Experimental Blood Cell Counting on Several Kinds of Animals with an Automated Hematology Analyzer

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We undertook the blood cell counting on blood from 15 kinds of animals including laboratory animals, such as rat, mouse and rabbit, and using the human blood measurement conditions of an automated hematology analyzer. We found the analyzer can count the numbers of leukocytes, erythrocytes, and platelets of the various animals except for the platelets of goats and sheep. Since some of the erythrocytes overlapped the cell size distribution of platelets, the numbers of platelets of goat and sheep could not be counted.

The cell size distribution curve of the leukocytes of dog, cat, ferret, hamster, and cheetah showed the single peak distribution curve, and other animals showed a double peak.

A hematocrit ratio (PCV/HCT) of the centrifugal hematocrit value (PCV) to HCT value in the human blood measurement conditions of automated hematology analyzer was 0.65 of minimum in the goat and 1.08 of maximum in the elephant.

Key Words Blood Cell Counting, Exotic Animals, Industrial Animals, Laboratory Animals, Wild Animals

Received 3 November, 2006; Accepted 6 November, 2006

INTRODUCTION

During the past several years we have received many inquiries from veterinary hospitals, university research laboratories, and animal-related public organizations about blood cell counting on animals other than dogs and cats.

We have obtained some useful information by making measurements on the blood cells of 15 types of animals, including exotic animals, laboratory animals, industrial animals, and wild animals etc. with an automated hematology analyzer, and report the results below.

MATERIALS AND METHODS

The automated hematology analyzer used in this study did not have inbuilt programs for measuring conditions for animals other than dogs and cats. Therefore, the blood samples of the different animals of this study were analyzed under the measuring conditions meant for human blood. The centrifugal hematocrit value was obtained with the micro hematocrit centrifuge 3220 (KUBOTA Corporation, Japan) run at 12,000 rpm for 6 min. The HCT, MCV, and MCHC values given in ***Table 1*** are values corrected manually by applying correction factors. These correction factors were the mean values of the hematocrit ratio (PCV/HCT) calculated from the centrifuge hematocrit value (PCV) and the HCT value measured by the automated hematology analyzer.

Note: This article is translated and republished from Sysmex Journal Vol. 29: 46-53, 2006.

