イヌ血液型判定用抗体の比較同定試験

誌名	日本獸醫學雜誌 = The Japanese journal of veterinary science
ISSN	00215295
著者	江島, 博康 黒川, 和雄 池本, 卯典
巻/号	42巻4号
掲載ページ	p. 435-441
発行年月	1980年8月

農林水産省 農林水産技術会議事務局筑波産学連携支援センター
Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council Secretariat

AgriKnowledge

Jpn. J. Vet. Sci. 42, 435~441 (1980)

Comparison Test of Antibodies for Dog Blood Grouping

Hiroyasu EJIMA and Kazuo KUROKAWA

First Department of Veterinary Surgery, Nippon Veterinary and Zootechnical College, Musashino-shi, Tokyo 180

Shigenori IKEMOTO

Department of Legal Medicine, Jichi Medical School, Minamikawachi-machi, Tochigi 329-04

(Received for publication November 19, 1979)

Abstract. A comparison test was carried out with 24 kinds of type specific antibodies prepared by the authors and Bull et al. for the purpose of standardizing dog blood grouping antisera in Japan. As a result, anti-D_1 and anti-E were identical with anti DEA-3, anti-A was with anti DEA-5, and anti-180a with anti DEA-8. Anti-M and anti DEA-5 possessed a common specificity. No antisera had the same specificity as anti DEA-1 • 1, 2, -1 • 1, -4, -6 or -7. Besides, anti-D_2, -B, -C, -F, -G, -L (-H, -I, -43), -44 and -2a prepared by the authors were identical with none of anti-DEA sera.

Studies of genetic markers on the dog red cell membrane have been performed by many researchers. These have been conducted by Iseki and Terashima [7], Hosoda [5], Kita et al. [11], Yoh [21], Yamada et al. [20], Iwasaki et al. [8, 9], Ikemoto et al. [6], Yoshida et al. [22], and Ejima et al [3] in Japan, and by Swisher and Young [15], Mears et al [12], Hall [4], Bowdler et al. [1], Suzuki et al. [14] and Vriesendorp et al. [19] in other countries. Recently, an international workshop was held, and 8 red cell antigens, consisting of DEA 1 • 1, 1 • 2, 3, 4, 5, 6, 7 and 8, were authorized in it [19].

The blood groups of dogs were classified into various systems by using naturally occurring antibodies, iso-immune antibodies, immune heterologous antibodies [3–9, 11, 12, 14, 15, 17, 19–21], naturally occurring heterologous antibody [1] and lectins [22].

The dog blood group systems have been taken into consideration in blood transfusion, organ transplantation, and judgment of the relationship between parents and offspring in the field of veterinary practice and experimental medicine [10, 11, 13, 15, 16, 18]. The authors applied the D blood group system [3] to judgment of the father from the blood type of puppies which had been delivered from bitches in experimental induction of superfecundation [16]. As a result, it was indicated that the D blood group system was useful for judgment of the relationship between parents and offspring.

On the other hand, no dog red cell typing antisera produced in Japan have been approved by the International Workshop of Canine Immunogenetics [17, 19]. The purpose of this paper is to compare antisera prepared by the authors with anti-DEA sera authorized by the workshop.

Materials and Methods

Blood grouping antisera: In this study, 16 antisera consisting of anti-D_1, -D_2, -A, -B, -C, -E, -F, -G, -H, -I, -L, -M, -2a, -43, -44 and -180a prepared by the authors, and 8 antisera consisting of anti DEA-1 • 1,2, -1 • 1, -3, -4, -5, -6, -7 and -8 kindly sup-

plied by Dr. R. W. Bull, of the Michigan State University, U.S.A., were examined.

Anti-D_1 and anti-D_2 were obtained from rabbits immunized with dog red cells [3]. Anti-A, -E, -F, -G, -L, -M and -2a were iso-immune antisera [3]. Anti-B [6], -H, -I, -44, -43 and -180a were naturally occurring antisera. Anti-C [22] was lectin extracted from the seed of *Clerodendron trichotomum*. Anti-C, -H, -44 and -43 were supplied by Ikemoto, one of the authors.

Panel red cells: Dog red cells were collected by venipuncture from 15 dogs, consisting of 11 beagles and 4 mongrel dogs. Clotted blood (without anticoagulant) and heparinized blood were prepared. The former was spun down to remove serum. In the latter, cells were washed three times with 0.85% physiological saline. Two 4% cell suspensions in fresh autologous serum and 0.85% physiological saline, respectively, were used for typing.

