大韓獸醫學會誌(1985) 第25卷 第1號
Korean J. Vet. Res., (1985) Vol. 25, No. 1, pp. 19~26

Studies on the Blood Protein Polymorphisms of Deer: *Cervus nippon, Cervus unicolor*

Young-jae Lim, Yung-yi Song*, Shozo Suzuki**, Kazue Thanaka**, Takashi Amano**, Yaetsu Kurosawa** and Makoto Katsumata**

Animal Hospital, Yong-in Farm Land, Korea; Dept. of Animal Husbandry, National Taiwan University, R.O.C.; Institute of Animal Serology, Tokyo University of Agriculture, Japan***
(Received March 15, 1985)

鹿의 血淸蛋白에 관한 硏究

林英在·宋永義*·鈴木正三**·田中一榮**·天野卓**·黑澤彌悅**·勝又誠**

龍仁自然農園 動物病院·臺灣大學 畜産學科*·東京農業大學**
(1984. 4. 2 授受)

抄錄: 韓國, 臺灣, 日本에서 飼育하고 있는 꽃사슴(Cervus nippon) 129頭와 물사슴(Cervus unicolor) 7頭에서 hemoglobin, transferrin, albumin carbonic anhydrase, slow-α_2 및 amylase型을 電氣泳動에 依하여 分析한 結果 다음과 같은 結論을 얻었다.

1. cellulose acetate에 依한 電氣泳動이 starch gel에 依한 電氣泳動보다 hemoglobin型 分離에 있어서 더 간편하며 시간이 적게 걸리고, 鮮明할 뿐만 아니라 永久保存이 可能하다.
2. 꽃사슴의 hemoglobin型은 Hb^F, Hb^{FS}, Hb^S型으로 分離되었으나 물사슴에 있어서는 全部 Hb^F型으로 나타났다.
3. hemoglobin β chain은 4가지型 즉 β-1, β-2, β-3 및 β-4로 分離되었다.
4. hemoglobin α chain은 α_1과 $\alpha_1\alpha_2$型으로 分離되었다.
5. slow-α_2型은 A型과 AB型으로 分離되었으며, 꽃사슴에 있어서는 AB型이 12% 出現하였으나 물사슴에서는 全部 A型으로 AB型은 없었다.
6. albumin型에서는 F型과 S型으로 分離되었으며 꽃사슴은 全部 F型이였고, 물사슴은 全部 S型이였다.
7. transferrin型, carbonic anhydrase型 및 amylase型은 全部 各各 1種類의 型이였다.

Introduction

Many invesitigations have been carried out concerning the blood type, saliva type, and serum type of many animals since Ehrlich and Morgenoth[11] who discovered individual differences in the erythrocytes of cattle and goat.[1,4,28~45]

In general it may be said that the modification of soluble protein can be found through electrophoresis, while the antigen of erytrocyte can be found through the discovery of antiserum taking place naturally. If the substitution of amino acid occurs in a protein as a result of mutation, it brings about the changes in the properties of the protein.

These changes influence the patterns of electr ophoresis. Many researches[39] have been conducted on protein polymorphisms and in recent years many studies have been made on the blood proteins such as transferrin (Tf), albumin (Alb), alkaline phosphatase (Akp), amylase (Am) and ceruloplasmin (Cp) of serum, and hemoglobin(Hb) and carbonic anhydrase (Ca) in the erythrocyte of various animals.

However, those of deer have not yet been clarified because of being hard to have the opportunities to obtain them. But lately in the Orient, especially in Korea, Taiwan, China, and the Soviet Union, young antlers of the deer have been consumed as a high-priced herb medicine. Accordingly, a large scale of deer breeding is now forming and attempts are being made to step up the production of young antlers of the deer by means of mixed breeding.

We have estabilished the blood scientific basis by analyzing polymorphisms of the serum proteins and hemoglobin and have acquired a general grasp of the component of genetic characters in the blood of the deer being raised in Korea, Taiwan, and Japan.

