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Detection of Antibodies Reacting with *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis* from Cats, Horses and Cattle in Korea

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Abstract : Antibodies to *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis* were detected by the immunofluorescent antibody (IFA) test in sera collected from cats, thoroughbred horses and Holstein cattle in Gwangju, Jeonju and Jeju Island of Korea. Two hundred fifty four sera (33 feral and pet cats, 92 grazing horses and 129 grazing cattle) were obtained from Republic of Korea. Antibodies to *A. phagocytophilum* (titer ≥ 80) were detected in 6 of the 33 feral and pet cats (18.2%), and 1 seropositive cat (3.0%) also had antibodies to *E. chaffeensis*. Only 1 of 129 (0.8%) cattle and 2 of 92 (2.2%) horses had antibodies to *A. phagocytophilum*. Antibodies to *E. chaffeensis* were not detected in either of these animals. This is the first report of serological evidence of *A. phagocytophilum* and *E. chaffeensis* from cats, cattle and horses in Korea. These rickettsial agents could have an important impact on human health or impact animal health with economic losses among industrial grazing animals in Korea.

Key words **:** *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis,* immunofluorescent antibody test, cat, horse, cattle.

Introduction

Anaplasma phagocytophilum and *Ehrlichia chaffeensis* are tick transmitted gram negative obligatory intracellular microorganisms, known as important agents of veterinary diseases (27). *A. phagocytophilum* is transmitted by *Ixodes* species ticks (5,23), whereas *E. chaffeensis* is transmitted primarily by the lone star tick (*Amblyomma americanum*) in the United States (1). Ehrlichial organisms infect blood cells of domestic and wild animals and may lead to a febrile systemic illness often accompanied by hematological abnormalities, lymphadenopathy and elevation of hepatic enzyme activities (4,27). Infection with *A. phagocytophilum* and *E. chaffeensis* has been reported in various animal species. *A. phagocytophilum* is the etiologic agent of tick-borne fever (TBF) of sheep, goats, and cattle, and is believed to occur predominantly in Europe and perhaps in Africa (14). *A. phagocytophilum* also causes disease in cats, dogs, horses and humans whereas *E. chaffeensis* is the cause of human monocytic ehrlichiosis, but may cause severe disease in naturally infected dogs, and natural subclinical infections in deer (6,20). The diagnosis of anaplasmosis and ehrlichiosis is usually based on clinical signs and examination for inclusions (morulae) in the leukocytes. Other diagnostic methods such as culture isolation, serologic tests, and molecular techniques are conducted to detect infection, but the indirect immunofluorescence antibody test (IFA) is commonly and widely used (13,28).

In Korea, Heo *et al.* (15) and Park *et al.* (24) reported the identification of the antibodies against *E. chaffeensis* and *A. phagocytophilum* among serum samples of patients with febrile illnesses of unknown etiology in Korea using the indirect fluorescent antibody (IFA) test. Also, Kim *et al.* (17) reported molecular evidence of *E. chaffeensis* and *A. phagocytophilum* using genus-specific TaqMan PCR and using a species-specific PCR on ticks collected from animals and grass vegetation in Korea (17). Recently, *E. chaffeensis rrs* (16S rRNA gene) fragments were identified from *Haemaphysalis longicornis* ticks in Korea by PCR (19), and *A. phagocytophilum* partial *gro*EL sequences were identified in the spleens of wild rodents (25). The striped field mouse probably plays a role as a reservoir for latent infections of

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Table 1. Percentage of *Anaplasma phagocytophilum* IFA-positive sera among in three animal species (cats, thoroughbred horses, and Holstein cattle) obtained from different regions of Republic of Korea

Animals	Provinces	Numbers of serum samples	Numbers of IFA positive	Seropositive rates (%)
Cat*	Gwangju/Jeonju	33	6	18.2
Thoroughbred horse	Jeju Island	92	2	2.2
Holstein cattle	Jeju Island	129	1	0.8
Total		254	9	3.5

*one cat reacted both *E. chaffeensis* and *A. phagocytophilum* (See Table 2)

various *Ehrlichia* or *Anaplasma* species in Korea (8).