Red cell typing: Two drops of each antiserum were combined with two drops of 4% appropriate red cell suspension in a round-bottomed glass test tube 10×75 mm in size. When the cell suspension in fresh autologous serum and antiserum were combined, the mixture was incubated at 37°C for 15 minutes, spun at 1500 rpm for 15 seconds, and read macroscopically for hemolysis and agglutination. Similarly, when the saline cell suspension and antiserum were combined, the mixture was incubated at 4°C for 30 minutes and spun at 1500 rpm for 15 seconds. The tubes were shaken weakly and then checked macroscopically for agglutination.

Absorption test: Equal volumes of antiserum or agglutinin prepared by the authors and washed packed red cells were placed together in a test tube and incubated successively at 37°C for 1 hour, at 4°C overnight, and at room temperature for 1 hour. They were centrifuged. The resulting supernatant was titrated with an appropriate red cell suspension. The absorption of anti-DEA sera was not carried out.

Indirect anti-globulin test: When the cell suspension in fresh autologous serum and antiserum were combined, tubes presenting no agglutination, trace or plus 1 reaction were treated with anti-dog whole serum rabbit serum obtained from rabbits immunized with dog serum. The cells in each tube were washed three times with saline. Then they were resuspended in saline. Two drops of the diluted anti-dog serum rabbit serum were added to each resulting cell suspension and mixed well. The tubes were incubated at room temperature for 5 minutes and spun at 1500 rpm for 15 seconds. They were shaken weakly and then checked macroscopically for agglutination.

Results

The reaction patterns were compared between antisera prepared by the authors and anti-DEA sera against 15 panel cells. The results obtained are shown in Tables 1A and 1B. They indicated that none of the former antisera had the same specificity as anti DEA-1 • 1, 2 or -1 • 1, because the reactivity of anti-2a against 15 panel red cells was not identical with anti DEA-1 • 1, 2 or -1 • 1.

The reaction patterns of anti-D_1 and -E were identical with that of anti DEA-3, that of anti-A was with that of anti DEA-5, and that of anti-180a with that of anti DEA-8 (Table 1B). Anti-H, -I, -L and -43 prepared by the authors had the same specificity (Table 1B).

DEA 5-positive red cells (Nos. 1, 5, 6, 12 and 14) reacted with anti-M strongly, and DEA 5-negative red cells (Nos. 7 to 11, and 13) with anti-M weakly (Table 1B). When anti-M was absorbed with DEA 5-positive red cells (Nos. 1, 5, 6, 12 and 14) of strong agglutinability against anti-M, the antibody activity disappeared against both red cells of strong and weak agglutinability. On the other hand, when the absorption was carried out on anti-M with DEA 5-negative red cells (Nos. 7 to 11, and 13) of weak agglutinability against anti-M, the antibody activity disappeared against DEA 5-negative red cells of weak agglutinability against anti-M (Nos. 7 to 11, and 13), but not reduced against DEA 5-positive red cells of strong agglutinability against anti-M (Nos. 1, 5, 6, 12 and 14). Moreover, no M(−) • DEA 5(+) type red cells were observed in this study. Namely, anti-M and anti DEA-5 had a common specificity (Table 2).

In the absorption test of various antisera with appropriate red cells, each antibody activity disappeared by the absorption of

Table 1A. Comparison of reaction pattern between anti-DEA and anti-2a for 15 types of dog panel red cells

Antiserum	Panel red cells (4% suspension in autologous serum)														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2a	0	0	2H 2+	3+	0	0	0	0	0	0	0	0	0	0	0
DEA-1·1,2	1+	0	3H 1+	3+	3+	3+	3+	0	0	0	0	0	3+	3+	3+
DEA-1·1	0	0	3+	3+	3+	3+	3+	0	0	0	0	0	3+	3+	3+

0 : No reaction. + : Agglutination. H : Hemolysis.
Tubes presenting no agglutination or plus 1 reaction were treated with anti-dog whole serum rabbit serum.

Table 1B. Comparison of hemagglutination between antisera prepared by the authors and anti-DEA for 15 types of dog panel red cells