Table 1 *Measured values of the different types of animals*

		WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT	Hematocrit ratio*
Ferret n=12	MIN	27	610	9.9	27.8	42.9	14.9	32.5	9.0	0.765
	MAX	155	1391	22.3	66.4	48.2	16.8	35.6	147.0	0.911
	AV	86.2	980.3	15.2	44.6	45.5	15.5	34.2	39.9	0.855
	SD	42.6	257.9	4.0	12.0	1.5	0.6	1.0	39.6	0.039
Prairie Dog n=10	MIN	39	485	7.4	24.5	50.6	15.3	28.7	31.1	0.887
	MAX	170	947	15.9	55.3	66.5	21.3	35.2	78.1	1.020
	AV	79.3	688.8	12.4	40.3	58.3	18.1	31.0	46.8	0.962
	SD	46.5	166.3	2.8	10.3	4.8	1.8	1.9	16.6	0.040
Rat n=20	MIN	14	726	13.5	41.9	50.5	15.9	30.7	69.2	0.889
	MAX	146	945	15.8	50.2	61.6	19.6	33.0	139.0	0.990
	AV	74.6	819.9	14.7	45.8	56.1	18.0	32.1	108.3	0.930
	SD	31.8	71.4	0.8	2.3	3.3	1.2	0.6	16.4	0.024
Mouse n=20	MIN	5	716	10.7	33.2	45.8	14.4	30.7	95.8	0.804
	MAX	46	920	14.3	43.8	49.5	16.3	33.7	183.4	0.902
	AV	23.3	830.6	12.8	39.6	47.7	15.4	32.2	146.1	0.859
	SD	12.1	55.7	0.9	2.5	1.1	0.5	1.0	29.8	0.026
Hamster n=12	MIN	23	728	15.3	46.7	57.8	19.3	32.4	38.8	0.911
	MAX	69	940	18.5	55.1	64.1	21.2	33.9	197.0	0.971
	AV	41.3	852.3	17.2	51.6	60.7	20.2	33.3	75.3	0.943
	SD	15.3	72.5	1.0	2.7	2.2	0.6	0.5	48.0	0.020
Rabbit n=20	MIN	54	563	10.7	34.4	57.3	17.8	31.1	27.3	0.896
	MAX	116	696	14.5	45.3	67.2	22.3	33.4	61.9	0.962
	AV	81.8	640.0	12.9	40.2	62.7	20.2	32.2	47.5	0.933
	SD	18.4	44.6	1.0	3.2	2.4	0.9	0.7	8.3	0.018
Horse n=36	MIN	55	663	10.8	29.7	41.6	15.0	33.3	9.7	0.870
	MAX	167	1165	19.7	55.1	48.7	17.4	37.7	19.8	1.050
	AV	88.8	976.5	15.8	44.5	45.5	16.2	35.5	13.1	0.926
	SD	19.7	108.1	1.8	5.2	1.7	0.6	1.0	3.0	0.032
Swine n=42	MIN	122	533	10.6	32.5	51.1	15.6	29.6	17.3	0.813
	MAX	262	853	15.5	47.3	63.4	21.6	35.5	131.0	0.911
	AV	180.0	733.5	13.3	40.6	55.6	18.3	32.9	37.3	0.869
	SD	37.0	83.4	1.0	3.3	3.4	1.4	1.1	23.2	0.022
Goat n=18	MIN	42	1170	8.5	24.3	20.2	6.6	30.8		0.613
	MAX	139	2109	15.9	50.3	25.1	8.0	35.6		0.756
	AV	88.8	1598.2	11.7	35.9	22.3	7.3	32.7		0.681
	SD	30.5	255.2	2.0	7.2	1.3	0.4	1.4		0.037
Sheep n=31	MIN	11	820	10.0	28.0	30.1	10.4	33.6		0.704
	MAX	96	1455	16.2	48.2	36.6	12.9	38.6		0.793
	AV	48.2	1079.0	12.4	34.7	32.1	11.5	35.7		0.742
	SD	16.0	138.6	1.4	4.6	1.5	0.6	1.4		0.022
Holstein n=55	MIN	37	468	8.5	23.9	33.6	9.8	29.0	24.2	0.791
	MAX	180	1150	13.4	41.7	53.6	19.8	37.3	175.5	0.983
	AV	88.5	737.5	10.9	32.2	44.7	15.2	33.9	56.9	0.915
	SD	25.4	159.5	0.9	3.6	5.5	2.7	2.3	36.5	0.042
Black Japanese n=46	MIN	32	695	9.1	26.2	32.2	10.3	31.5	15.6	0.795
	MAX	94	1134	18.6	48.1	54.0	20.9	38.9	51.8	0.947
	AV	64.0	924.7	13.0	37.2	40.9	14.4	34.8	28.6	0.868
	SD	15.9	133.8	2.1	4.8	7.2	3.4	2.2	8.9	0.039
Elephant n=5	MIN	103	252	11.2	32.9	123.0	41.4	33.6	18.7	1.044
	MAX	230	310	13.3	39.3	135.0	46.3	34.5	36.8	1.098
	AV	147.4	282.6	12.4	36.5	129.4	44.1	34.1	27.3	1.080
	SD	52.4	22.1	0.8	2.3	4.6	2.0	0.4	6.9	0.023
Bharal n=8	MIN	54	1365	12.9	35.7	24.5	8.4	33.5	43.0	0.643
	MAX	127	2031	18.6	55.5	27.3	9.6	36.6	90.3	0.804
	AV	87.5	1586.4	14.6	41.1	25.9	9.2	35.5	71.1	0.700
	SD	22.5	213.2	1.8	6.3	1.0	0.4	1.1	19.0	0.047
Cheetah n=2	MIN	92	796	13.3	42.1	43.9	13.9	31.6	25.7	0.767
	MAX	106	959	14.5	44.4	55.7	18.7	33.6	28.7	0.789
	AV	99.0	877.5	14.1	43.2	49.8	16.3	32.6	27.2	0.778
	SD	9.9	115.3	1.1	1.6	8.4	3.4	1.4	2.1	0.016

Hematocrit ratio*: Centrifuge hematocrit value/HCT value obtained under the measeurement conditions for human blood

[Units]

WBC: $\times 10^2/\mu L$ MCV: fL MIN: Minimum
RBC: $\times 10^4/\mu L$ MCH: pg MAX: Maximum
HGB: g/dL MCHC: g/dL AV: Average
HCT: % PLT: $\times 10^4/\mu L$ SD: Standard deviation

1. Exotic animals

1) Ferret
The blood of 12 ferrets, 9 males and 3 females (aged 1-6 years), which were brought to the Exotic Pet Clinic was sampled by cutting a nail and collecting the dripping fresh blood directly into a Capiject blood collection tube containing Na2EDTA (TERUMO Corporation, Japan ; hereinafter "Capiject tubes").

