A Novel Method for Estimating the Adaptive Ability of Guide Dogs Using Salivary sIgA

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ABSTRACT. Salivary secretory immunoglobulin A (sIgA) concentrations of prospective guide dogs for the blind were determined to clarify whether salivary sIgA is useful in evaluating the potential suitability of guide dogs for the blind. Saliva was collected from 73 prospective guide dogs in the kennel on day 1 (the day of separation from volunteer puppy-raisers), 2, 3, 7 and 14 during the estimation period (at about 1 year old). We selected particularly suitable dogs (superior dogs) and unsuitable dogs (inferior dogs) on the basis of the trainers' estimation. All dogs were divided into two groups, those were acceptable dogs would become the guide dogs and rejected dogs could not become guide dogs. The sIgA concentrations in superior dogs gradually increased from day 1 to 14 and those in inferior dogs remained at low levels. Moreover, the sIgA concentrations on day 14 in the acceptable dogs were significantly higher than those in rejected dogs. The cut-off point of sIgA concentrations on day 14 using an ROC curve was 90 EU/ml, and the specificity of the estimation at this point (70.4%) was higher than that of trainers' estimation (50%). Moreover, parallel testing using both trainers' estimation and sIgA estimation, showed that specificity was further improved (79.5%). The present study showed that sIgA concentration was extremely useful in estimating the adaptive ability for guide dogs for the blind.

KEY WORDS: canine, guide dog, saliva, sIgA, stress.

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In Japan, 895 guide dogs are working and provide physical and mental support to the sight impaired. The raising of guide dogs is not easy, and the cost and labor involved is huge. Therefore, it is difficult to train more guide dogs, although many sight impaired need their own guide dogs.

Usually, prospective guide dogs are raised in the homes of volunteer puppy-workers from 7 weeks to 1–2 years old. After that, they return to the guide dogs associations and are estimated in terms of temperament for 1 week to 1 month (estimation period). Only the adaptive dogs are trained to become guide dogs for 1–2 years, including training with their users. They finish training and begin work as guide dogs when they are 2 years old. The appropriate method and time for the estimation of temperament is controversial, as most of the reasons for the rejection of guide dogs are related to behavioral problems rather than physical problems [5, 17, 22].

With regard to the estimation period, most guide dogs associations estimated prospective dogs' behavior by several trainers. However, in many cases, the results from the various trainers were inconsistent due to the subjectivity of the trainers' estimation and the variations in the dogs' behavior in response to environmental stimuli. Therefore, most dogs thought to have potential as guide dogs are trained, and are re-estimated during the training period. In consequence, the dogs that subsequently fail to qualify as guide dogs are handed over to alternative homes at 2 years or older. As one of the prime reasons why the prospective guide dogs are rejected is nervousness and fearfulness [3, 5,

16, 17], it is more difficult to adapt to a new environment for rejected adult dogs than for younger animals. The problem of animal welfare and cost and labor efficiency would be improved, if the adaptive ability of guide dogs for the blind could be estimated objectively at an earlier age.

Recently, salivary sIgA concentration has been shown to be changed by psychological stresses in humans [1, 2, 6, 7, 9, 11, 12, 19–21, 23], and the mechanism of this response was suggested as sympathetic-adrenal-medullary activation [15, 21]. As samples are easily obtained without causing undue distress to the subject and only a little volume of sample to determine is required, salivary sIgA has been applied as an ideal markers for psychological disorders [14], quality of life [4, 7], and personality [13] in humans. In dogs, previous study has suggested salivary sIgA to be useful as a stress marker [10]. In addition, Skandakumar *et al.* showed that adaptive ability in police dogs was associated with sIgA levels [18]. This gives rise to the view that sIgA concentrations might also be of use in estimating potential guide dogs for the blind and other service dogs.

In the present study, to clarify the efficacy of salivary sIgA in estimating guide dogs for the blind, we practically determined sIgA concentrations in prospective guide dogs during their raising. Moreover, we also examined practical screening method using salivary sIgA.

MATERIALS AND METHOD

Study 1. Correlation between sIgA concentration during the estimation period and adaptive ability as guide dogs

Animals: Seventy-three healthy prospective guide dogs (35 male and 38 female) from the Hokkaido Guide Dogs for

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the Blind Association in Japan were used. The dogs were either Labrador Retrievers or Labrador $\times$ Golden Retriever crosses. These were 11 to 14 months old, after puppy walking, and were housed individually in a well-ventilated kennel (1 $\times$ 1.2 m) of the association for estimation of their behavior and temperament for about 14 days (estimation period).

Saliva sampling and care: Saliva was collected on day 1 (the day of separation from volunteer puppy-raisers), 2, 3, 7 and 14 during the estimation period. The time of saliva sampling was between 1,230 hr and 1,300 hr on day 1, and between 0900 hr and 0930 hr on the other days. Food was given at 0700 hr and water was provided *ad libitum*, though the day of sampling, all animals were not allowed to drink water for 1 hr before each sampling.

