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Western Immunoblot Analysis of *Ehrlichia chaffeensis*, *E. canis*, or *E. ewingii* Infections in Dogs and Humans

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***Ehrlichia chaffeensis*, *E. canis*, and *E. ewingii* are genetically closely related, as determined by 16S rRNA gene base sequence comparison, but they exhibit biologic differences. *E. chaffeensis* is the etiologic agent of human ehrlichiosis. *E. canis* and *E. ewingii* cause two distinctly different forms of canine ehrlichiosis and infect different types of leukocytes, monocytes and granulocytes, respectively. *E. chaffeensis* can also infect dogs. In the study, Western immunoblot analysis of sera from dogs inoculated with *E. chaffeensis*, *E. canis*, or *E. ewingii* was performed to determine antigenic specificity and the intensities of the reactions to purified *E. chaffeensis* and *E. canis* antigens. At 2 to 3 weeks postexposure, antisera from four dogs inoculated with *E. chaffeensis* reacted with 64-, 47-, 31-, and 29-kDa proteins of *E. chaffeensis* but reacted poorly with *E. canis* antigen. In contrast, at 2 to 3 weeks postexposure, antisera from four *E. canis*-inoculated dogs reacted strongly with the 30-kDa major antigen of *E. canis* but reacted poorly with proteins from *E. chaffeensis*. At 4 weeks postexposure, the sera from three *E. ewingii*-inoculated dogs showed weak binding to 64- and 47-kDa proteins of both *E. chaffeensis* and *E. canis*. Convalescent-phase sera from human ehrlichiosis patients and sera from dogs chronically infected with *E. ewingii* strongly reacted with similar sets of proteins of *E. chaffeensis* and *E. canis* with similar intensities. However, sera from dogs chronically infected with *E. canis* reacted more strongly with a greater number of *E. canis* proteins than with *E. chaffeensis* proteins. The protein specificity described in the report suggests that dogs with *E. canis* infections can be distinguished from *E. chaffeensis*-infected animals by Western immunoblot analysis with both *E. canis* and *E. chaffeensis* antigens.**

Organisms of the tribe *Ehrlichieae* are obligate intracellular bacteria of the family *Rickettsiaceae*. They are minute, gram-negative, and highly pleomorphic cocci which replicate within phagosomes in the cytoplasms of either monocytes, granulocytes, or thrombocytes (13). Of several known *Ehrlichia* spp., *E. chaffeensis*, *E. canis*, and *E. ewingii* are most closely related, as demonstrated by 16S rRNA gene base sequence comparison (more than 98% homology) (1, 2), and all are transmitted by ticks. Each ehrlichial species coexists in certain parts of the United States (e.g., Oklahoma, Missouri, Tennessee, Arkansas, and California) (7, 11, 17).

E. chaffeensis was isolated from a human patient in Arkansas (3). Human ehrlichiosis is characterized by fever, headache, myalgia, anorexia, chills, leukopenia, thrombocytopenia, anemia, and an elevation in hepatic aminotransferase levels in serum. The severity of the disease ranges from asymptomatic seroconversion to death, and severe morbidity is documented (7, 10, 11). Since the discovery of the disease in 1986 (10), more than 300 cases have been identified within 27 states in the United States (2a). Inoculation of dogs with *E. chaffeensis* does not produce any clinical signs other than mild, transient fever, even though the organisms establish infection and may be isolated from peripheral blood monocytes between 7 and 26 days postinoculation (4). It is unknown whether dogs are infected with *E. chaffeensis* in nature.

E. canis infects members of the family *Canidae* and is not known to infect other vertebrate species. Canine ehrlichiosis exhibits both acute and chronic phases. In the acute phase of the disease, susceptible dogs manifest transient fever, anorexia, depression, and mild thrombocytopenia. Most dogs recover from the acute phase of the disease but remain infected. A few months or years after the acute phase of the disease, some dogs develop the chronic form of the disease, characterized by severe thrombocytopenia, weight loss, emaciation, and hemorrhage (8, 18). The chronic and acute phases of ehrlichiosis are frequently seen all over the United States and are especially common in the southern states.