Antiserum	Panel red cells (4% suspension in saline)														
	1	2	3	2	5	6	7	8	9	10	11	12	13	14	15
D_1	0	2+	2+	2+	0	0	2+	0	0	0	0	0	0	0	0
D_2	0	3+	3+	0	0	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
A	2+	0	0	0	2+	2+	0	0	0	0	0	2+	0	2+	0
B	1+	0	0	0	1+	0	0	1+	1+	1+	1+	1+	0	0	0
C	0	0	0	1+	0	0	0	0	0	0	0	0	0	$1+_w$	$1+_w$
E	0	1+	1+	1+	0	0	1+	0	0	0	0	0	0	0	0
F	0	0	0	0	1+	1+	0	0	1+	1+	1+	1+	1+	1+	1+
G	0	1+	0	1+	0	0	1+	0	0	0	0	0	0	0	1+
L (H,I,43)	1+	0	1+	0	1+	1+	0	0	1+	1+	1+	1+	1+	1+	0
M	3+	0	0	0	3+	3+	1+	1+	1+	1+	1+	3+	1+	3+	0
44	0	1+	0	0	1+	0	0	0	0	0	0	0	0	0	1+
180a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1+
DEA-3	0	2+	2+	2+	0	0	2+	0	0	0	0	0	0	0	0
DEA-4	1+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+
DEA-5	1+	0	0	0	1+	1+	0	0	0	0	0	1+	0	1+	0
DEA-6	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+
DEA-7	2+	2+	2+	2+	2+	2+	2+	0	2+	$1+_w$	2+	2+	2+	2+	2+
DEA-8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	$1+_w$

0 : No reaction. + : Agglutination. $+_w$: Weak agglutination.

Table 2. Absorption test of anti-M with M(+)·DEA 5(+) type, M(+)·DEA 5(−) type, and M(−)·DEA 5(−) type red cells

Antiserum	Type of absorbing cells	Indicator cells		
		M(+)·DEA 5(+)*	M(+)·DEA 5(−)**	M(−)·DEA 5(−)
Anti-M	M(+)·DEA 5(+)*	−	−	−
	M(+)·DEA 5(−)**	⧻	−	−
	M(−)·DEA 5(−)	⧻	+	−

* M(+)·DEA 5(+) type red cells show strong agglutinability with anti-M.
** M(+)·DEA 5(−) type red cells show weak agglutinability with anti-M.

Table 3. List of red cell antisera

Antiserum prepared by the authors	Specificity
D_1, E	DEA 3
A	DEA 5
M	DEA 5+extra
180a	DEA 8
D_2	
B	
C	
F	
G	
L (H, I, 43)	
44	
* 2a	

positive red cells, but not at all by negative red cells.

In this study, there were no antisera with the same specificity as anti DEA-1 · 1, 2, -1 · 1, -4, -6 or -7. Besides, none of anti-D_2, -B, -C, -F, -G, -H, -I, -L, -2a, -43 and -44 prepared by the authors were identical with any of anti-DEA sera (Table 3).

Discussion

The realities of investigation on dog blood groups have developed into a request for arrangement of blood typing antisera.

Comparison tests of antisera and antigens for dog blood grouping have been advanced in the International Workshops [17, 19]. Table 4 shows dog red cell antigen systems arranged by interchanges of organization by Vriesendorp et al. [17, 19] and Bull et al. [2] as leaders.

It has not been determined, however, whether the blood grouping antisera produced in Japan correspond to the antisera approved by the International Workshop or not. The authors could obtain 8 antisera which had been supplied by Dr. R. W. Bull and compared 16 antisera prepared by the authors with the anti-DEA sera for the purpose of standardizing dog blood grouping antisera.

Table 4. Nomenclature of dog red cell typing antisera authorized by International Workshops

Old	New
Anti–A	Anti DEA–1 · 1, 2
–A1	–1 · 1
–B	–3
–C	–4
–D	–5
–F	–6
–Tr	–7
–He	–8

Table 5. Phenotype and genotype of the D system

Phenotype	Genotype	Anti–D_1*	Anti–D_2*
D_1	D^1/D^1	+	–
D_2	D^2/D^2	–	+
D_1D_2	D^1/D^2	+	+

* Anti–D_1 and –D_2 are immune antibodies obtained from rabbits following immunization with dog red cells.

The reaction patterns were compared among 24 dog blood grouping antisera and 15 samples of dog panel red cells. None of the antisera prepared by the authors had the same specificity as anti DEA-1 · 1, 2 or 1 · 1. Anti-D_1 and -E were identical with anti DEA-3, anti-A was with anti DEA-5, and anti-180a with anti DEA-8. Anti-M and anti DEA-5 contained a common specificity.

In this comparison test, it was very interesting to note that the specificity of anti-D_1 was identical with that of anti DEA-3. Furthermore, there was no anti-DEA that had the same specificity as anti-D_2 prepared by the authors. Anti-D_1 and -D_2 were immune antibodies obtained from rabbits following immunization with dog red cells. Genetically, it was confirmed that D_1 and D_2 antigens in the D system were co-dominant factors (Table 5) [3, 7]. This D system is a historic system in Japan.