Materials and Methods

Blood samples were collected from deer being raised in Korea, Taiwan and Japan (Formosan deer; *Cervus nippon*, Sambor deer; *Cervus unicolor*) as shown in Table 1. The method of analysis of Hb was referred to Lim *et al.*[31], Chernoff and pettit,[8] and Shimaoka *et al.*[40] as shown in Table 2. The analysis of Tf and Alb were done by the method of Abe *et al.*[38], Kim,[24,26] and Voglino.[19] The method of Ferguson and Wallace[12] was used in analysing slow-alpha 2 globin and amylase was analyzied by the method of Ashton.[2] The analysis of carbonic anhydrase was referred to show and Prasad[16] and Hopkinson *et al.*[14]

Results and Discussion

The electrophoretic results of this experiment were shown in Fig. 1, 2 and 3 on Hb, in Fig. 4 on Alb, in Fig. 5 on Slow-alpha 2 globin, in Fig. 6 on Tf, in Fig. 7 on Am and in Fig. 8 on CA. The schematic diagrams of electrophoretic patterns of each protein were shown in Fig. 9.

Electrophoretic pattern of the hemoglobin phenotypes was similar to the results obtained by cellulose acetate and starch gel electrophoresis. From the results of scanning (Gelman DCD-16/575nm) of cellulose acetate electrophoresis, S-type is called to be the quantities more than 60% at the slower mobility, F-type to be the quantities more than 80% at the faster mobility, and SF-type is between S-type and F-type.

As shown in Table 3, 44 of 90 Formosan deer in Korea were F-type and all Formosan deer in Taiwan and in Japan were F-type; and one sambar deer in korea and six in Taiwan also were F-type.

The types of hemoglobin beta-chain were divided into β-1, β-2, β-3 and β-4 as shown in Fig. 9, All types of β-chains were composed of two chains namely the faster and slower one. β-1 was that the faster chain had 60% or more quantities than slower as a result of densitometry(Gelman DCD-16/572nm). β-2 was that the faster chain and the slower had about equally quautities, β-3, the slower chain had the quantities more than 60% with markedly preponderant, and β-4, the slower had the quantities more than 60% with the extensive width.

The hemoglobin S-type was indentical to hemoglobin β-3. But the relations between Hb β-chain (β-1, β-2, β-4) and F-type or FS-type were variable, Thus, we consider that the relations ought to be studied in the future.

The hemoglobin alpha-chain, as shown in Fig. 9, had two types:the one (α-1) migrated faster from origin to cathode than the other (α-2), thus there were α_1 type and $\alpha_1\alpha_2$ type. All Formosan deer and Samber deer in Korea were $\alpha_1\alpha_2$ type, and all deer in Japan and Taiwan were α_1 type. These facts are considered to be the result of the genetic differences between regions.

Albumin type was classified into F ad S type;F type was migrated faster and S-type slower. All the Formosan deer(Korea, Japan and Taiwan) were F-type. On the contrary all the Samber deer were S-type.

On the slow-α_2, the type which moved fast from origin to anode, was named B-type and slow A-type. B type was not present, but AB type and A

type were present that the present rate is same that as the following Table 3. Among 125 Formosan deer, AB type was 12%(15 deers), while 13 Formosan deer(14.4%) in Korea were AB type. Therefore, Formosan deer in Korea show a higher distribution rate of AB type than those in Japan (5.8%) and Taiwan(5.5%). All the types of transferrin, carbonic anhydrase, and amylase didn't show polymorphisms as shown in Fig. 6, 7, 8 and table 3. Carbonic anhydrase of deer moved more slowly than that of sheep, that is to say, it migrated less from origin.

Conclusion

The studies on phenotypes of hemoglobin, transferrin, albumin, carbonic anhydrase, slow-alpha 2 globin and amylase of 129 Formosan deer and 7 Sambar deer in Korea, Taiwan, and Japan were carried out by electrophoresis. The results obtained were as follows.

1. For the identification of hemoglobin phenotypes, cellulose acetate electrophoresis was simpler and clearer than starch gel electrophresis. The results obtained from the former method was able to be preserved for a longer time.

2. The Hb phenotypes of Formosan deer, were Hb^F, Hb^{FS} and Hb^S type on the other hand, that of sambar deer was only F type.

