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Research Note

Antigenic Cross Reactivity Among *Dirofilaria immitis* and Four Intestinal Parasite-species in the Dog

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The immunological cross-reactivity has been reported between *Dirofilaria immitis* and *Toxocara canis* (Dzimianski and McCall, 1986; Grieve *et al.*, 1981; Hayasaki, 1981; Matsumura *et al.*, 1984; Ott *et al.*, 1985; Thilsted *et al.*, 1987). Therefore, immunodiagnostic test to *D. immitis* infection may mislead to be positive by *T. canis* infection, as a false-positive reaction. Thus, more strict analysis is necessary for the study of antigenic cross-reactivities. However, little information is available between *D. immitis* and other intestinal parasites. From this point of view, the present study was performed to analyse a similarity of antigenicity among *D. immitis* and four intestinal parasite-species of dogs.

Somatic antigens used in this study were extracted from adult worms of *D. immitis* (DiEX), *T. canis* (TcEX), *Ancylostoma caninum* (AcEX), *Trichuris vulpis* (TvEX) and *Dipylidium caninum* (DcEX). These worms were homoginized by tissue homoginizer and ultrasonicator, and then allowed to incubate overnight at 4°C. After centrifugation at 18,000 × g, the supernatants as antigen were obtained and kept at –80°C until use.

Analysis of the antigens was carried out with a minislab gel consisting of 12.5% acrylamide and 0.1% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) as described by Kaneko *et al.* (1990). Briefly, the sample were heated in the sample buffer (0.0625M Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, 0.00125% bromophenol blue) for 5 min. Ten micrograms of protein were then loaded per well, followed by electrophoresis at 100 volts for 120 min. The gels were subsequently stained with coomassie brilliant blue R-250. Approximate molecular weights of the separated bands were estimated using molecular weight markers (Sigma Chemical Co., St. Louis, MO, USA).

Each SDS-PAGE and immunoblotting analysis was simultaneously loaded by using 10 columns and each separated bands in the columns was statistically identified based on Student's *t* test after tracing peaks of curve by densitometry. The molecular weight of each separated bands were calculated by comparing with the markers.

Following SDS-PAGE, the antigenic bands were then analyzed by immunoblotting as described by Kaneko *et al.* (1990). The protein bands in polyacrylamide gel were transferred electrophoretically to a nitrocellulose membrane. The nitrocellulose membrane was blocked overnight in Tris-buffered saline (TBS) (0.02M Tris-HCl, pH 7.5, 0.5M NaCl) containing 3% gelatin. The blocked membrane was treated with mouse sera immunized with extractive antigen of *D. immitis* or *T. canis* at dilution of 1:125. The membrane was then treated with peroxidase-conjugated goat anti-mouse IgG (Cappel Lab. Inc., Malvern, PA, USA) diluted 1:125. Antigen bands on the nitrocellulose membrane were developed with a substrate solution (0.5 mg/ml 4-chloro-1-naphthol, 0.015% H_2O_2 in TBS).

In SDS-PAGE analysis, many bands appeared to

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Table 1 Protein bands (kilodalton) in extractive antigens of *D. immitis* and four intestinal parasite species as detected by coomassie blue staining

	DiEX	TcEX	AcEX	TvEX	DcEX
	230	253	261	213	234
	224	225	246	171	221
	215	223	181	143	201
	199	212	171	108	192
	183	182	159	77	174
	169	172	144	73	161
	149	156	133	65	154
	143	148	125	63	139
	125	137	102	61	121
	113	130	91	57	113
	102	123	77	55	97
	95	114	72	46	93
	91	105	63	44	88
	81	96	60	43	76
	72	92	58	41	64
	66	87	52	38	58
	63	82	51	36	55
	60	73	49	34	52
	59	68	47	30	49
	57	65	46	28	47
	54	61	44	27	44
	52	59	42	25	41
	50	58	41	23	38
	48	54	39	21	36
	47	51	37	20	34
	45	50	36	18	32
	43	47	34	17	30
	40	46	31	16	28
	39	42	29	15	25
	38	40	28	13	24
	37	38	26		23
	35	36	24		20
	33	35	23		19
	31	34	21		17
	29	33	19		16
	26	32	18		14
	25	30	16		13
	23	27	14		12
	20	26			11
	19	25			
	17	23			
	16	22			
	15	20			
	14	18			
		16			
		15			
		13			
		12			
Total No.	44	48	38	30	39

be stained with coomassie brilliant blue, including 44 bands in DiEX, 48 bands in TcEX, 38 bands in AcEX, 30 bands in TvEX, and 39 bands in DcEX, ranging in 11 to 261 kilodalton (kDa) of molecular weights (Table 1).

Antigenicity of the bands fractionated from DiEX, TcEX, AcEX, TvEX and DcEX was assessed by immunoblotting with the anti-DiEX mouse sera. The antigenic molecules were recognized in range of 19 to 262 kDa (Table 2), indicating that the total 19 bands were recognized as having a similar molecular weight. In these 19 bands, the similar molecular weight was found in 18 bands to 2 species and 1 band to 3 species, but no band was similar to 4 or 5 species.

Antigenicity of the bands in these extracts was assessed by immunoblotting with the anti-TcEX mouse sera. Twenty-four antigenic bands in DiEX, 44 bands in TcEX, 20 bands in AcEX, 30 bands in TvEX, and 25 bands in DcEX were recognized in 12 to 273 kDa (Table 3). The total number of 22 bands were recognized. Among them, 18 bands to 2 species and 4 bands to 3 species were similar in molecular weight. However, no bands was similar to 4 or 5 species.

No band was recognized between these five extracts and a normal mouse serum when it was assessed by immunoblotting.