In this study, we examined the prevalence of antibodies reactive with *A. phagocytophilum* and *E. chaffeensis* in serum samples from cats, horses and cattle in Republic of Korea using the IFA test. The results of this study suggest that the etiologic agents of the ehrlichioses are distributed in various animal species in Republic of Korea.

Materials and Methods

Serum samples

Two hundred fifty four whole blood samples were collected in tubes without anticoagulant from 33 cats, 92 horses and 129 cattle. Twenty cat sera were collected from feral cats in Gwangju Province and 13 sera were obtained from pet cats that had been at the Veterinary Medical Teaching Hospital in Chonbuk National University from Jeonju City during 2002 to 2004. Sera from thoroughbred horses and Holstein cattle were obtained from meadows on Jeju Island. Blood samples were centrifuged and sera were collected and stored at -80°C until analyzed by IFA test.

In vitro culture

A. phagocytophilum was propagated in HL-60 cells (human promyelocytic leukemia cell line) in RPMI 1640 medium (GIBCO-BRL) supplemented with 1% fetal bovine serum (FBS) and 2 mM L-glutamine. *E. chaffeensis* was cultivated in DH82 cells (dog macrophage cell line) in MEM medium (Gibco-BRL) supplemented with 1% FBS and 2 mM L-glutamine. These agents were cultured in a humidified 37°C incubator.

IFA test

The indirect immunofluorescence antibody test was performed as described previously (30). Antigen slides were made with highly infected HL-60 cells and DH82 cells fixed in cold-acetone and stored at -80°C until used. For the IFA, animal sera were diluted 1 : 80 in PBS (pH 7.4) with 0.5% nonfat skim milk (PBSM) and added to antigen slides for 1 hour at room temperature (RT). After washing with PBS, fluorescein isothiocyanate (FITC)-labeled goat anti-horse immunoglobulin G (IgG), IgA, and IgM (Kirkegaard & Perry laboratories; KPL) for horse sera, FITC-labeled goat anti-bovine IgG, IgA, and IgM (KPL) for cattle, and FITC-labeled goat anti-cat IgG, IgA, and IgM (KPL) for cat sera were added as a secondary antibody at a dilution of 1 : 50 and incubated at RT for 1 hour. After that, antigen slides were counterstained with Evans blue (0.005%) and mounted for examination by fluorescence microscopy. The serum antibody titer was determined in twofold serial dilutions of animal sera, starting at a dilution of 1 : 80 in 0.5% PBSM.

Results

A total of 254 animal sera from feral cats, thoroughbred horses and Holstein cattle in Republic of Korea were tested for *A. phagocytophilum* and *E. chaffeensis* by IFA test (Table 1). In total, 9 sera reacted with *A. phagocytophilum* (3.5%) and 1 reacted with *E. chaffeensis* (0.4%). Five sera of 20 feral cats and 1 of 13 pet cat sera were reactive with *A. phagocytophilum*. One serum from a feral cat reacted with *E. chaffeensis*. Two of the 57 thoroughbred horses and 1 of the 129 Holstein cattle were seropositive for *A. phagocytophilum*; however, all were seronegative for *E. chaffeensis*. Table 2 shows the IFA titer of sera reactive with *A. phagocytophilum* and *E. chaffeensis*. The ranges of IFA titer were 160 to 640. Only 1 serum was considered reactive with both *A. phagocytophilum* and *E. chaffeensis* which was from a feral cat and the titer was 640 for both organisms.