2) Prairie dog
The blood was drawn from the femoral vein of 10 prairie dogs, 4 males, 4 females, and 2 castrated males (aged 2-8 years) that were brought to the Exotic Pet Clinic, with a manual syringe, and transferred into Capiject tubes.

2. Industrial animals

1) Cattle
The blood was drawn under vacuum from the jugular vein of 46 head of Black Japanese and 55 head of Holsteins of the Hyogo Prefectural Technology Center for Agriculture, Forestry and Fisheries into 5mL vacuum blood collection tubes containing K2EDTA (TERUMO Corporation, Japan ; hereinafter "2K-5mL tubes").

Black Japanese:
28 growing steers, castrated males (aged 9-10 months)
18 breeding bulls (aged 3-6 years)

Holstein:
19 heifers (uncalved) (aged 0 to 28 months)
36 adult cows (aged 2-8 years)

2) Horse
The blood was drawn under vacuum from the jugular vein of 36 thoroughbreds of the Hyogo Prefecture Horse Racing Union into 5mL vacuum blood collection tubes containing Na2EDTA (TERUMO Corporation, Japan).

Thoroughbred:
19 males (aged 3-6 years)
15 females (aged 3-6 years)
2 castrated males (aged 3-6 years)

3) Swine
The blood was drawn under vacuum from the jugular vein of 42 pigs of the Hyogo Prefectural Technology Center for Agriculture, Forestry and Fisheries, into 2K-5mL tubes.

Large Yorkshire:
4 males (aged 5-7 months), 2 males (aged 4 and 5 years), and 5 females (aged 5-7 months)
Landrace:
2 males (aged 4 and 5 years) and 3 females (aged 7 months)
Duroc:
4 males (aged 2-5 months), 5 females (aged 2-6 months), and 2 females (aged 1 and 2 years)
Crossbreeds of the above 3 varieties:
7 males (aged 3-8 months), 4 females (aged 5-8 months), and 4 females (aged 1-4 years)

4) Goat
The blood was sampled from the jugular vein of a total of 18 goats from 3 farms given below. Manual syringes were used to collect blood from goats of the Kobe City Rokkousan Pasture and the Awaji Farm Park England Hill and the sampled blood was transferred to 2K-5mL tubes. For goats of the Himeji Central Park, the blood was drawn under vacuum into the collection tubes of the same type.

Kobe City Rokkousan Pasture
Saanen: 1 male (aged 7 years)
Crossbreeds: 7 females (aged 4-9 years) and 2 castrated males (aged 6 and 7 years)
Awaji Farm Park England Hill
Angora goat: 2 males (aged 6 years)
Yakushima goat: 2 females (aged 8 and 9 years)
Himeji Central Park
Crossbreeds: 1 male and 3 females (aged 1-4 years)

5) Sheep
The blood was collected with manual syringes from 31 sheep of the Awaji Farm Park England Hill, and transferred to 2K-5mL tubes.

Corriedale: 23 males (aged 1-6 years) and 5 castrated males (aged 2 and 3 years).
Suffolk: 1 female (aged 8 years)
Southdown: 1 female (aged 2 years)
Suffolk crossbreeds: 1 castrated male (aged 2 years)

3. Laboratory animals

1) Rabbit
The blood was collected from an auricular posterior vein of 20 male Std.NZW rabbits (aged 11-44 weeks) with manual syringes and transferred to 2mL vacuum collection tubes containing K2EDTA (TERUMO Corporation, Japan ; hereinafter "2K-2mL tubes").

2) Rat
The blood was collected from a total of 20 SPF/VAF rats, 10 males and 10 females, (aged 8 and 40 weeks). The animals were given ether anesthesia, their abdomen opened, and the blood collected from the heart with a manual syringe and transferred to 2K-2mL tubes. The animals were killed after cardiac puncture without regaining consciousness.

