vet Pathol. 40: 149-156.
3. Baker, D.C., Gaunt, S.D., Babin, S.S. (1988) Anemia of inflammation in dogs infected with *Ehrlichia platys*. Am J Vet Res. 49: 1014-1016.
4. Boyd, J.W. (1984) The interpretation of serum biochemistry test results in domestic animals. In: Veterinary Clinical Pathology, Veterinary Practice Publishing Co. 13(2).
5. Bradfield, J.F., Vore, S.J., Pryor Jr., W.H. (1996) *Ehrlichia platys* infection in dogs. Lab Anim Sci. 46: 565-568.
6. Chang, A.C., Chang, W.L., Lin, C.T., Pan, M.J., Lee, S.C. (1996) Canine infectious cyclic thrombocytopenia found in Taiwan. J Vet Med Sci. 58: 473-476.
7. Dumler, J.S., Barbet, A.F., Bekker, C.P.J., Dasch, G.A., Palmer, G.H., Ray, S.C., Rikihisa, Rurangirwa, F.R. (2001) Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of Ehrlichia with Anaplasma, Cowdria with Ehrlichia and Ehrlichia with Neorickettsia, descriptions of six new species combinations and designation of Ehrlichia equi and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. Int J Syst Evol Microbiol. 51: 2145-2165.
8. Duncan, J.R., Prasse, K.W. (1986) Veterinary Laboratory Medicine-Clinical Pathology. (2nd ed.) Iowa State University Press. Ames. USA.
9. Gaunt, S., Beall, M., Stillman, B., Lorentzen, L., Diniz, P., Chandrashekar, R., Breitschwerdt, E. (2010) Experimental infection and co-infection of dogs with *Anaplasma platys* and *Ehrlichia canis*: hematologic, serologic and molecular findings. Parasit Vectors. 3: 10.1186/1756-3305-3-33.
10. Harrus, S., Aroch, I., Lavy, E., Bark, H. (1997) Clinical manifestations of infectious canine cyclic thrombocytopenia. Vet Rec. 141: 247-250.
11. Harvey, J.W., Simpson, C.F., Gaskin, J.M. (1978) Cyclic thrombocytopenia induced by a Rickettsia-like agent in dogs. J Infect Dis. 137: 182-188.
12. Inokuma, H., Beppu, T., Okuda, M., Shimada, Y., Sakata, Y. (2003) Epidemiological survey of *Anaplasma platys* and *Ehrlichia canis* using ticks collected from Dogs in Japan. Vet Parasitol. 115: 343-348.
13. Inokuma, H., Raoult, D., Brouqui, P. (2000) Detection of *Ehrlichia platys* DNA in brown dog ticks (*Rhipicephalus sanguineus*) in Okinawa Island, Japan. J Clin Microbiol. 38: 4218-4221.
14. Kim, C.M., Yi, Y.H., Yu, D.H., Lee, M.J., Cho, M.R., Desai, A.R., Shringi, T.A., Klein, H.C., Kim, J.W., Song, L.J., Baek, S.T., Chong, M.L., O`Guinn, J.S., Lee, I.Y., Lee, J.H., Park, J., Foley, Chae, J.S. (2006) Tick-borne Rickettsial pathogens in ticks and small mammals in Korea. Appl Environ Microb. 72: 5766-

5776.

15. Otranto, D., Testini, G., Dantas-Torres, F., Latrofa, M.S., de Caprariis, D., Lia, R.P., Mencke, D., Stanneck, G., Capelli, Breitschwerdt, E.B. (2010) Diagnosis of canine vector-borne diseases in young dogs: a longitudinal study. J Clin Microbiol. 48: 3316-3324.
16. Parola, P., Roux, V., Camicas, J.L., Baradji, I., Brouqui, P., Raoult, D. (2000) Detection of ehrlichiae in African ticks by polymerase chain reaction. Trans R Soc Trop Med Hyg. 94: 707-708.
17. Suksawat, J., Xuejie, Y., Hancock, S.I., Hegarty, B.C., Nilkumhang, P., Breitschwerdt, E.B. (2001) Serologic and molecular evidence of coinfection with multiple vector-borne pathogens in dogs from Thailand. J Vet Int Med. 15: 453-462.
18. Wen, B., Cao, W., Pan, H. (2003) Ehrlichiae and ehrlichial diseases in china. Ann N Y Acad Sci. 990: 45-53.
19. Ybañez, A.P., Matsumoto, K., Kishimoto, T., Inokuma, H. (2012) Molecular analyses of a potentially novel Anaplasma species closely related to Anaplasma phagocytophilum detected in sika deer (*Cervus nippon yesoensis*) in Japan. Vet Microbiol. 157: 232-236.

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اولین گزارش از آلودگی سگ‌ها به آناپلاسما پلیتیس در فیلیپین

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چکیده

Anaplasms platys با نام قبلی *Ehrlichia platys* که عامل ایجاد ترمبوسیتوپنی دوره‌ای عفونی در سگ است تاکنون بطور گسترده در جنوب شرقی آسیا مورد مطالعه قرار نگرفته است. تازه ترین گزارش های علمی برای این منطقه محدود به یک گزارش آلودگی کنه از تایلند و همچنین فیلیپین می باشد. در گزارش فعلی قطعات DNA مربوط به *Anaplasms platys* در سگ های فیلیپین شناسایی گردید. همچنین این توله سگ مبتلا به پان سیتوپنی (٪۱۱/۵, RBC: ۱/۲ x۱۰^{۶} µL, HGB: ۳/۷g/dL, platelet count: ۲۴ x۱۰^{۳} µL) هیاتیت، افزایش میزان آنزیم الانین ترانسفراز (۱۲۷ U/L) و بزرگ شدن طحال بود. بعلاوه گنجیدگی های داخل سلولی در پلاکت‌ها نشان داده شد. سکانس قطعات DNA تشابه ۱۰۰ درصدی را با کنه *Rhipicephalus sanguineus* بیشتر گزارش شده از فیلیپین نشان داد. گزارش فعلی بعنوان اولین مورد از آلودگی *Anaplasms platys* در سگ های فیلیپینی می باشد که بایستی به منابع مرتبط با اپیدمیولوژی vector-borne منطقه جنوب شرقی آسیا اضافه شود.

واژه‌های کلیدی: *Anaplasms platys*، سگ، اپیدمیولوژی

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