To collect the saliva, cotton swabs, approximately 1 cm in diameter, were placed bilaterally in each dog's cheek pouches and left for 1 min, with the handler gently holding the muzzle to prevent swallowing. Upon removal, the pair of absorbent cotton swabs was immediately placed in a pipette tip (5 ml), and that was put in the centrifugation tube. The tube was covered and kept in a cooler box for about one hour during delivered to our laboratory. The saliva was extracted by centrifugation at 2,500 g for 30 min at 4°C. The saliva samples were stored at -20°C until analysis.

Determination of sIgA concentration: Salivary sIgA concentration was determined by sandwich ELISA with a quantitation kit (Dog IgA Quantitation kit, Bethyl, Texas). Results are expressed in ELISA units (EU), where the concentration of the reference serum (1 mg/ml) was defined as 100 EU/ml. Six different saliva samples were analyzed, with 20 repeats each, and the inter-assay variation was between 4.3% and 10.8% (mean 7.2%).

Estimation of temperament (Trainers' test): Three trainers walked with the prospective guide dogs on the street and in a park, and estimated the dogs' behavior and temperament. They also observed the dogs' behavior in the kennel. After the estimation period, the dogs were divided into two groups; those considered suitable and those thought to be unsuitable. The unsuitable dogs could not be trained as guide dogs, and they were given to volunteers.

We chose the superior dogs that all three trainers estimated as adaptive, and the inferior dogs which were estimated as unsuitable by all trainers.

Determination of guide dog ability: The rejected dogs were these regarded as unsuitable during the estimation period and the dogs which could not adapt to the guide dog training. The acceptable dogs were those that could adapt to all procedures necessary for guide dogs.

Statistical analysis: To evaluate variations of sIgA concentration in acceptable dogs or rejected dogs from day 1 to day 14, repeated-measure ANOVA was used.

To estimate differences in sIgA concentration between acceptable dogs and rejected dogs on each sampling day, student's *t*-test was used.

$P < 0.05$ was considered significant.

To estimate the efficiency of the trainer's test, sensitivity,

specificity, positive predictive value, negative predictive value, accuracy, and diagnostic efficiency were calculated.

To establish the cut-off point of sIgA concentration on day 14, a receiver operating characteristics curve (ROC curve) was used. Estimation of the efficiency of the estimation of adaptive ability using sIgA concentrations (sIgA test), sensitivity, specificity, positive predictive value, negative predictive value, accuracy, likelihood ratio and diagnostic efficiency were calculated.

To estimate the efficacy of the trainer's test and sIgA test conducted simultaneously (parallel test), sensitivity, specificity, positive predictive value, negative predictive value, accuracy, and diagnostic efficiency were calculated. The parallel test gave priority to specificity, and unsuitable dogs estimated by whichever test were taken to be unsuitable.

Study 2. Correlation between sIgA concentration in puppy period and adaptive ability for guide dogs

Animals: Twenty-six of the healthy prospective guide dogs (15 male and 11 female) from Hokkaido Guide Dogs for the Blind Association in Japan that were used in study 1 were used. These dogs were cared for by volunteer puppy-workers in their homes.

Saliva sampling and sIgA concentrations: Saliva was collected when the prospective guide dogs were 8 weeks (8 W), 6 months (6 M), and 12 months (12 M) old. After explanation of the method for saliva sampling (follow the method on study 1), saliva was collected by each puppy-worker between 0630 hr and 0730 hr before walking and feeding. The centrifugation tubes containing the cotton swabs were stored at 4°C in puppy-workers' refrigerator. The saliva was extracted by centrifugation at 2500 g for 30 min at 4°C within 8 hr and stored at -20°C until analysis. The determination of salivary sIgA concentration followed

Fig. 1. The sIgA concentration in acceptable dogs (n=25) or rejected dogs (n=44) during the estimation period (average $\pm$ SE). A significant difference was shown on day 14 (** $P < 0.001$).

Fig. 2. Variations in sIgA concentration in superior dogs during the estimation period.

the procedure used in study 1.

Statistical analysis: To estimate differences in sIgA concentration between acceptable dogs and rejected dogs for each sampling, student's *t*-test was used.

$P < 0.05$ was considered significant.

RESULTS

Study 1. Correlation between sIgA concentration during the estimation period and adaptive ability as guide dogs.

During the estimation period, sufficient saliva could be collected from 69 dogs (94.52%). Among these dogs, 25 dogs were acceptable (36%), 44 were rejected (64%), 7

Fig. 3. Variation in sIgA concentration in inferior dogs during the estimation period.

Table 1. Trainers' test and ability as guide dogs

Trainers' test	Ability as guide dogs		
	acceptable(head)	rejected(head)	total
suitable	25	22	47
unsuitable	0	22	22
total	25	44	69

were superior, and a 9 were inferior.