Discussion

Infections caused by members of the *Anaplasmataceae* family are recognized in animals and humans (3,28). The aim of this study was to examine the existence of ehrlichial infection of domestic and companion animals in Republic of

Table 2. *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis* IFA titers for seropositive cats, horses, and cattle from Republic of Korea

Animals	Location	IFA titer (number of positive sera)	
		E. chaffensis	*A. phagocytophilum*
Cat	Gwangju (feral cat)	1 : 640 (1)	1 : 640 (3)
	Gwangju (feral cat)	< 1 : 80 (0)	1 : 160 (2)
	Jeonju (Pet cat)	< 1 : 80 (0)	1 : 160 (1)
Horse	Jeju Island	< 1 : 80 (0)	1 : 320 (1)
	Jeju Island	< 1 : 80 (0)	1 : 160 (1)
Cattle	Jeju Island	< 1 : 80 (0)	1 : 160 (1)

Korea. We tested for the presence *A. phagocytophilum* and *E. chaffeensis* antibodies in 3 animal species: cats, thoroughbred horses, and Holstein cattle. None had unique signs such as fever, rigors, muscle tenderness, vomiting, as typically observed with ehrlichiosis and granulocytic anaplasmosis. Although we are unaware of hematological changes of the animals tested in our survey, these results suggest that some animals in the Republic of Korea were exposed to the *Anaplasmataceae*, *A. phagocytophilum* and *E. chaffeensis,* or serologically similar organisms. In our prior studies, rickettsial pathogens were detected in ticks from 9 provinces of Republic of Korea (17). We also detected several rickettsial pathogens in wild small mammals collected from Army training sites (9,18). Recently, Yu *et al*. (33) reported about *E. chaffeensis* infections in Korean dogs. Based on these results, it is likely that these rickettsial pathogens are enzootic in Korea.

In this study, we found that 1 serum from a cat had antibodies to both *A. phagocytophilum* and *E. chaffeensis*. It is possible that this represents prior infection with both pathogens transmitted by ticks, although cross-reactivity is well recognized between these species and cannot been excluded.

The IFA test is rapid and convenient, and is the most widely used method for screening of *A. phagocytophilum* and *E. chaffeensis* exposures, although there are both advantages and disadvantages. It is the most sensitive test to detect ehrlichiosis or granulocytic anaplasmosis, but could give inconsistent results because of pathogen antigenic diversity and various technical factors that differ among laboratories (2). Also serological cross-reactivity between *Anaplasmataceae* genera and species can pose a serious problem for interpretation of IFA results (22) leading to potential misdiagnoses (7,26). It has been reported that serum from patients with human granulocytic anaplasmosis (HGA, *A. phagocytophilum* infection) cross-react with *E.* chaffeensis (11,32); *E. canis* can also cross-react with *A. phagocytophilum* (31). Other reports demonstrate cross-reactivity between *E. canis* and *A. phagocytophilum*; however, until recently this has been considered rare and generally only a problem with hyperimmunized sera (12).

Western immunoblot is generally thought to be more specific than IFA, since it provides information about specific reactive antigens (29). Western immunoblot analysis with purified whole organisms or recombinant protein antigens has been used to differentiate *A. phagocytophilum* from *E. chaffeensis*. It is reported that 24 of 30 (80%) sera reactive with *A. phagocytophilum* react with a 44 kDa antigen, presumably major surface protein 2 (Msp2), and 25 of 39 sera reactive with *E. chaffeensis* reacted with a 28 kDa antigen (24). Previous studies showed that anti-*E. chaffeensis* sera do not react with recombinant *A. phagocytophilum* p44, but sera from patients with HGA have antibodies that specifically react to this protein (34). Despite this, there has been little corroboration or evidence to support Western blot diagnostic utility.

PCR is another important method to detect ehrlichiosis and granulocytic anaplasmosis. This assay is performed by amplification of bacterial DNA in the blood of suspected hosts or vectors (6,21). Recently *E. chaffeensis, A. phagocytophilum* and *A. bovis* were detected in *H. longicornis* and *Ixodes persulcatus* ticks from Korea (17). In other molecular comparison studies, *gro*EL of *Anaplasma* species and an *rrs* (16S rRNA gene) fragment of *E. chaffeensis* were detected in mammalian and human samples from Korea (19,25).

and granulocytic anaplasmosis have been studied and identified in many countries including in North America, Europe, Asia and others. *Ehrlichia* and *Anaplasma* spp. are reported in China and Japan (10,16). In Republic of Korea, a serologic survey for *A. phagocytophilum* and *E. chaffeensis* in human patients was performed (15,24). But, infection of horses, cattle and cats with *A. phagocytophilum* and *E. chaffeensis* in Korea has not been reported.