DEA 3 antigen was recognized by the International Workshop. It is presumed that

Table 6. Comparison of phenotype frequencies of D_1 and DEA 3 antigen on dogs reared in Japan and U.S.A.

Red cell antigen	Present authors			Swisher & Young*		
	No. of dogs tested	No. of positive dogs	%	No. of dogs tested	No. of positive dogs	%
D_1	543	39	7.2			
DEA 3(B)				867	48	5.5

* Cited from *Physiol. Rev.* **41**, 495–520 (1961).

Table 7. Frequencies of D antigens in dogs of various breeds reared in Japan

Phenotype	% Positive dogs (No. of dogs tested)					
	Beagle	Shepherd	Mongrel	Shiba	Akita	Terrier
D_1	0.0 (346)	0.0 (40)	7.2 (543)	7.7 (13)	57 (7)	14 (7)
D_2	99.4 (346)	95.0 (40)	63.9 (543)	15.4 (13)	14 (7)	86 (7)
D_1D_2	0.6 (346)	5.0 (40)	28.9 (543)	76.9 (13)	29 (7)	0 (7)

DEA 3 may have the same antigenic membrane structure as D_1 determinants. D_1-positive mongrel dogs bred in Japan exhibited almost the same frequency of appearance as DEA 3-positive dogs bred in the U.S.A. (Table 6). In the future, if anti-D_2 serum is presented to the workshop, the allelic relationship between D_1 and D_2 antigen will be authorized.

On the dog blood groups detected by immune rabbit sera several investigators performed studies with different results. Namely, Suzuki et al. [14] described that immune antibodies obtained from rabbits immunized with dog red cells were not so sufficiently available for use in the preparation of dog blood grouping antiserum. In Japan, Iseki and Terashima [7] and Yoh [21] divided dog blood groups into 3 types, D_1, D_2 and D_1D_2, Yamada et al. [20] into 4 types, D_1, D_2, D_1D_2 and X by using anti-D_1 and -D_2, and Iwasaki et al [8, 9] into 10 types from the combinations of antigens by using anti-D_1 to anti-D_7. From the results mentioned above, it is assumed that the International Workshop may have possessed no sufficient number of valuable materials.

The frequency of appearance of D antigen in dogs bred in Japan is shown in Table 7. That of D_2 antigen is higher in beagles, shepherds, and terriers than in mongrels, shiba and akita. That of D_1 antigen is higher in mongrels, shiba, akita and terriers than in beagles and shepherds. On the other hand, Vriesendorp et al. [19] observed no difference in that of DEA 3 antigen between beagles and mongrels.

From the results mentioned above, anti-D_2, -B, -C, -F, -G, -L (-H, -I, -43), -44 and -2a seem to be new antibodies at present, because none of them have the same specificity as any of anti-DEA sera, also each antibody activity was absorbed by appropriate positive red cells, but not by negative red cells. Anti-C was lectin extracted from the seed of *Clerodendron trichotomum*. It may be new distinguishable agglutinin against dog red cell antigens.

Vriesendorp et al. [19] found anti-N1 to -N8 sera to be new antisera for dog blood grouping. Further investigation may be necessary for the preparation of dog blood grouping antisera.

Acknowledgments. The authors are grateful to Drs. R. W. Bull and S. N. Swisher, of the Michigan State University, East Lansing, Mich., U.S.A., for their supply of dog blood typing antisera.

References

[1] Bowdler, A. J., Bull, R. W., Slating, R., and Swisher, S. N. (1971). Tr: A canine red cell antigen related to the A-antigen of human red cells. *Vox. Sang.* **20**, 542–554.

[2] Bull, R. W. (1978). Personal communicaiton.

[3] Ejima, H., Tagawa, M., Kurokawa, K., and Tanaka, H. (1976). Experimental studies on blood transfusion of dogs. I. Survey on blood group antigens by agglutination test with hetero- and iso-immune sera. *Bull. Nippon Vet. Zootech. Coll.*, No. 25, 163–168 (in Japanese).

[4] Hall, D. E. (1970). A naturally occurring red-cell antigen-antibody system in beagle dogs. *J. Small Anim. Pract.* **11**, 543–551.

[5] Hosoda, T. (1943). On the common antigens between horse and other animals' blood corpuscles. *Jpn. J. Vet. Sci.* **3**, 599–612 (in Japanese).

[6] Ikemoto, S., Sakurai, S., and Ejima, H. (1976). Genetic marker in beagle blood. Individual difference within blood groups detected by isohemagglutinin. *Jpn. J. Vet. Sci.* **38**, 647–649.