3) Mouse
The blood was collected from a total of 20Crj: CD mice, 10 males (aged 10 and 28 weeks) and 10 females (aged 10 and 32 weeks). The animals were given ether anesthesia, the abdomen opened, and the blood collected from the heart with a manual syringe and transferred to 2K-2mL tubes. The animals were killed after cardiac puncture without regaining consciousness.

4) Hamster
The blood was collected from 6 male and 6 female Syrian hamsters (all aged 10 weeks). The animals were given ether anesthesia, the abdomen opened, and the blood collected from the heart with a manual syringe and

transferred to 2K-2mL tubes. The animals were killed after cardiac puncture without regaining consciousness.

4. Wild animals

1) Elephant
The blood was drawn under vacuum from the auricular vein of a total of 5 African elephants, 2 males (aged 13 and 23 years) and 3 females (aged 22-23 years), of the Himeji Central Park into 2K-5mL tubes.

2) Bharal
The blood was drawn under vacuum from the jugular veins of a total of 8 bharals, 3 males (aged 2-5 years) and 5 females (aged 3 and 12 years) of the Himeji Central Park into 2K-5mL tubes.

3) Cheetah
The blood was drawn under vacuum from the caudal veins of 2 male cheetahs (aged 6 and 8 years) of the Himeji Central Park into 2K-5mL tubes.

RESULTS AND DISCUSSION

We examined whether it was possible to measure WBC, RBC, and platelets from the cell size distribution curves. ***Fig. 1*** shows typical blood cell size distribution curves of the different animal species, and compares them with those of humans, dogs, and cats. ***Table 1*** shows the minimum, maximum, average, and standard deviation of the measured values and the hematocrit ratio for each animal type.