In contrast, *E. ewingii* causes a milder disease characterized by a mild fever and thrombocytopenia, and in the chronic phase it has been associated with polyarthritis (6, 17). The disease is sporadically seen in several states, especially in Oklahoma, Arkansas, and Missouri (6, 17). *E. ewingii* has been found only in neutrophils and eosinophils, unlike *E. canis* and *E. chaffeensis*, which are found primarily in circulating monocytes.

Despite considerable serologic cross-reactivity between *E. canis* and *E. chaffeensis* by indirect fluorescent-antibody assay (IFA) (5, 10, 11), previous inoculation of a dog with *E. chaffeensis* cannot prevent the development of clinical signs of canine ehrlichiosis after challenge with *E. canis* (4). Likewise, there is a serologic cross-reactivity between *E. canis* and *E. ewingii* (14). However, prior inoculation of a dog with *E. canis* and the persistence of *E. canis* in peripheral blood monocytes does not prevent infection of granulocytes with *E. ewingii* upon challenge with *E. ewingii* (14). Serologic cross-reactivity and cross-protection between *E. ewingii* and *E. chaffeensis* have not been tested. The previous studies used unfractionated antigens; thus, they did not address the molecular sizes of the reacting antigenic proteins. Although Western blot (immunoblot) analysis of *E. canis* antigens has been reported (14), that of *E. chaffeensis* antigen has not been done, and the reactivities

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of sera from dogs and humans infected with *E. chaffeensis* have not been examined by Western blot analysis.

In the study described here, we compared sera serially collected from dogs experimentally infected with *E. chaffeensis*, *E. canis*, and *E. ewingii* by Western immunoblot analysis by using purified *E. canis* and *E. chaffeensis* antigens. Sera from human ehrlichiosis patients were also evaluated against purified *E. chaffeensis* and *E. canis* antigens.

MATERIALS AND METHODS

Culturing and purification of *E. canis* and *E. chaffeensis*. *E. canis*, Oklahoma isolate, and *E. chaffeensis*, Arkansas isolate, originally provided by the Centers for Disease Control and Prevention (CDC), Atlanta, Ga., were propagated in the continuous canine macrophage cell line DH82 as described previously (4, 12, 14). *E. canis* and *E. chaffeensis* were purified from 5 to 10 150-cm^2 flasks of infected DH82 cells as described previously (12, 14). Briefly, when more than 90% of the cells were heavily infected, the cells were gently dislodged from the bottom of the flasks by scraping with a rubber policeman. The cell suspension was centrifuged at 300 × *g* for 5 min. The pellet was resuspended in minimum essential medium (MEM; GIBCO, Grand Island, N.Y.) at 5×10^6 cells per ml, and the suspension was sonicated at setting 2 for 5 s by using a W-380 sonicator (Heat Systems-Ultrasonics, Inc., Farmingdale, N.Y.). This procedure was repeated two to three times, until Diff-Quik-stained cytocentrifuged preparations of infected DH82 cells showed complete host cell lysis. Unbroken cells and the large cellular debris were removed by centrifugation at 1,500 × *g* for 15 min.

Host cell-free ehrlichial organisms were obtained by centrifugation of the supernatant at 10,000 × *g* for 10 min. The pellet was resuspended in 2 ml of phosphate-buffered saline (2× PBS; 19 mM K_2HPO_4, 12 mM KH_2PO_4, 300 mM NaCl). The suspension was applied to the top of a chromatography column containing packed sterile Sephacryl S-1000 (height, 5 cm; diameter, 1.6 cm; Pharmacia, Uppsala, Sweden) and was eluted in 2× PBS. A flowthrough fraction containing ehrlichial organisms was collected. The protein content of the isolated ehrlichial organisms was determined by using the bicinchoninic acid (BCA) method described by Smith et al. (16) with a bovine serum albumin standard (BCA protein assay kit; Pierce, Rockford, Ill.).