3. On hemoglobin β chain, β-1, β-2, β-3, and β-4 type were recogniged; and on hemoglobin α chain, α_1 and $\alpha_1\alpha_2$ type.

4. Slow-α 2 globin types were A and AB type. On Formosan deer, the frequency of appearance of Ab type was 12%, but on Sambar deer, only A type was present.

5. On albumin, F type and S type were clarified;

Table 1. Blood Sampling Places and Number of Individuals Examined

Species \ Area	Korea	Taiwan	Japan	Total
Formosan deer (Cervus nippon)	90	22	17	129
Sambar deer (Cervus unicolor)	1	6	0	7
Tatal	91	28	17	136

Table 2. List of Blood Proteins Examined

Proteins	Reference
Hemoglobin phenotype (Hb)	Lim and Suzuki (1984)
Hemoglobin-α (Hb-α)	Chernoff and Pettiti (1964)
Hemoglobin-β(Hb-β)	
Serum transferrin (Tf)	Abe et al. (1968)
Serum albumin (Alb)	Voglino, (1969) Abe et al. (1968)
Serum slow-α_2-macroglobin (slow-α_2)	Ferguson and Wallace (1961)
Serum amylase (Amy)	Ashton (1965)
Carbonic anhydraze (CA)	Shaw and Prasad (1970) Hopkinson et al. (1974)

Table 3. Phenotype Frequencies in Deer

Species	Population	No	Hb			Hb-α		Alb		slow-α_2		Tf	CA	Amy
			F	FS	S	α_1	$\alpha_1\alpha_2$	F	S	A	AB			
Formosan deer (*Cervus nippon*)	Korea	90	44	44	2	0	90	90		77	13	90	90	90
	Taiwan	22	22			22		*15		**17	**1	**18	**18	**18
	Japan	17	17			17		17		16	1	17	17	17
Sambar deer (*Cervus unicolor*)	Korea	1	1			0	1		1	1		1	1	1
	Taiwan	6	6			6			6	6		6	6	6
Total		136	90	44	2	45	91	122	7	117	15	132	132	132

*The Number of deer was 15. **The Number of deer was 18.

that of Formosan deer was F type and that of Sambar deer, S type.

6. On transferrin, carbonic anhydrase and amylase, no variations were recogniged, respectively.

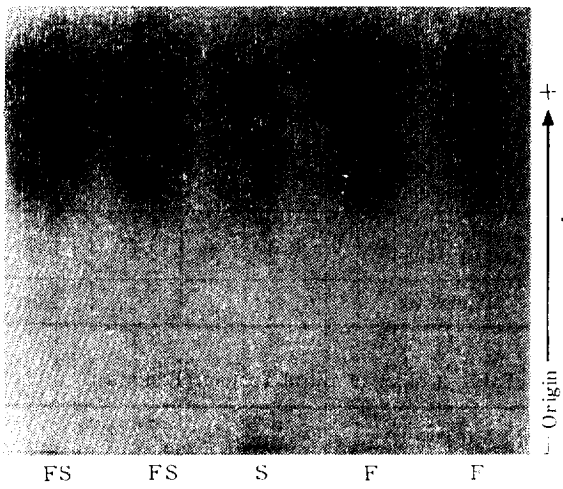

Fig 1. Hemoglobin phenotypes in deer by cellulos acetate electrophoresis.

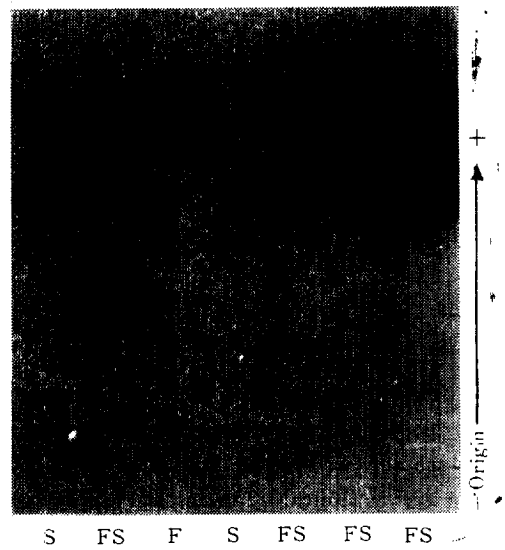

Fig 2. Hemoglobin phenotypes in deer by starch gel electrophoresis.