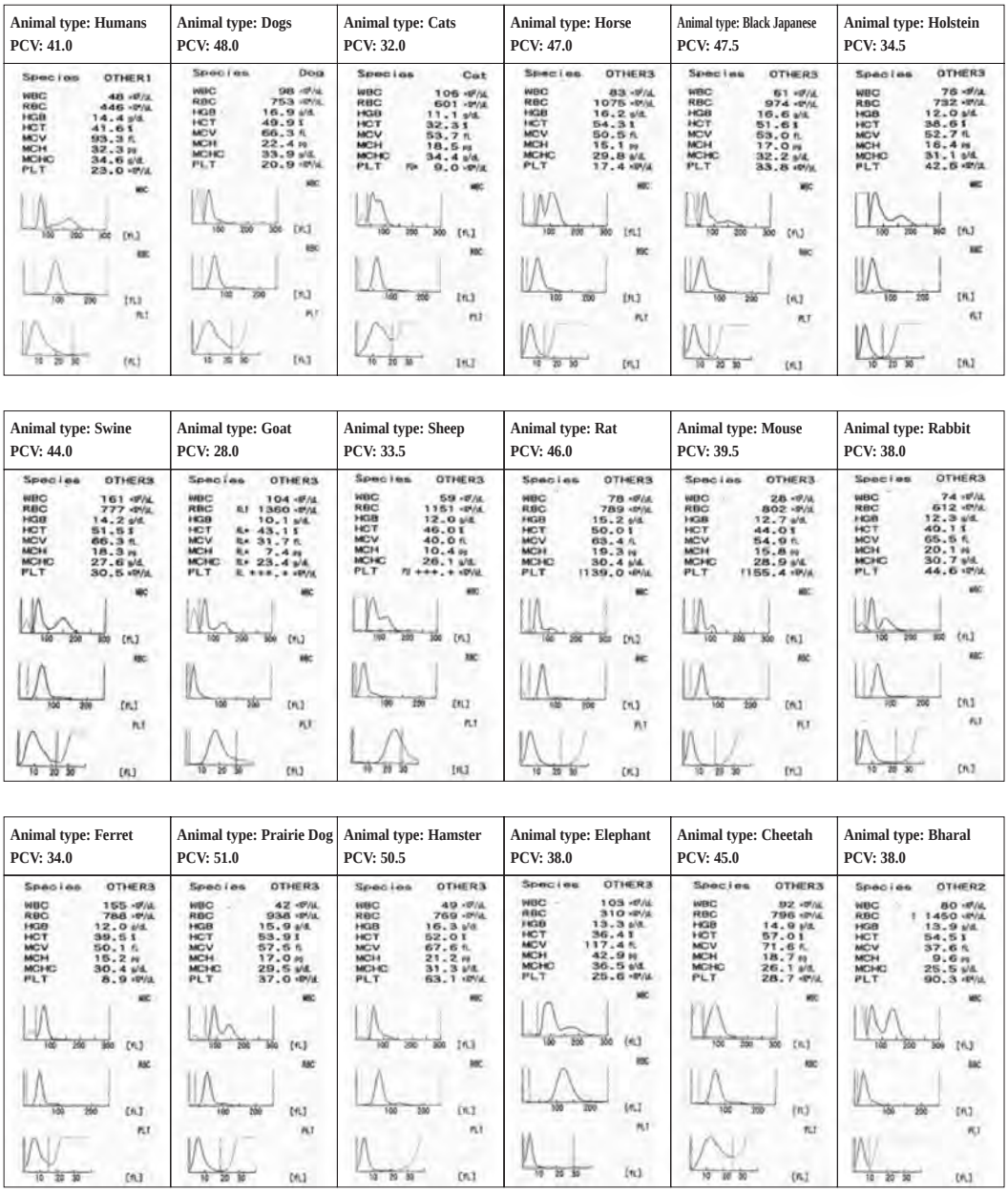


Fig. 1 *Blood cell size of the different types of animals*

1. WBC counting

It was possible to determine the WBC counts from the cell size distribution curves of the animals other than dogs and cats, without major problems. As can be seen from ***Fig. 2***, in bovine blood, there were some RBCs that were not completely lysed by the hemolyzing reagent. Because of this, the cell size distribution of RBC ghosts partly overlapped the cell size distribution of the WBCs, possibly affecting the WBC counts in some samples. WBCs of the control animals (dogs and cats) and the ferrets, hamsters and cheetahs did not have high resistance against the hemolyzing reagent and therefore the cell size distribution had a single peak. But the rest of the animals showed two-peak distributions. Among the 15 types of animals whose blood was analyzed in the present study, mice had the lowest WBC counts at 5-46 x 10^2/uL and pigs had the highest at 122-262 x 10^2/uL.

2. RBC counting

Judging from the cell size distribution curves, there appears to be no problem in measuring RBCs, except with goat's RBCs. In the case of goats, some RBCs were apparently missed while counting at the set sensitivity of the analyzer. The RBC count was the highest in goats, at 1170-2109 x 10^4/uL. It was the lowest in elephants, at 252-310 x10^4/uL. MCV was the highest in elephants at 123-135fL and smallest in goats at 20.2-25.1fL. ***Fig. 3*** is a photomicrograph that compares the RBCs of elephants with those of goats.

The ancestors such as goats and sheep had lived near the tops of high altitude mountains. Therefore, the RBCs became smaller and more numerous to increase the surface area, to achieve a higher gas exchange capacity. The bharal, which was distributed in mountainous regions, had an RBC count of 1365-2031 x 10^4/uL and MCV of 24.5-27.3fL, which were similar to the levels of goats.