An uninfected DH82 cell lysate was used as a control antigen. The cells suspended in MEM at 5×10^6 cells per ml were sonicated and centrifuged as described above for infected cells. The pellet obtained by centrifugation at 10,000 × *g* for 10 min was used as the antigen, since Sephacryl S-1000 chromatography of an uninfected DH82 cell lysate did not yield a sufficient amount of protein for analysis.

Antisera used. Sera were collected from four dogs intravenously inoculated with either 4.2×10^7 intact (dogs 1124 and 1125) or 8.0×10^8 Dounce-homogenized (dogs 1117 and 1120) *E. chaffeensis*-infected DH82 cells, from four dogs intravenously inoculated with either 4.5×10^7 intact (dogs 1116 and 1123) or 9.0×10^8 Dounce-homogenized (dogs 1115 and 1118) *E. canis*-infected DH82 cells, from three dogs (dogs 1122, 1090, and 1091) intravenously inoculated with *E. ewingii* by transfusion of 10 ml of whole blood from another chronically infected dog, or from two dogs intravenously inoculated with either 4.9×10^7 intact (dog 1121) or 8.0×10^8 Dounce-homogenized (dog 1119) uninfected DH82 cells. Dog 1125, which had initially been inoculated with *E. chaffeensis*, was challenge exposed to 10 ml of blood from *E. canis*-infected dog 1123 on day 28 after initial inoculation to examine cross-protection and in vivo cross-reacting antigens. Establishment of infection was confirmed by reisolation of organisms in DH82 cells from the peripheral blood buffy coat fractions (4) and by light microscopic observation of *E. ewingii* organisms in the peripheral blood neutrophils or *E. canis* organisms in monocytes smeared on glass slides (4, 14). Dogs 303, 011, and 307 were inoculated with 10^7 *E. canis*-infected DH82 cells, and blood was collected from these dogs at 2 months postinfection as reported previously (15). The clinical signs and IFA titers of all dogs except dog 1122 were reported previously (4, 14, 15). Convalescent-phase sera from humans with clinical signs of human ehrlichiosis were kindly provided by CDC (one serum specimen) and the Oklahoma State Department of Health, Oklahoma City (two serum specimens). These patients had clinical signs compatible with human ehrlichiosis (fever, anorexia, headache, myalgia, lymphadenopathy, leukopenia, thrombocytopenia, and elevated liver enzyme activity) (7, 10, 11). The patients showed serologic evidence of recent infection with *E. chaffeensis*. The rabbit antiserum against *E. chaffeensis* was raised by injecting 100 μg (initially, four times) and 200 μg (four subsequent times) of protein of Sephacryl S-1000-purified ehrlichiae with an equal volume of Freund adjuvant (complete the first time; incomplete the remaining seven times) into three rabbits every 2 weeks for a total of eight times. The antiserum was preabsorbed with uninfected DH82 cells at a ratio of 10^6 DH82 cells per 10 ml of serum at room temperature for 1 h.

Western immunoblot analysis. Purified *E. chaffeensis*, *E. canis*, and uninfected DH82 cells (40 μg of protein per lane) were separated by sodium dodecyl sulfate (SDS)–10 to 20% polyacrylamide gradient gel electrophoresis, and Western immunoblotting was performed with various dog, human, or rabbit sera as described previously (12, 14). Antigens electroblotted onto nitrocellulose membranes were reacted with the primary antibody at a 1:100 (1:20 for the rabbit serum) dilution and were then incubated with alkaline phosphatase-conjugated affinity-purified anti-dog, anti-rabbit, or anti-human immunoglobulin G (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.) at a 1:1,000 or 1:500 dilution. The enzyme reaction was followed for color development as described previously (12, 14).