Ott *et al.* (1985) also reported the presence of cross-reactive and uncross-reactive antigens between extracts of *D. immitis* and *T. canis*. Weil (1987) also had indicated that a single antigen epitope is being distributed in wide range of molecular bands. Therefore, these data indicate a detail analysis on characteristics of antigenic cross reactivity in terms of an availability in immunodiagnosis and an understanding of control of parasitic infection.

This study revealed that *D. immitis* possess a partial antigenic cross reactivity to *T. canis, A. caninum, T. vulpis* and *D. caninum*, and similarly *T. canis* is also. This cross-reactivity may be dependent on many antigenic epitopes existing in somatic components of the parasites, although it is not re-

DiEX: *D. immitis* extract. TcEX: *T. canis* extract. AcEX: *A. caninum* extract. TvEX: *T. vulpis* extract. DcEX: *D. caninum* extract.

Table 2 Antigenic bands (kilodalton) in extractive antigens of *D. immitis* and four intestinal parasite species as detected by sera from mice immunized with extractive antigen of *D. immitis*

	DiEX	TcEX	AcEX	TvEX	DcEX
	239	257	257	262	259
	217	241	228	242	245
	207	217	216	234	228
	206	207	202	220	203
	184	166	182	188	191
	173	155	170	161	181
	166	139	150	147	168
	141	124	142	136	159
	126	111	129	124	151
	109	99	115	112	141
	96	86	105	98	132
	86	72	94	89	121
	79	62	84	78	110
	73	57	70	75	95
	65	55	60	67	89
	59	50	58	63	81
	57	47	54	60	69
	54	45	48	58	
	53	43	46	54	
	49	40	44	52	
	48		42	47	
	46			45	
	42			43	
	32			39	
	22			38	
	19				
Total No.	26	20	21	25	17

DiEX: *D. immitis* extract. TcEX: *T. canis* extract. AcEX: *A. caninum* extract. TvEX: *T. vulpis* extract. DcEX: *D. caninum* extract.

vealed in this study, because previous report had indicated that *D. immitis* consisted of many protein components and it's antigenicity was very complex (Hayasaki *et al.*, 1994). From these results, it is conceivable that these five parasites may originated from a same progenitor and still possess partially a similar antigenicity, although they passed a long biological history of natural selection in biological evolution and adaptation to their hosts.

Table 3 Antigenic bands (kilodalton) in extractive antigens of *D. immitis* and four intestinal parasite species as detected by sera from mice immunized with extractive antigen of *T. canis*

	DiEX	TcEX	AcEX	TvEX	DcEX
	242	261	259	273	273
	230	254	225	268	264
	219	245	219	243	246
	207	213	203	234	230
	182	201	181	225	221
	173	199	170	206	209
	142	191	147	189	200
	128	181	130	172	193
	112	170	121	146	181
	109	158	107	134	168
	96	148	92	125	160
	86	136	88	115	153
	78	133	77	110	145
	73	119	62	97	135
	65	100	58	89	121
	59	92	55	80	110
	57	87	53	75	93
	56	79	50	66	83
	53	67	47	64	69
	51	61	41	60	63
	48	58		57	60
	45	54		53	56
	41	52		51	54
	33	49		49	52
		47		44	
		43		43	
		40		41	
		39		38	
		35		36	
		32			
		30			
		29			
		28			
		26			
		24			
		21			
		20			
		18			
		16			
		15			
		14			
		13			
		12			
Total No.	24	44	20	30	25

DiEX: *D. immitis* extract. TcEX: *T. canis* extract. AcEX: *A. caninum* extract. TvEX: *T. vulpis* extract. DcEX: *D. caninum* extract.

References

1) Dzimianski, M. T. and McCall, J. W. (1986): Evaluation of adult antigen diagnostic test kits using well-defined dogs sera from laboratory and field trials. In Proceedings of the Heartworm Symposium '86, Otto, G. F. eds., American Heartworm Society, Washington, 83–86.
2) Grieve, R. B., Mika-Johnson, M., Jacobson, R. H. and Cypess, R. H. (1981): Enzyme-linked immunosorbent assay for measurement of antibody responses to *Dirofilaria immitis* in experimentally infected dogs. Am. J. Vet. Res., 42, 66–69.
3) Hayasaki, M. (1981): Indirect hemagglutination test for diagnosis of canine filariasis. Jpn. J. Vet. Sci., 43, 21–26.
4) Hayasaki, M., Nanamura, F. and Konno, K. (1994): Immunoblotting analysis of somatic components of *Dirofilaria immitis*. J. Vet. Med. Sci., 56, 1181–1183.
5) Kaneko, H., Hayasaki, M. and Ohishi, I. (1990): Antigenic identification of excretory-secretory products of adult *Dirofilaria immitis*. Jpn. J. Vet. Sci., 52, 995–1000.
6) Matsumura, K., Kazuta, Y., Endo, R. and Tanaka, K. (1984): Detection of circulating toxocaral antigens in dogs by sandwich enzyme-immunoassay. Immunology, 51, 609–613.
7) Ott, R. A., Staples, M., Weekley, M. and Maggio, E. T. (1985): Demonstration of both immunogenically unique and common antigenic determinants in *Dirofilaria immitis* and *Toxocara canis* using monoclonal antibodies. Vet. Immunol. Immunopathol., 10, 147–153.
8) Thilsted, J. P., Whorton, J., Hibbs, C. M., Jillson, G. P., Steece, R. and Stromei, M. (1987): Comparison of four serotests for the detection of *Dirofilaria immitis* infection in dogs. Am. J. Vet. Res., 48, 837–841.
9) Weil, G. J. (1987): *Dirofilaria immitis*: Identification and partial characterization of parasite antigens in the serum of infected dogs. Exp. Parasitol., 64, 244–251.