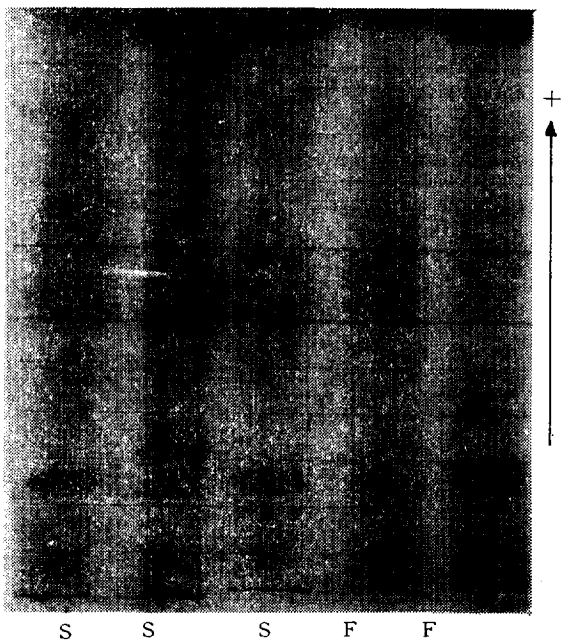

Fig 3. Hemoglobin types in deer by urea gel electrophoresis.

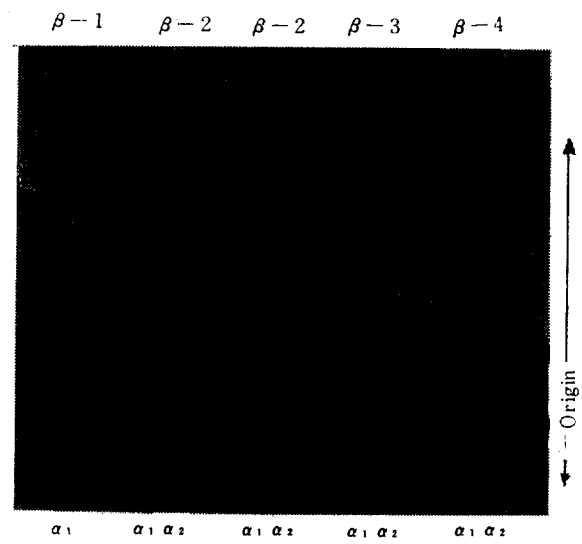

Fig 4. Albumin phenotypes in deer by starch gel electrophoresis.

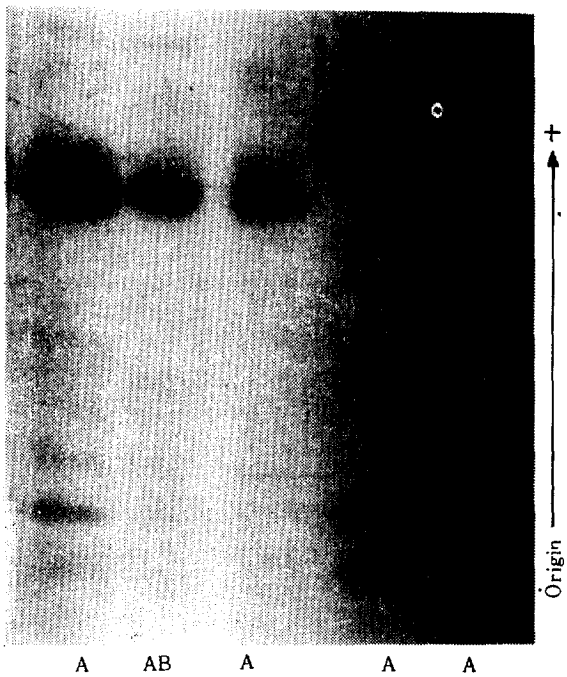

Fig 5. Slow-α_2 globin types in deer by starch gel electrophoresis.