[7] Iseki, S., and Terashima, M. (1940). Canine blood types D_1 and D_2 demonstrated by immuno-agglutinin. *Tokyo Med. J.*, No. 3192, 1373–1374 (in Japanese).

[8] Iwasaki, Y., Mukoyama, H., Ikemoto, S., and Ichiki, H. (1969). The study on the canine blood groups. I. Identification of canine blood groups with iso-hemagglutination. *Jpn. J. Vet. Sci.* **31** (Suppl.), 97 (in Japanese).

[9] Iwasaki, Y., Ichiki, H., Ikemoto, S., and Oshima, H. (1969). The study on the canine blood groups. II. Classification of dog blood types with immunoantibody. *Jpn J. Vet. Sci.* **31** (Suppl.), 190–191 (in Japanese).

[10] Kelly, G. E. (1977). Current prospects for renal transplantation in veterinary practice. *Aust. Vet. J.* **53**, 53–60.

[11] Kita, T., Takahashi, M., Shibanai, O., Tazawa, M., and Fukano, T. (1957). Studies on the blood transfusion to dogs. (2) Investigation on the relation with the blood pressure and blood type of dogs and the applying of dried plasma to the transfusion. *Bull. Azabu Vet. Coll.* **4**, 89–107 (in Japanese).

[12] Mears, D. C., Sheil, A. G. R., and Barnett, A. L. (1969). Preparation of canine anti-A serum. *Aust. Vet. J.* **45**, 13–14.

[13] Schall, W. D., and Perman, V. (1975). Disease of the red cells. *In* Textbook of Veterinary Internal Medicine. Ettinger, S. J., editor, W.B. Saunders, Philadelphia, 1602–1605.

[14] Suzuki, Y., Stormont, C., Morris, B. G., Shifrine, M., and Dobrucki, R. (1975). New antibodies in dog blood groups. *Transplant. Proc.* **7**, 365–367.

[15] Swisher, S. N., and Young, L. E. (1961). The blood grouping systems of dogs. *Physiol. Rev.* **41**, 495–520.

[16] Tsutsui, T., Ozaki, R., Kogure, T., Nishiu, K., Ejima, H., and Hoshi, S. (1976). Studies on reproduction in the dog. XIV. Experimental induction of superfecundation. The 83rd Meet. of Jpn. Soc. Vet. Sci.

[17] Vriesendorp, H. P., et al. (1973). Joint report of the First International Workshop on Canine Immunogenetics. *Tissue Antigens* **3**, 145–172.

[18] Vriesendorp, H. M., Duyzer den Hortog, B., Smid-Mercx, B. M. J., and Westbroek, D. L. (1974). Immunogenetic markers in canine paternity cases. *J. Small Anim. Pract.* **15**, 693–699.

[19] Vriesendorp, H. M., et al. (1976). Joint report of the Second International Workshop on Canine Immunogenetics. *Transplant. Proc.* **8**, 289–314.

[20] Yamada, M., Makita, M., and Shinoda, K. (1958). Studies on the blood type of dogs. *Jpn. J. Blood Transfusion* **5**, 98–101 (in Japanese).

[21] Yoh, R. (1955). Studies on blood groups and serum protein types in Japanese mongrel dogs. *Jpn. J. Breeding* **5**, 7–14 (in Japanese).

[22] Yoshida, H., Sakurai, Y., Muto, S., Sato, S., Fukui, M., Ando, R., and Ikemoto, S. (1997). Individual difference of dog blood groups detected by *Clerodendron trichotomum* extract. *J. Vet. Med.*, No. 691, 85–87 (in Japanese).

要　　約

イヌ血液型判定用抗体の比較同定試験：江島博康・黒川和雄（日本獣医畜産大学第1外科学教室），池本卯典（自治医科大学法医学教室）——日本におけるイヌ血液型判定用抗体の標準化を目的として，Bull らより入手した抗 DEA 抗体8種ならびに著者らにより作製分類された抗体16種，計24種類について比較同定試験を行った．その結果，抗 D_1 および抗 E は抗 DEA-3 と同一型特異性を有し，抗 A は抗 DEA-5 と，抗 180a は抗 DEA-8 と同一型特異性を示した．また，抗 M は抗 DEA-5 と共通な抗体を含有していた．著者らの作製分類した抗体の中で抗 DEA-1·1, 2, -1·1, -4, -6, -7 と同一型特異性を示す抗体は認められなかった．また，抗 D_2, B, C, F, G, L (H, I, 43), 44 および 2a はいずれの抗 DEA 抗体とも符合しなかった．