TECHNICAL NOTE

James Harrington,[1] M.S.; Herbert Heaney,[2] B.S.; Cynthia McSweeney,[1] M.S.; Lawrence Quarino,[3] M.S.; Ted Schwartz,[1] M.S.; and Joselito Versoza,[2] B.S.

Haptoglobin Typing in Canine Bloods

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ABSTRACT: Haptoglobin typing by vertical electrophoresis in a discontinuous polyacrylamide gel was conducted on 47 dog blood samples, of which 19 were from Doberman pinschers, 20 from German shepherds, and 8 from pit bullterriers. Two phenotypes were common in the three breeds and could not be used to differentiate between them. Canine haptoglobin phenotypes were, however, sufficiently different from those of humans to warrant using haptoglobin typing as a method for determining the origin of bloodstains.

KEYWORDS: forensic science, serology, haptoglobin, canines, canine blood

Frequently, because of the standard operating procedures in most forensic science laboratories, polymorphic protein determinations are not performed on bloodstains that have been identified as nonhuman. This is unfortunate, since animal bloodstains can yield information that can be useful in a forensic science investigation. Bowen has shown that polymorphic protein determinations can be used in identifying the species of origin of bloodstains [*1*]. Since the facts and case histories of forensic science investigations are not always accurate as to the species of origin of bloodstains, polymorphic protein studies may be useful in determining the origin. Similarly, Hill has found that deoxyribonucleic acid (DNA) fingerprinting can be used in identifying the species of origin of unknown animal material [*2*].

The following study was performed to see if polymorphism in canine blood exists with regard to the serum protein haptoglobin and if such polymorphism can be used to distinguish a particular dog breed and to differentiate between human and canine blood.

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[1]Senior forensic scientists, Department of Biochemistry, North Regional Laboratory, New Jersey State Police, Little Falls, NJ.

[2]Principal forensic scientists, Department of Biochemistry, North Regional Laboratory, New Jersey State Police, Little Falls, NJ.

[3]Senior forensic scientist, Department of Biochemistry, North Regional Laboratory, New Jersey State Police, Little Falls, NJ, and forensic scientist, Department of Forensic Biology, Office of the Chief Medical Examiner, New York, NY.

This study was conducted on blood samples from three breeds of dogs that are among those that are likely to be encountered in physical attacks and other situations of interest to forensic science: Doberman pinschers, German shepherds, and pit bullterriers.

Preparation of Samples

The serum samples used for haptoglobin typing were prepared from whole dog and whole human bloods by isolating the serum fractions of the bloods by centrifugation. Forty-seven whole blood samples from dogs were obtained from two veterinarians and a local animal shelter, of which 19 were from Doberman pinschers, 20 from German shepherds, and 8 from pit bullterriers. The bloods were drawn from the animals into tubes containing no additive. After each tube was centrifuged, the serum was removed and frozen. The samples were thawed just before analysis. It should be noted, however, that some of the samples had been retained at the veterinarians' offices for periods of 1 to 2 weeks at 4°C before they were analyzed. Control serum samples were prepared from human whole blood having the known haptoglobin phenotypes 2-1, 1, and 2.

Procedure

Haptoglobin typing of the blood samples was performed using the method of Budowle and Chow [3]. Basically, samples were placed on prepared discontinuous polyacrylamide gels (composed of resolving and stacking gels) and vertically electrophoresed for a period of approximately 5 h with a constant current (20 mA when the samples were migrating through the resolving gel and 60 mA when they were migrating through the stacking gel). When the samples reached approximately 1 cm from the anode, the gel was developed by staining with a solution containing dilute hydrogen peroxide, *o*-toluidine, ethanol, and glacial acetic acid.

Results and Discussion

The haptoglobin patterns of the dog serums were easily distinguishable from those of human haptoglobin types. In all cases, the haptoglobin bands from canine bloods ran anodic to the Human 1 band.

The haptoglobin banding of the three dog species did not differ among the breeds. Two phenotypes were observed in all three species. Both phenotypes share a common band with one of the phenotypes exhibiting a second, more anodic band (see Figs. 1 and 2). It is not certain whether this doublet banding is an actual phenotype or just the result of some sort of degradation.

Table 1 shows the number of samples with each type of banding for the three breeds examined. Conclusive results were obtained on 40 of the 47 bloods tested. Combining the results of all three breeds, the number of samples yielding singlet haptoglobin banding equaled the number of samples giving doublet banding. Examining the data for each breed, the authors noted that doublet banding was twice as prevalent among samples originating from Doberman pinschers, while singlet banding was twice as prevalent among samples from German shepherds. However, because of the limited sample size in this study, nothing can be concluded about the relative percentage of each apparent phenotype in either dog breed.