We report the first serologic evidence of *A. phagocytophilum* and *E. chaffeensis* infections in animals in Korea. More study and molecular surveys are needed to confirm these initial data and to isolate *Ehrlichia* and *Anaplasma* spp. from animals in Korea and Asia. Moreover, it is necessary to identify whether novel *Ehrlichia* and *Anaplasma* species could account for some infected animals or humans in Korea. These rickettsial agents could have an important impact on human health or impact animal health with economic losses among industrial grazing animals in Korea.

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References

1. Anderson BE, Sims KG, Olson JG, Chiles JE, Piesman JE, Happ CM, Maupin GO, Johnson BJB. *Amblyomma americanum*: a potential vector of human ehrlichiosis. Am J Trop Med Hyg 1993; 49: 239-244.
2. Asanovich KM, Bakken JS, Madigan JE, Aguero-Rosenfeld M, Wormser GP, Dumler JS. Antigenic diversity of granulocytic *Ehrlichia* isolates from humans in Wisconsin and New York and a horse in California. J Infect Dis 1997; 176: 1029-1034.
3. Bakken JS, Dumler JS, Chen SM, Eckman MR, Van Etta LL, Walker DH, Human granulocytic ehrlichiosis in the upper Midsest United States. A new species emerging? JAMA 1994; 272: 212-218.
4. Bakken JS, Krueth J, Wilson-Nordskog C, Tilden RL, Asanovich K, Dumler JS. Clinical and laboratory characteristics of human granulocytic ehrlichiosis. JAMA 1996; 275:199-205.
5. Baumgarten BU, Rollinghoff M, Bogdan C. Prevalence of *Borrelia burgdorferi* and granulocytic and monocytic *Ehrlichiae* in *Ixodes ricinus* ticks from southern Germany. J Clin Microbiol 1999; 37: 3448-3451.
6. Breitschwerdt EB, Hegarty BC, Hancock SI. Sequential evaluation of dogs naturally infected with *Ehrlichia canis, Ehrlichia chaffeensis, Ehrlichia equi, Ehrlichia ewingii*, or