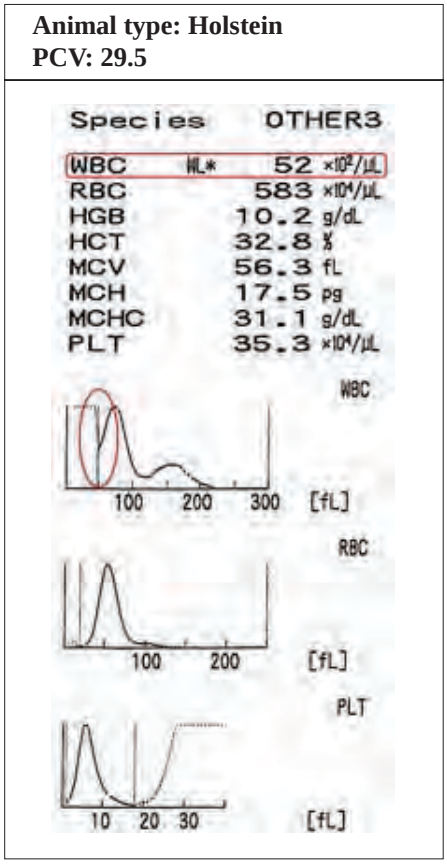
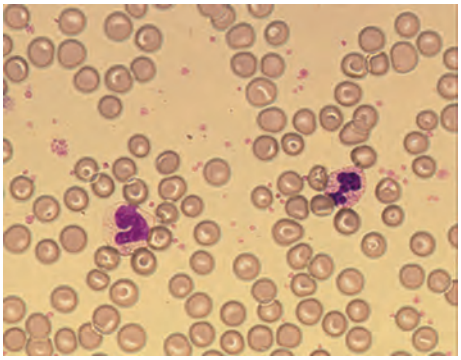
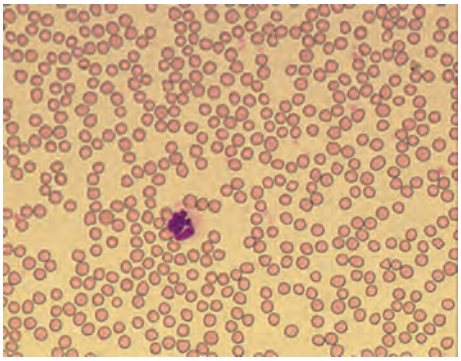


Fig. 2 *RBC ghosts*



Elephant



Goat

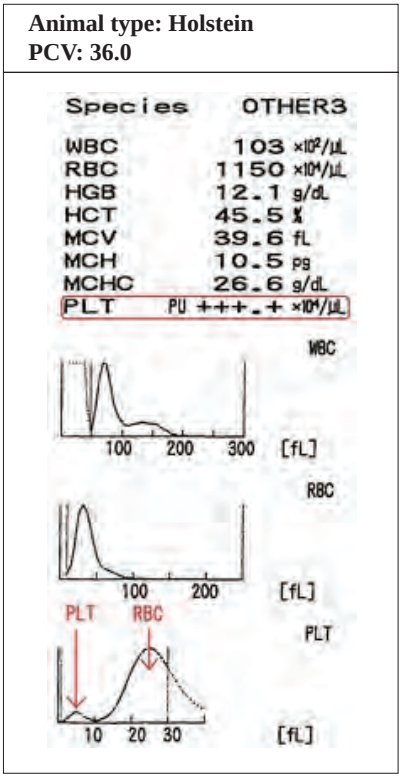
Fig. 3 *Photomicrograph*

3. Platelet counting

With goats and sheep, the platelet counts went beyond the measurement range because the RBCs overlapped their measuring range, making it impossible to accurately count the platelets. The reason was not only the small size of the RBCs but the fact that the relative sizes of the RBCs and platelets caused the overlapping of their cell size distributions. It was possible to measure platelets in bharals, which had MCV in almost the same range (24.5-27.3fL) as in goats because their cell size distribution of platelets did not overlap with that of RBCs. Platelet counting was also impossible with many calves that were less than one year old (***Fig. 4***) because their cell size distribution curves was very similar to that of goats and sheep. However, a small peak of platelets could be seen, and it is assumed that measurement may become possible if the automatic search range of the platelet fraction is suitably adjusted. There was no problem with platelet counting in the other animals. Animal blood gets coagulated more easily than human blood. Therefore, in some samples that were suspected, from the shapes of their cell size distribution curves shown in ***Fig. 5***, to have had platelet aggregation at the time of sampling, the aggregation was confirmed with smear samples and the data from samples that showed aggregation was excluded from the calculation of the minimum, maximum, average, and standard deviation given in ***Table 1***.