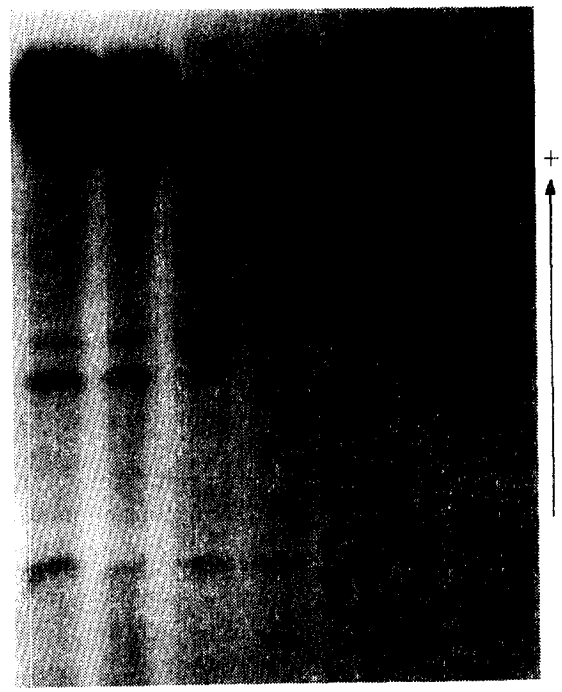

Fig 6. Transferrin phenotype in deer by starch gel electrophoresis.

Fig 7. Amylase type in deer by starch gel electrophoresis.

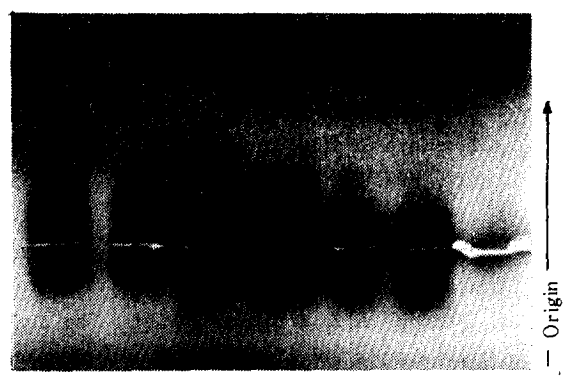

Fig 8. Carbonic anhydrase type in deer by starch gel electrophoresis.

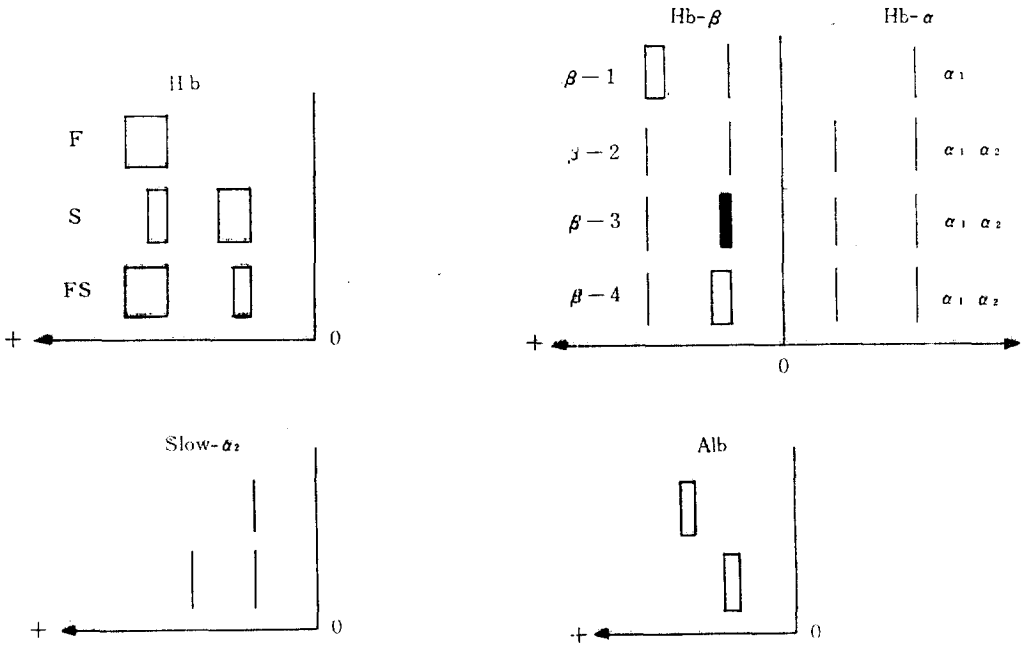

Fig 9. Electrophoretic patterns of blood protein variations in deer.