Bartonella vinsonii. J Clin Micribiol 1998; 36: 2645-2651.
7. Brouqui P, Dumler JS. Serologic evidence of human monocytic and granulocytic ehrlichiosis in Israel. Emerg Infect Dis 2000; 6: 314-315.
8. Chae JS, Kim CM, Kim EH, Hur EJ, Klein TA, Kang TK, Lee HC, Song JW. Molecular epidemiological study for tick-borne disease (*Ehrlichia* and *Anaplasma* spp.) surveillance from wild rodents and mustelids at selected U.S. military training sites/installations in Korea. Ann N Y Acad Sci 2003; 990: 118-125.
9. Chae JS, Yu do H, Shringi S, Klein TA, Kim HC, Chong ST, Lee IY, Foley J. Microbial pathogens in ticks, rodents and a shrew in northern Gyeonggi-do near the DMZ, Korea. J Vet Sci. 2008; 9: 285-293.
10. Cao WC, Zhao QM, Zhang PH, Dumler JS, Zhang XT, Fang LQ, Yang H. Granulocytic ehrlichiae in *Ixodes persulcatus* ticks from an area in China where Lyme disease is endemic. J Clin Microbiol 2000; 38: 4208-4210.
11. Comer JA, Nicholson WL, Olson JG, Childs JE. Serologic testing for human granulocytic ehrlichiosis at a national referral center. J Clin Microbiol 1999; 37: 558-564.
12. Dumler JS, Asanovich KM, Bakken JS, Richter P, Kimsey R, Madigan JE. Serologic cross-reactions among *Ehrlichia equi, Ehrlichia phagocytophila*, and human granulocytic ehrlichia. J Clin Micribiol 1995; 33: 1098-1103.
13. Engvall EO, Pettersson B, Persson M, Artursson K, Johansson KE. A 16S rRNA-based PCR assay for detection and identification of granulocytic *Ehrlichia* species in dogs, horses, and cattle. 1996; 34: 2170-2174.
14. Gordon WS, Broenlee A, Wilson DR, MacLeod J, "Tick-borne fever." (A hitherto undescribed disease of sheep). J Comp Pathol Therap 1932; 65: 301-307.
15. Heo EJ, Park JH, Koo JR, Park MS, Park MY, Dumler JS, Chae JS. Serologic and molecular detection of *Ehrlichia chaffeensis* and *Anaplasma phagocytophila* (human granulocytic ehrlichiosis agent) in Korean patients. J Clin Microbial 2002; 40: 3082-3085.
16. Inokuma H, Nane G, Uechi T, Yonahara Y, Brouqui P, Okuda M, Onishi T. Survey of tick infestation and tick-borne ehrlichial infection of dogs in Ishigaki Island, Japan. J Vet Med Sci 2001; 63: 1225-1227.
17. Kim CM, Kim MS, Park MS, Park JH, Chae JS. Identification of *Ehrlichia chaffeensis, Anaplasma phagocytophila*, and *A. bovis* in *Haemaphysalis longicornis* and *Ixodes persulcatus* ticks from Korea. Vector Borne Zoonotic Dis. 2003; 3: 17-26.
18. Kim CM, Yi YH, Yu DH, Lee MJ, Cho MR, Desai AR, Shringi S, Klein TA, Kim HC, Song JW, Baek LJ, Chong ST, O'Guinn ML, Lee SS, Lee IY, Park JH, Foley J, Chae JS. Tick-borne rickettsial pathogens in ticks and small mammals in Korea. Appl Environ Microbiol 2006; 72: 5766-5776.
19. Lee SO, Na DK, Kim CM, Li YH, Cho YH, Park JH, Lee JH, Eo SK, Klein TA, Chae JS. Identification and prevalence of *Ehrlichia chaffeensis* infection in *Haemaphysalis longicornis* ticks from Korea by PCR, sequencing and phylogenetic analysis based on 16S rRNA gene. J Vet Sci 2005; 6: 151-155.
20. Lockhart JM, Davidson WR, Stallknecht DE, Dawson JE, Howerth EW, Isolation of *Ehrlichia chaffeensis* from wild white-tailed deer (*Odocoileus virginianus*) confirms their role as natural reservoir hosts. J Clin Microbiol 1997; 35: 1681-1686.
21. Massung RF, Slater K, Owens JH, Nicholson WL, Mather TN, Solberg VB, Olson JG. Nested PCR assay for detection of granulocytic ehrlichiae. J Clin Microbiol 1998; 36: 1090-1095.
22. Neer TM. Ehrlichiosis. Canine monocytic and granulocytic ehrlichiosis. In: Greene CE. (Ed.). Infectious disease of the dog and cat. Philadelphia: Saunders. 1998: 139-154.
23. Pancholi P, Kolbert CP, Mitchell PD, Reed KD, Dumler JS, Bakken JS, Telford III SR, Persing DH. *Ixodes dammini* as a potential vector of human granulocytic ehrlichiosis. J Infect Dis 1995; 172: 1007-1012.
24. Park JH, Heo EJ, Choi KS, Dumler JS, Chae JS. Detection of antibodies to *Anaplasma phagocytophila* and *Ehrlichia chaffeensis* antigens in sera of Korean patients by Western immunoblotting and indirect immunofluorescence assay. Clin Diag Lab Immunol 2003; 10: 1059-1064.
25. Park HS, Lee JH, Jeong EJ, Park TK, Kim TY, Chae JS, Park JH, Klein TA, Jang WJ, Park KH, Lee SH. Differentiation of Anaplasmataceae through partial *gro*EL gene analysis. Microbiol Immunol 2005; 49: 655-662.
26. Parola P, Raoult D. Ticks and tick-borne bacterial diseases in humans: an emerging infectious threat. Clin Infect Dis 2001; 32: 897-928.
27. Rikihisa Y. New findings on members of the family Anaplasmataceae of veterinary importance. Ann N Y Acad Sci 2006; 1078: 438-445.
28. Rikihisa Y. The tribe Ehrlichieae and ehrlichial diseases. Clin Microbial Rev 1991; 4: 286-308.
29. Unver A, Felek S, Paddock CD, Zhi N, Horowitz HW, Wormser GP, Cullman LC, Rikihisa Y. Western blot analysis of sera reactive to human monocytic ehlrichiosis and human granulocytic ehrlichiosis agents. J Clin Microbiol 2001; 39: 3982-3986.
30. Walls JJ, Aguero-Rosenfeld M, Bakken JS, Goodman JL, Hossain D, Johnson RC, Dumler JS. Inter- and intralaboratory comparison of *Ehrlichia equi* and human granulocytic ehrlichiosis (HGE) agent strains for serodiagnosis of HGE by the immunofluorescent-antibody test. J Clin Microbiol 1999; 37: 2968-2973.
31. Waner T, Strenger C, Keysary A, Harrus S. Kinetics of serologic cross-reactions between *Ehrlichia canis* and the *Ehrlichia phagocytophila* genogroups in experimental *E. canis* infection in dogs. Vet Immunol Immunopathol 1998; 66: 237-243.
32. Wong SJ, Brady GS, Dumler JS. Serological responses to *Ehrlichia equi, Ehrlichia chaffeensis*, and *Borrelia burgdorferi* in patients from New York State. J Clin Microbiol 1997; 35: 2198-2205.
33. Yu DH, Li YH, Yoon JS, Lee JH, Lee MJ, Yu IJ, Chae JS, Park JH. *Ehrlichia chaffeensis* infection in dogs in South Korea.Vector Borne Zoonotic Dis. 2008; 8: 355-358.
34. Zhi N, Ohashi N, Rikihisa Y, Horowitz HW, Wormser GP, Hechemy K. Cloning and expression of the 44-kilodalton major outer membrane protein gene of the human granulocytic ehrlichiosis agent and application of the recombinant protein to serodiagnosis. J Clin Microbiol 1998; 36: 1666-1673.