4. Hematocrit ratio (PCV/HCT)

The volume of blood cells changes greatly, depending on the environment they are placed in. The centrifugal hematocrit value (PCV) is the ratio of the volume of RBC cells packed by applying physical pressure. On the other hand, the hematocrit value (HCT) calculated by the hematology analyzer is measured on blood cells that are in an expanded or a shrunk state because of the osmotic pressure and pH of the diluting solution or the chemicals in it, etc. Thus, the measuring principle is very different in PCV and HCT. But, as a whole, PCV was correlated with HCT, although they were not correlated in some samples. Currently, because of the efficiency, the HCT values measured with hematology analyzers are used more often than the centrifugal hematocrit values. In practice, the rate of change in the RBC volume caused by the physical and chemical factors in the environment varies greatly depending on the animal species. Therefore, we have to apply a correction for each animal species, by obtaining a conversion factor that would bring the HCT values close to the centrifugal hematocrit values. In the present study, the number of samples used appeared to be not large enough in some animal species for determining the proper conversion factors. But when we calculated PCV/HCT, i.e., the ratio of centrifugal hematocrit value/the HCT value under the conditions of the automated hematology analyzer meant for analysis of human blood, goats had the lowest average ratio of 0.68



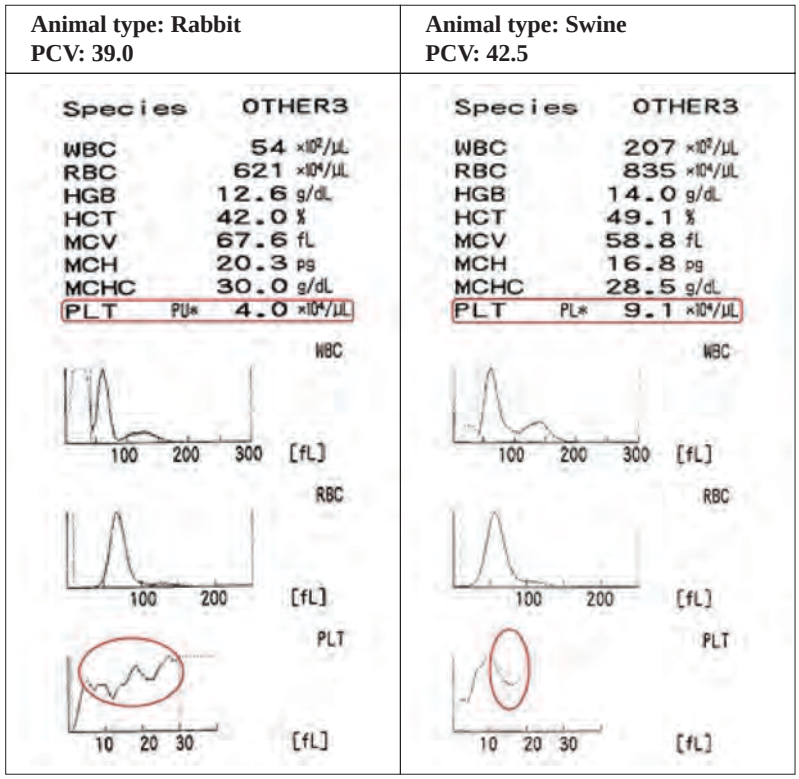


Fig. 4 *Sample of calves*

Fig. 5 *Sample of platelet aggregation*

and elephants the highest (1.08). Only prairie dogs, in some cases, and elephants had ratios of 1.00 or higher, and the others had lower than 1.00.

CONCLUSION

Measurements with the multiparameter automated hematology analyzer on the blood of 15 types of animals, under measurement conditions which had been optimized for human blood, showed that it was generally possible to measure the parameters except for the platelet counts in goats, sheep, and less than one year old bovines. The number and size of blood cells differed from one species to another. Therefore, it is necessary to set the measurement thresholds for each type of animal and correct the hematocrit value. With the blood of different types of animals, it was not possible to make accurate measurements of the parameters directly with the hematology analyzer set for human blood. The hematocrit ratio (the correction factor) shown in ***Table 1*** would differ within the same animal species, depending not only on the sex and age but also on the automated hematology analyzer used for the measurements. Therefore, it is necessary to determine the correction factor for the automated hematology analyzer used. On the basis of the results obtained in this study, we plan to make the next version of the analyzer capable of making measurements on the blood cells of a number of different animal species.

ACKNOWLEDGEMENT

We would like to express our sincere gratitude to the Exotic Pet Clinic, Hyogo Prefectural Technology Center for Agriculture, Forestry and Fisheries (Livestock Technology Institute and Awaji Agricultural Institute), Hyogo Prefecture Horse Racing Union, Himeji Central Park, Kobe City Rokkousan Pasture, and Awaji Farm Park England Hill, and the staff of these organizations, who provided the animal blood samples for the present study.

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