RESULTS

The protein profiles of Sephacryl S-1000-purified *E. chaffeensis* and *E. canis* are shown in Fig. 1. Major proteins of approximately 74, 64, 43, and 30 kDa were shared between *E. chaffeensis* and *E. canis*. The major antigens recognized by the serum from a rabbit inoculated eight times with Sephacryl S-1000-purified *E. chaffeensis* and sera from chronically *E. canis*-infected dogs at 2 months postinfection are shown in Fig. 1. The rabbit anti-*E. chaffeensis* serum detected one major 64-kDa protein of *E. chaffeensis* and *E. canis*. In addition, minor 45- and 28-kDa proteins of *E. chaffeensis* reacted with the rabbit antiserum. The rabbit antiserum used was preabsorbed with DH82 cells and did not react with uninfected DH82 lysate (data not shown). After absorption with uninfected DH82 cells, the IFA titer of the rabbit anti-*E. chaffeensis* serum to *E. chaffeensis* was 1:320 (preabsorption titer, 1,280). On the other hand, the sera from three dogs chronically infected with *E. canis* preferentially reacted with *E. canis* antigen (Fig. 1). The approximate molecular sizes of the major *E. canis* antigens that commonly reacted with the sera from chronically infected dogs were 74, 47, and 30 kDa. The *E. chaffeensis* 47-kDa antigen was commonly recognized with these sera (Fig. 1; Table 1).

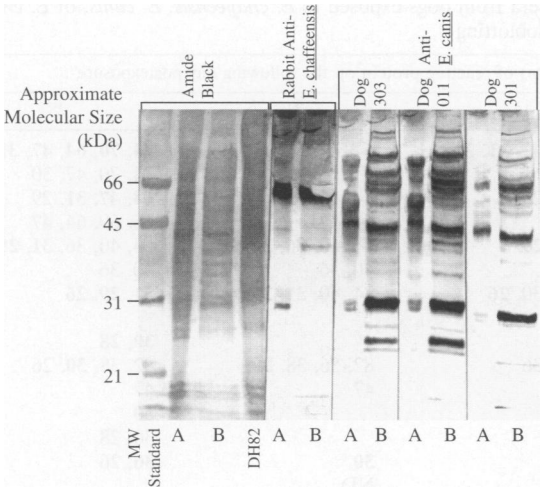

FIG. 1. Western blot analysis of *E. chaffeensis* (A), *E. canis* (B), and uninfected DH82 lysate (DH82) on an SDS–10 to 20% polyacrylamide gradient gel. Amido black staining for protein profiles was used. Samples were incubated with the rabbit anti-*E. chaffeensis* serum (1:320) or sera from dogs with chronic canine ehrlichiosis (1:5,120 homologous titer).

The sera collected weekly for up to 4 weeks from four dogs infected with *E. chaffeensis* reacted to the homologous antigen starting at week 2 postexposure, and all four dogs were positive for proteins of 64, 47, 40, 31, or 29 kDa of *E. chaffeensis* antigen (Table 2; Fig. 2). The same sera reacted poorly with *E. canis* proteins in three dogs, but positive reactions to 64- and 47-kDa proteins of *E. canis* were seen only in dog 1120.

The sera collected weekly for up to 4 weeks from four dogs infected with *E. canis* essentially showed similar reaction patterns by Western immunoblot analysis (Table 2; Fig. 3). Reactions to the homologous antigen were demonstrated starting at week 1 or 2 postexposure, and the strongest reaction was to the 30-kDa protein, as reported previously (14). In contrast, reactions to *E. chaffeensis* proteins were weak to nonexistent. Sera from two dogs reacted with the 30- and 28-kDa proteins of *E. chaffeensis* only at week 4 postinfection.

One dog (number 1125) was initially infected with *E. chaffeensis* and was then challenge exposed to *E. canis* on week 4. The dog developed fever, anorexia, and thrombocytopenia, and morulae were found in peripheral blood films 17 days after exposure to *E. canis*, indicating a lack of cross-protection against *E. canis* (4). The reaction of the serum to *E. chaffeensis* antigen was first detected at week 2 postinfection and persisted until week 6 postexposure, when the experiment was terminated. The significant reaction of the serum to *E. canis* antigen was seen only at week 6 after *E. chaffeensis* infection (week 2 after *E. canis* challenge). The major reacting protein of *E. canis* antigen was the 30-kDa protein (Fig. 2).