The sIgA concentration in acceptable dogs gradually increased from day 1 to day 14, and the variation was significant ($P < 0.001$) (Fig. 1). This pattern of sIgA variation was shown in all superior dogs, and the lowest sIgA concentration on day 14 was 151.9 EU/ml (Fig. 2). On the other hand, the sIgA concentration in inferior dogs remained low, and

the highest sIgA concentration on day 14 was 81.5 EU/ml (Fig. 3). The difference between sIgA concentration in acceptable dogs and that in rejected dogs was significant on day 14 ($P < 0.001$) (Fig. 1).

The parameters for evaluating the efficiency of the trainer's test showed sensitivity was 100%, specificity was 50%, positive predictive value was 46.8%, negative predictive value was 100%, accuracy was 68.1%, likelihood ratio was 2.0, and diagnostic efficiency was 50% (Table 1).

The cut-off point of sIgA test was 90 EU/ml, and the sensitivity was 92.0%, specificity was 70.4%, positive predictive value was 63.8%, negative predictive value was 93.9%, accuracy was 78.3, likelihood ratio was 3.1, and diagnostic efficiency was 64.8% (Fig. 4).

Regarding the efficacy of the parallel test, sensitivity was 92.0%, specificity was 79.5%, positive predictive value was 71.9%, negative predictive value was 94.6%, accuracy was 84.1%, likelihood ratio was 3.6, and diagnostic efficiency was 73.1% (Table 2).

Study 2. Correlation between sIgA concentration during the puppy period and adaptive ability as guide dogs.

Among the 26 dogs, 6 dogs were acceptable and 20 rejected.

No significant differences were found on any sampling day (Fig. 5).

DISCUSSION

The present study showed that sIgA concentration on day 14 of the estimation period was significantly different between acceptable dogs and rejected dogs. These results are thought to show the potential of salivary sIgA concentration as a marker of adaptive ability in prospective guide dogs.

Although the sensitivity and negative predictive value of the trainer's test were complete (100%), the rejection of unsuitable dogs during the estimation period is very important as evaluation is followed by a long period of intense training. On this point, the specificity of sIgA test (70.4%) was higher than that of trainer's test (50%), and this result suggests that more unsuitable dogs could be rejected, if the sIgA test was used. Moreover, using both the trainer's test and sIgA test as a parallel test giving priority to specificity, specificity was 79.5%, and efficacy was improved. Therefore, combination of the sIgA test with the trainer's test was extremely useful in estimating adaptive ability as guide dogs for the blind.

During the estimation period, sIgA concentration was lowest on day 1 when the prospective guide dogs were separated from their puppy-workers and their environment was changed largely. The low sIgA concentration suggested a stress response, as many studies have shown that sIgA concentration is decreased by stress in human and dogs [6, 7, 10, 12, 19]. A difference in sIgA concentration between superior dogs and inferior dogs gradually became observable, though the value and degree of stimulus were the same in that the prospective guide dogs were housed in the identi-

Table 2. Parallel test (trainers' test and sIgA test were conducted simultaneously) and ability as guide dogs

Parallel test	Ability as guide dogs		total
	acceptable(head)	rejected(head)	
suitable	23	9	32
unsuitable	2	35	37
total	25	44	69

Fig. 4. The receiver operating characteristics curve (ROC curve) for sIgA concentration on day 14.

Fig. 5. The sIgA concentration in acceptable dogs ($n=6$) or rejected dogs ($n=20$) during the puppy period (average $\pm$ SE).

cal kennels and had a same care. Therefore, the sIgA levels during the estimation period indicated sensitivity and adaptability to stress, such as changes in environment and various environmental stimuli, in each dog. Specifically, the dogs whose sIgA concentration was low appear to lack adaptability to stress and be of unstable temperament.

On the other hand, sIgA concentration during the puppy period (during the prospective guide dogs were housed in each puppy raisers' houses) was shown to have no correlation to the adaptive ability of the guide dogs for the blind. In the present study, the saliva samples were collected in the morning, before feeding and walking, because the effects of sampling time, feeding, and activity on salivary sIgA concentration have been reported in dogs and humans [8, 10, 15]. However, it was impossible to collect saliva under standardized condition and the value and degree of stimuli on each dog was different. Therefore, if standardized condition like the estimation period can be set up for puppies, it may be possible to estimate the adaptability earlier. As earlier estimation is very important and improves the efficiency of training guide dogs, further studies are necessary.

The present study showed that sIgA concentration is an objective marker of the adaptive ability of guide dogs for the blind, and the dogs whose sIgA concentration is lower than 90 EU/ml are suggested to be unsuitable as guide dogs because of their lack of adaptability to stress. The sIgA test is also suggested to be useful in improving animal welfare. Moreover, the cost and labor involved in raising guide dogs could be reduced.

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