References

1. Abe, T.: The phlogeny of the East Asian cattle studied by blood typing. Japan Agr. Res Quart. (1970) 5:353.
2. Ashton, G.C.: Serum transferrin alleles in Australian cattle. Austr. J. Biol. Sci. (1965) 18.
3. Bangham, A.D. and Blumberg, B.S.: Distribution of electrophortically different hemoglobins among some cattle breeds of Europe and Africa. Natured. (1958)181:1551.
4. Braend, M.: Hemoglobin and transferrin type in the American buffralo. Nature. (1963) 197:910.
5. Braend, M.: Hemoglobin variants cattle. Animal Blood. Grps biochem. (1971) 2:15.
6. Cabannes, R. and Serain, C.: Heterogeneited 1. Hemoglobine des Bovides, Identification electrophoretique de deux Hemoglobines bovines. C.R., Soc. Biol. Paris(1955) 149:7.
7. Carr, W.R.: The hemoglobins of indigenous breeds cattle in central Africa, Rhod. J. agri. e. (1964) 9:94.
8. Chernoff, A.Z. and pettiti, N.M.: The amino acid composition of hemoglobin. III, A quantitative method for identifying abnormalities of hemoglobin. Blood (1964) 24:750-756.
9. Crockett, J.R., Koger, M. and Chapman, Jr. H, L.: Genetic variation in hemoglobin of beef cattle. J. Anim. Sci. (1963) 22:173.
10. Efremov, G. and Braend, M.: Hemoglobines, transterres and albumins of sheep and goats. Europ. Anim. Blood Group. Conf. (1964) 9:313.
11. Ehrlich, p. and Morenoth, T.: Aus dem Institut fur experimentelle therapie in Frankfurt a M. Uber Haemolysine. Berliner Klinische Wochemschrift. (1900) 37: 453.
12. Ferguson, K.A. and Wallace. A.L.C: Starch gel electrophoresis of anterior pituitary hormones. Nature(1961) 190:629-630.
13. Han, Sang Kee, and Suzki, S.: Studies on hemoglobin variants in Korean cattle, anim. Blood Grps biochem. Genet(1976) 7:21.
14. Hopkinson, D.A., Coppock, J.S., Muhlemann, M.F. and Edwards, Y.H.: The detectionn and differentiation of the products of the human carbonic anhydrase loci, CAI and CAII, using forogenic Substrates. Amm. Hum, Genet, London(1974) 38:155-162.
15. Oishi, T., Abe, T., Suzuki, S. and Namikawa, T.: Hemoglobin pholymorphisms in East Asia cattle and Formosan Water Buffalo. Imm-

