TECHNICAL NOTE

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Isoelectrophoretogram of Gazelle Hemoglobin—A Suggested Tool for Proving Hunting Offenses

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ABSTRACT: Hunting gazelle is an offense according to Israeli law. When comparative isoelectric focusing was performed on bloodstains made from gazelle, goat, sheep, and cow blood, the pattern obtained from gazelle hemoglobin differed from those of the other animals tested. The use of this difference in hemoglobin pattern is suggested as a means to identify gazelle blood in hunting offense cases.

KEYWORDS: criminalistics, isoelectric focusing, blood, big game animals, gazelles, hemoglobin, hunting offenses

The genus *Gazella* belongs to the family *Bovidae* which also includes the genera *Capra* (goat), *Ovis* (sheep), and *Bos* (cow) [*1*].

There are three forms of gazelle in Israel. The most common one is the mountain gazelle (*Gazella gazella gazella*) [*2*]. Less common is the Dorcas gazelle (*G. dorcas*). The third gazelle, *G. gazella ssp.*, is very rare [*3*].

All the gazelle species in Israel are protected wild animals and hunting gazelle is an offense [*4*].

In cases of suspected gazelle hunting, all the relevant exhibits were collected. Since we had not found a commercial anti-sera specific against gazelle, bloodstains were of little forensic science value. To date, physical evidence for hunting gazelle was based on hair and bone examination only. As bloodstains were common exhibits in hunting cases, we decided to investigate the possibility of using gazelle hemoglobin as a specific marker.

The use of hemoglobin for species determination was proposed as early as the end of the last century [*5*]. Conventional electrophoresis was not found useful [*6, 7*]. When substituted by isoelectric focusing, good results were obtained for hemoglobin derivatives [*8*] and origin determination of bloodstains from deer [*9*] and other animals [*10*].

Materials and Methods

Bloodstains of the animals tested in this work were prepared from fresh blood applied to white gauze, cotton fabric, or absorbent paper. The stains were received in the laboratory by

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mail several days after preparation. Thereafter, the samples were stored at −20°C. Isoelectrophoresis, with a pH gradient 7 to 9, was performed with an LKB Multiphor system on a 245- by 110- by 0.5-mm polyacrylamide gel ($T = 5\%$, $C = 3\%$ where T = concentration of total acrylamide [acrylamide + cross-linker] and C = concentration of the cross-linker [*NN'*-methylenebisacrylamide]) containing: 10% v/v final concentration of glycerol, 7.3% v/v final concentration of LKB Ampholine 1809-036, 550 μL of 1% stock solution of ammonium persulfate, and 5 μL of *N,N, N',N'*-tetramethylethylenediamine (TEMED) in a total volume of 20.5 mL. 0.5*M* acetic acid was used as anolyte and 0.5*M* sodium hydroxide as catholyte. With the power supply set to 2000 V, 10 mA, and 15 W, the gel was prerun for 30 min and the samples were then electrophoresed for 2 h at 4°C. Pieces of bloodstained gauze, fabric, or paper, 1 by 8 mm, were applied 2 cm from the anodic side of the gel.

Anti-sera against goat/sheep, cow, and deer were purchased from Wellcome Diagnostics (KA 12, KA 02, and KA 03, respectively). Precipitin reactions were carried out by one-dimensional double electroimmunodiffusion [*11*].

Results and Discussion

Gazelle bloodstains showed cross-reaction with anti-goat/sheep in the precipitin reaction. Negative results were obtained when anti-cow or anti-deer anti-serum were used.

Figure 1 shows the banding pattern of hemoglobin from bloodstains of Bovidae members which are commonly found in Israel. Since the bands are distinctly clear as a result of the intensity of the hemoglobin color, no special staining is needed.

The pattern of gazelle hemoglobin can be distinguished from that of goat, sheep, cow, and Nubian ibex (*Carpa ibex nubiana*) by the presence of additional cathodic bands. None of the isoelectrophoretogram patterns of other animals tested in this study contained hemoglobin bands corresponding to the cathodic bands of the gazelle. Bloodstains from 95 mountain gazelle from 3 geographically isolated areas of Israel were examined. All bloodstains showed a similar pattern. Three blood samples from Dorcas gazelles also showed cathodic bands at the same position as the mountain gazelle pattern. Bloodstains from 53 goats (white goats and black goats [*C. aegerus hircus and C. aegerus ssp.*, respectively]) were examined. The hemoglobin band patterns were all similar and did not contain the cathodic bands seen in gazelle.

Sheep (*Oris aries*) (13 samples), cow (*Bos taurus*) (1 sample), and Nubian ibex (3 samples) also lacked the cathodic bands.

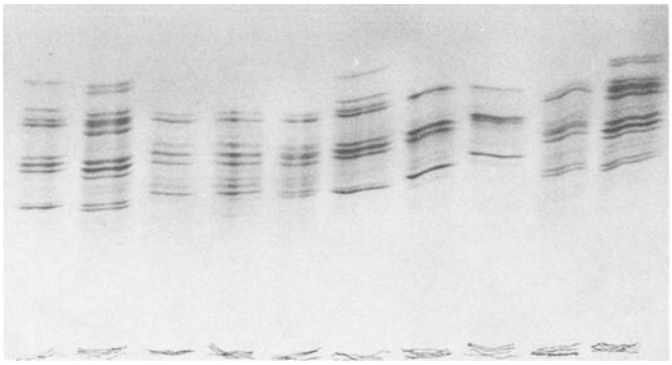

FIG. 1—*Hemoglobin isoelectrophoretogram of some common Bovidae in Israel. Lanes* (from left to right, cathode at the top): *(1) Dorcas gazelle, (2) mountain gazelle, (3) interbreed between goat and Nubian ibex, (4) Nubian ibex, (5) goat, (6) Dorcas gazelle, (7) sheep, (8) cow, (9) goat, and (10) mountain gazelle.*

The hemoglobin pattern obtained from the tested animals was stable even after incubating bloodstains at 37°C for 40 days. No additional bands in the cathodic zone of hemoglobin pattern of goat, sheep, cow, and Nubian ibex appeared nor did cathodic bands in the gazelle hemoglobin pattern disappear. This stability is important in a country with a hot climate like Israel, as it ensures the examiner against false positive and false negative results due to the effects of extreme heat.

Furthermore, we obtained clear electrophoretograms from bloodstains of the tested animals after storage in a deep-freeze (−20°C) for over one year. Hence, samples can be stored for long periods before examination or for reexamination.

Bloodstains from four actual cases were examined by this method and found to contain gazelle hemoglobin. These results were confirmed by other evidence, namely, hairs or parts from the animal body or a suspect's confession. Our results demonstrate that hemoglobin patterns provide a valuable tool for identifying gazelle blood in cases where illegal hunting is suspected. Isoelectric focusing of bloodstains should be performed after obtaining a positive reaction with anti-goat/sheep anti-serum. Based on the hemoglobin patterns, gazelle blood can now be distinguished from that of goat, sheep, cow, and Nubian ibex.

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