The sera collected weekly from three dogs infected with *E. ewingii* reacted weakly to both *E. canis* and *E. chaffeensis* at week 3 or 4 postexposure (Table 2). At 2 weeks postexposure, the serum from dog 1090 reacted with *E. canis* antigen but not with *E. chaffeensis* antigen (Fig. 4). However, the serum from dog 1091 reacted more strongly with *E. chaffeensis* antigen than with *E. canis* antigen, and the serum from dog 1122 did not react with antigen from either ehrlichial species at 2 weeks postexposure. At week 4 postexposure, the antisera did not react strongly with the 30-kDa protein of *E. canis* or *E. chaffeensis* but reacted with 64- and 47-kDa proteins of both *E. canis* and *E. chaffeensis* at similar intensities at week 4 postexposure. At 2 to 3 months postexposure to *E. ewingii*, reactions of the antisera became much stronger and more bands were seen (Fig. 4; see Fig. 6; Table 1). After several weeks postexposure, the sera reacted with antigens from *E. chaffeensis* and *E. canis* in a similar pattern (Fig. 4; Table 1; see Fig. 6). The sera collected weekly from two dogs experimentally exposed to uninfected DH82 cells as controls did not show these reactions (data not shown). Sera from none of these dogs reacted with DH82 lysate (Fig. 5).

Convalescent-phase sera from three human ehrlichiosis patients strongly reacted with both *E. chaffeensis* and *E. canis* antigens (Table 1; Fig. 6). The sera from two human patients in Oklahoma reacted to *E. canis* and *E. chaffeensis* in an identical manner. The positive banding patterns of these two human serum specimens were similar to those of three serum specimens from dogs infected with *E. ewingii* but were different from that of one patient serum specimen obtained from CDC (Fig. 6), which resembled that of an *E. canis*-infected dog serum specimen (Fig. 1 and 3). None of these reacting bands was seen when these sera were incubated with DH82 cell lysates (Fig. 6; data for the serum specimen from CDC are shown).

DISCUSSION

The present study demonstrated that *E. canis* infection of dogs can be distinguished from *E. chaffeensis* and *E. ewingii* infections by simultaneous Western immunoblot analysis by using both *E. canis* and *E. chaffeensis* as the antigens. Sera from dogs infected with either *E. canis* or *E. chaffeensis* preferentially reacted with homologous antigens during the initial 4

TABLE 1. Molecular sizes of *E. chaffeensis* and *E. canis* proteins reacting with sera from human ehrlichiosis patients and sera from dogs chronically infected with *E. ewingii* and *E. canis*

Serum specimen	Molecular size (kDa) of reacting antigen[a]	
	E. chaffeensis	*E. canis*
CDC, human	74, **64**, **47**, **40**, **30**, **28**, 23	**64**, **47**, 40, **30**, **26**, 23
Oklahoma, human 72088	90, **74**, **64**, **47**, 46, **40**, 30, 23	110, **74**, **64**, **47**, **45**, **40**, 26, 23
Oklahoma, human 1-1055	90, **74**, **64**, **47**, 45, **40**, 30, 23	110, **74**, **64**, **47**, **45**, 43, **40**, 26, 23
E. chaffeensis (dog 1117), 28 days postexposure	110, **74**, **70**, **64**, **47**, **31**, **29**	**74**, **70**, **47**, **30**
E. ewingii (dog 1090), 63 days postexposure	**74**, **64**, **47**, 45, 43, 40, 30, 23	**74**, **64**, **47**, 45, 43, 40, 26, 23
E. canis (dog 303), 2 mo postexposure	90, **47**, 30	110, 74, **47**, 40, **30**, **26**
E. canis (dog 011), 2 mo postexposure	**90**, 74, **56**, **47**, 30	110, 74, **47**, 40, **30**, **26**
E. canis (dog 307), 2 mo postexposure	90, 74, **47**	110, 74, **47**, 40, **30**

[a] Major antigens are shown in boldface.