unogenetics Letter(1968) 5:170.
16. Shaw, C.R. and prasad, R.: Starch gel electrophresis of enzymes: A complation of recipes. Biochemical Genetics(1970) 4:297-320.
17. Suzuki, S. and Watanabe, S.: Studies on the serolgical constitution of goats. Blood graps biochem. (1968) 7:513.
18. Shimaoka, T., Tanaka, K. and Suzuki, S.: Availability as paternity tost of nine loci on blood protein poymorphisms in six sheep breeds. J. Agr. Sci. (1981) 3:251.
19. Voglino, G.F.: Personal communication(1969)
20. Watanabe, S., Nozawa, K. and Suzuki, S.: Studies on the transferrin of goat. 1. Typing of transferrin of goat serum by starch gel electrophoresis. Furhats. M.J.A. (1965) 4:326.
21. Watanabe, S. and Suzuki, S.: Studies on the transferrin of goat. II. Inheritance mode of serum transferrin types. Proceedings of Japan Academy(1966) 42:178.
22. Watanabe, S. and Suzuki, S.: Studies on the transferrins of goats 3. Evidence for a third transferrin allele. Anim. Blood Grops biochem. (1973) 4:23.
23. 金宇權：犬의 血液型에 關한 硏究. 農漁村開發硏究(1970) 5:123.
24. 金宇權：개의 Hemoglobin, Albumin 및 Transferrin의 型에 關한 硏究. 大韓獸醫學會誌(1974) 14:11.
25. 金宇權, 李載洪, 金五南, 林貞澤, 韓邦根：反芻動物의 Hemoglobin型에 關한 硏究. 農漁村開發硏究(1977) 12:33.
26. 金宇權, 李載洪, 林貞澤, 韓邦根：乳牛의 血液蛋白質型에 關한 硏究. 全南大論文集(1979) 25:355.
27. 李載洪：韓牛의 血液型에 關한 硏究. 農漁村開發硏究(1970) 5:151.
28. 李載洪, 金宇權：山羊의 血淸 Transferrin型에 關한 硏究. 農漁村開發硏究(1975) 10:219.
29. 李載洪：澱粉 gel 電氣泳動法에 의한 염소의 血淸 Albumin型에 관한 硏究. 大韓獸醫學會誌(1975) 15:19.
30. 林鳳鎬：血淸蛋白質分劃에 對한 硏究. 大韓獸醫學會誌(1965) 5:1.
31. 林英在, 鈴木正三：꽃사슴(Formosan Deer)의 Hemoglobin型에 關한 硏究. 大韓獸醫學會誌(1964) 24(1)31.
32. 勝又誠, 天野卓, 田中一榮, 野澤謙, 朴根植, 朴鳳祚, 李載洪：韓國在來山羊における 血液蛋白の遺傳子 構成. 日畜會報(1982) 53:521.
33. 日本在來家畜調査團：日本在來家畜調査團報告(1969) 3:92.
34. 日本在來家畜調査團：日本在來家畜調査團報告(1970) 4:49.
35. 赤木昭治, 渡邊誠喜, 鈴木正三：緬羊の 血淸學的體質に關する硏究. 1. 血球抗原の分類. 日本畜産學會報(1968) 39:511.
36. 赤木昭治, 渡邊誠喜, 鈴木正三：緬羊の 血淸學的體質に關する硏究. Ⅳ. 緬羊血球と 他種動物 血球間の 共通性抗原について. 日本畜産學會報(1970) 41:63.
37. 赤木昭, 渡邊誠喜, 鈴木正三：緬羊の血淸學的 體質に關する硏究. Ⅳ. 血液型抗原およびそれの 抗體の 免疫化學的 性狀について. 日畜會報(1971) 42:513.
38. 阿部恒夫, 大酵雄, 鈴木正三, 天野卓, 近藤恭司, 野澤謙, 並河鷹夫, 態崎一雄, 古賀修. 林田重幸. 大塚閏一：東亞の在來家畜に關する硏究. 1. 東アジアにおける 牛の血液型ならびに蛋白質の 多型現象について. 日畜會報(1968) 39:523.
39. 佐佐木淸綱監修：家畜の血液型とその應用(1971)養賢堂, 東京.
40. 島岡達朗, 田中一榮, 角田健司, 鈴木正三：めん羊における 血球中の Hemoglobin α, β X-protein及び NADH diaphorase並び血漿中の Alkaline phosphatase及び Transferrinの傳的遺變異. 東京農業大學 農學集報(1980) 25:145.
41. 池本卯典：人類の 血液型 と 動物の 血液型(上). 獸醫畜産新報(1966) 434:1212.
42. 池本卯典：人類の 血液型と 動物の 血液型(中) 獸醫畜産新報(1966) 437:1388.
43. 池本卯典：人類の 血液型と 動物の 血液型(下) 獸醫畜産新報(1967) 439:72.
44. 鈴木正三. 渡邊誠喜：緬羊の 血液型に關する 硏究. 1. 血球抗原の 分類と 型特異的 抗原物質の血淸化學的 性質. 東京農業大學 家畜育種學硏究室創設 15周年記念論文集(1965)：29.
45. 鈴木正三, 茂木一重, 細田達雄：山羊の 血液型に關する硏究. Ⅱ. 型物質の 血淸化學的 性狀並びに山羊の 血淸型. 日畜會報(1956) 27:191.