Although it is apparent from this limited study that haptoglobin typing cannot differentiate between bloods from different dog species, it may be used to distinguish between canine and human bloods. This can be particularly useful in bloodstain samples of limited size that may contain a mixture of dog and human blood. In such a case, the analyst

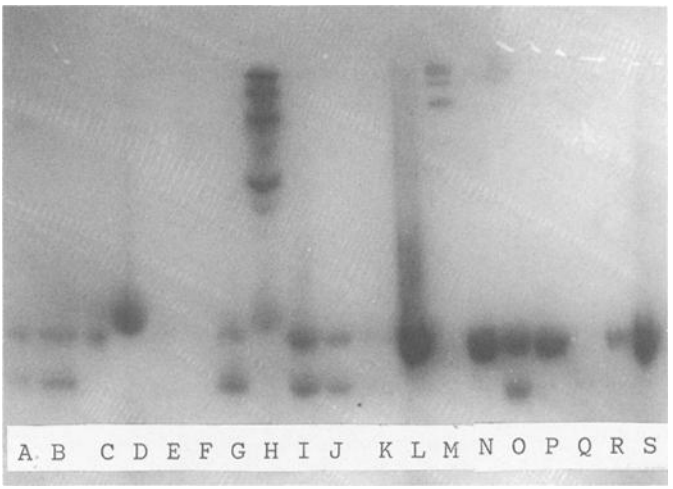

FIG. 1—*Canine and human haptoglobin types: A, canine doublet—Doberman pinscher; B, canine doublet—German shepherd; C, canine singlet—Doberman pinscher; D, human 1; E, inconclusive result—Doberman pinscher; F, empty; G, canine doublet—German shepherd; H, human 2-1; I, canine doublet—Doberman pinscher; J, canine doublet—pit bullterrier; K, weak canine doublet—Doberman pinscher; L, canine singlet (smear)—pit bullterrier; M, human 2; N, canine singlet—Doberman pinscher; O, canine doublet—Doberman pinscher; P, canine singlet—German shepherd; Q, empty; R, canine singlet—German shepherd; S, canine singlet (smear)—German shepherd.*

should not hesitate to attempt haptoglobin typing, because the banding patterns of canine and human blood are sufficiently different to identify a mixture easily.

Additional tests on other canine samples are planned to see if other phenotypes exist and perhaps to determine the nature of the doublet pattern. Furthermore, tests on blood samples from other species of animals will be performed to determine whether the phenotypes exhibited in this limited study are unique to canine blood. Once these questions are resolved, studies are planned to determine population frequencies and to investigate the effects of contamination and environmental factors.

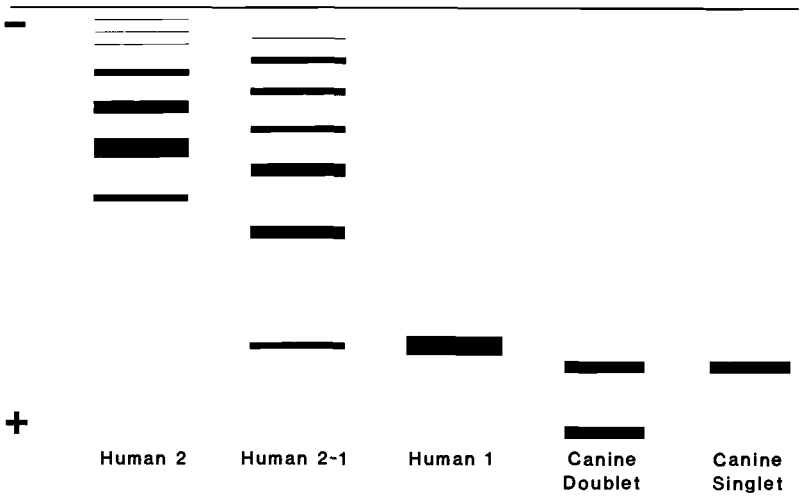

FIG. 2—*Human and canine haptoglobin types.*

TABLE 1—*Number of canine blood samples exhibiting haptoglobin singlet and doublet banding according to species.*

Dog Breed	Singlet Banding, No.	Doublet Banding, No.	Inconclusive Result, No.
Doberman pinscher	6	12	1
German shepherd	11	6	3
Pit bullterrier	3	2	3
Total	20	20	7

References

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Address requests for reprints or additional information to
Lawrence Quarino, Forensic Scientist
Department of Forensic Biology
Office of the Chief Medical Examiner
520 First Avenue
New York, NY 10016