TABLE 2. Molecular sizes of *E. chaffeensis* and *E. canis* proteins reacting with sera from dogs exposed to *E. chaffeensis*, *E. canis*, or *E. ewingii* detected by Western immunoblotting

Dog no.	Dog exposed to:	Western blot antigen	Molecular size (kDa) of reacting protein on the following day postexposure[a]:			
			7	14	21	28
1117	*E. chaffeensis*	*E. chaffeensis*		**64**, **47**, 40, **31**, **29**	**64**, **47**, 40, **31**, **29**	**74**, **70**, **64**, **47**, **31**, **29**
		E. canis				74, 70, 47, 30
1120	*E. chaffeensis*	*E. chaffeensis*		**64**, 31, 29	**64**, 40, 31	**64**, **47**, **31**, 29
		E. canis		**64**	**64**, **47**	75, 69, 64, **47**
1124	*E. chaffeensis*	*E. chaffeensis*	40, 36, 31, 29	64, 31, **29**	**64**, 40, 36, **31**, **29**	64, 40, 36, **31**, **29**
		E. canis	40, 36	40, 36	40, 36	40, 36
1125	*E. chaffeensis*	*E. chaffeensis*	ND	**74**, **40**, 30, **26**	74, 40, **31**, **29**	**31**, **29**, **26**
		E. canis	ND			
1115	*E. canis*	*E. chaffeensis*				**30**, **28**
		E. canis	47, 38, 36	47, 38, 36	82, 56, 38, **30**	82, 38, **30**, 26
1116	*E. canis*	*E. chaffeensis*	47	47	47	47
		E. canis				**30**
1118	*E. canis*	*E. chaffeensis*				30, 28
		E. canis		**30**	**30**	**30**, 26
1123	*E. canis*	*E. chaffeensis*			ND	
		E. canis		**30**	ND	**30**, **26**
1122	*E. ewingii*	*E. chaffeensis*			40	ND
		E. canis			43, 40	ND
1090	*E. ewingii*	*E. chaffeensis*	ND		ND	74, **64**, **47**, 40
		E. canis	ND	70, 64, 56 (day 12)	ND	78, 74, 64, **47**
1091	*E. ewingii*	*E. chaffeensis*	ND	64, 47 (day 15)	ND	64, 47 (day 33)
		E. canis	ND	47		74, 64, 47

[a] Major antigens are shown in boldface. ND, not determined. No antigens were detected on day 0.

weeks postexposure. During the chronic stages of infection in dogs infected with *E. canis*, the preferential reaction to homologous antigen was distinct. In contrast, sera from *E. ewingii*-infected dogs reacted weakly to similar protein bands of both *E. canis* and *E. chaffeensis* antigens, and the reaction was somewhat similar to that of the sera from *E. chaffeensis*-inoculated dogs. *E. ewingii* has not been cultivated in sufficient quantities to purify antigens for Western immunoblot analysis (2). However, 16S rRNA gene sequence comparisons with the 16S rRNA sequences of other ehrlichial species identify *E. ewingii* as a distinct ehrlichial species that is closely related to *E. canis*, *E. chaffeensis*, and some other ehrlichial species (2). The 16S rRNA gene base sequence comparison result coincides with the previously noted biologic differences of *E. ewingii* from *E. canis* (e.g., *E. ewingii* infects granulocytes rather than monocytes and it induces different clinical responses in dogs [6, 17]). The protein which induced the earliest antibody response in dogs infected with *E. canis* was the 30-kDa protein, an observation that is in agreement with the results of our previous experiment (14). In contrast, several larger proteins (viz., 64, 47, and 40 kDa) and an approximately 30 kDa protein of *E. chaffeensis* induced early antibody production in dogs. Interestingly, proteins of approximately 30 kDa of *E. canis* and *E. chaffeensis* were strongly recognized at 2 to 4 weeks postexposure by the sera from dogs infected with homologous *Ehrlichia* spp. but weakly or not recognized by the sera from dogs infected with heterologous species (*E. canis*, *E. chaffeen-*