한국에서 사육되는 고양이, 말, 소로부터 *Anaplasma phagocytophilum*과 *Ehrlichia chaffeensis*에 대한 항체 검출

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요 약 : 국내 전주, 광주 그리고 제주에서 고양이, 더러브렛 말, 홀스테인 소로부터 진드기 매개성 인수공통 병원체인 *Anaplasma phagocytophilum*과 *Ehrlichia chaffeensis*에 대한 항체가 조사를 위하여 면역형광항체법을 이용하였다. 본 연구를 위하여 254마리의 혈청(33마리의 애완 및 길거리 고양이, 92마리의 방목장 말 그리고 129마리의 방목장 소)을 수집하였다. 33마리의 고양이 중에서 6마리가 *A. phagocytophilum*(titer ≥ 80)에 대한 양성항체가 검출되어 18.2%의 양성율을 나타내었으며, 1마리에 있어서는 *E. chaffeensis*에 대한 항체가 검출되어 3%의 양성율을 나타내었다. 소에 있어서는 129마리 중에서 1마리에서 그리고 말에 있어서는 92마리 중에서 2마리에서 *A. phagocytophilum*에 대한 양성항체가 검출되어 각각 0.8%와 2.2%의 양성율을 나타내었으나 *E. chaffeensis*에 대한 항체는 모두 음성 결과를 나타내었다. 이 결과는 국내 사육 고양이, 말 그리고 소에 있어서 *A. phagocytophilum*과 *E. chaffeensis*에 대한 혈청학적 양성결과로서 국내에서 처음 보고되는 자연감염 예이며, 진드기 서식지역의 방목동물에서는 이들 질병의 감염으로 경제성 손실이 우려되며 예방대책이 마련되어야 할 것으로 판단된다.

주요어 : *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis*, 면역형광항체법, 고양이, 말, 소.