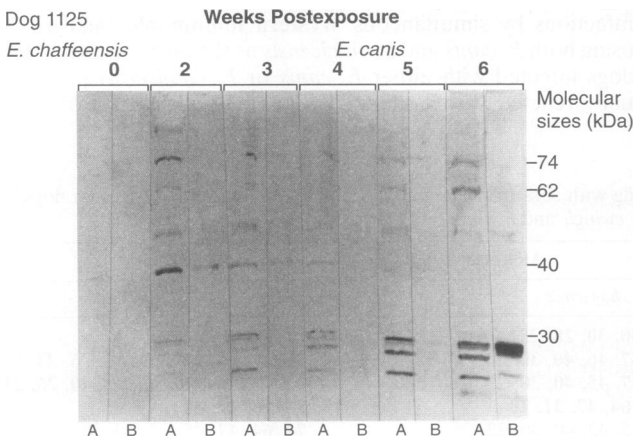

FIG. 2. Western immunoblot analysis of sera from dog 1125 infected with *E. chaffeensis* and challenge exposed on day 28 postinfection with *E. canis*, with *E. chaffeensis* (A) and *E. canis* (B) used as antigens.

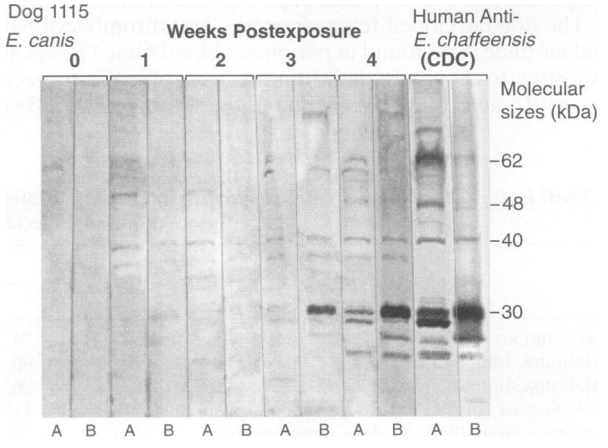

FIG. 3. Western immunoblot analysis of sera collected weekly from dog 1115 infected with *E. canis* and convalescent-phase sera (CDC) from humans with ehrlichiosis, with *E. chaffeensis* (A) and *E. canis* (B) used as antigens.

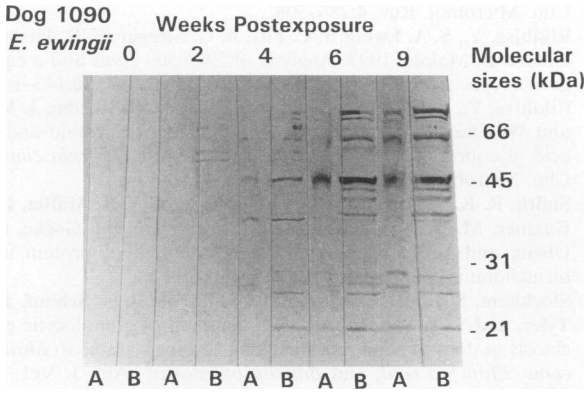

FIG. 4. Western immunoblot analysis of sera from dog 1090 infected with *E. ewingii*, with *E. chaffeensis* (A) and *E. canis* (B) used as antigens.

sis). This finding suggests that proteins of approximately 30 kDa (major proteins of *E. canis* and *E. chaffeensis*) are antigenically distinct proteins with similar molecular sizes. In support of this speculation is the humoral immune response to the *E. canis* 30-kDa protein in dog 1125. It was not faster when dog 1125, which was initially infected with *E. chaffeensis*, was challenged with *E. canis*. The failure of *E. chaffeensis* infection to cross-protect this dog from *E. canis* infection (4) would be consistent with our Western immunoblot results. In contrast, the antisera from *E. ewingii*-infected dogs reacted with common epitopes of *E. canis* and *E. chaffeensis* antigens. Thus, these antisera could not distinguish between these two ehrlichial species. The common antigens between *E. canis* and *E. ewingii*, however, also do not provide *E. canis*-infected dogs with protection from *E. ewingii* challenge (14).

Convalescent-phase sera from human ehrlichiosis patients also strongly reacted with both *E. chaffeensis* and *E. canis* antigens in a similar pattern. Two human serum specimens obtained from the Oklahoma State Department of Health reacted to both *E. chaffeensis* and *E. canis* antigens, as did dog anti-*E. ewingii* sera (i.e., they primarily reacted with proteins

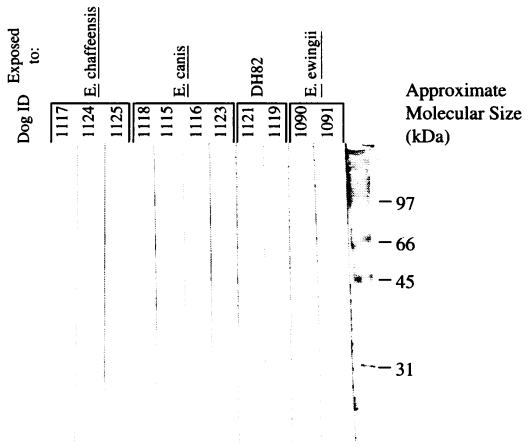

FIG. 5. Western immunoblot analysis of sera from dogs exposed to *E. canis*, *E. chaffeensis*, *E. ewingii*, or uninfected DH82 cells against uninfected DH82 cell lysate antigen. Sera were obtained at day 28 postexposure (sera from dog 1125 were obtained at 6 weeks postexposure to *E. chaffeensis* and at 2 weeks postexposure to *E. canis*).

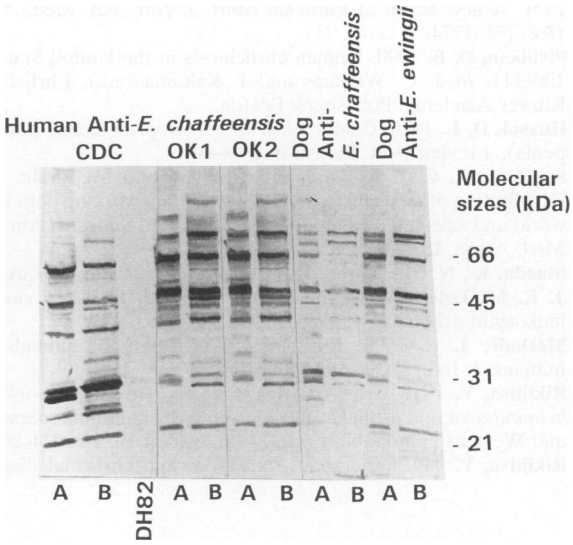

FIG. 6. Western immunoblot analysis of sera from human ehrlichiosis patients (CDC, OK1 = 72088, OK2 = 1-1055), dog 1117 anti-*E. chaffeensis* serum (day 28 postexposure), and dog 1090 anti-*E. ewingii* serum (day 63 postexposure), with *E. chaffeensis* (A), *E. canis* (B), and uninfected DH82 cell lysate (DH82) used as antigens.

larger than 40 kDa). However, the human serum specimen obtained from CDC reacted to both *E. chaffeensis* and *E. canis* antigens, much as the dog anti-*E. canis* serum specimens did (i.e., they reacted more strongly to proteins of 30 kDa or less). This suggests that there may be different strains of *E. chaffeensis* which vary in their antigenic compositions or the human may have been exposed to *E. canis* or *E. canis*-like ehrlichial spp.

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