PAPER

ANTHROPOLOGY

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The Use of Crossover Immunoelectrophoresis to Detect Human Blood Protein in Soil from an Ambush Scene in Kosovo*,†

ABSTRACT: This study examines the survivability of human blood proteins in soils from a year and a half old ambush scene in Kosovo. A total of 72 soil samples were collected, a number of which were directly associated with bone fragments or bullet projectiles. The samples were examined using crossover immunoelectrophoresis (CIEP) to determine the presence of blood protein and species affiliation. Human blood proteins were identified in 44 of the 72 samples (61%) with the majority of the positive observations (29 of 44) found 0.0–4.5 cm below ground surface (65%). Chi-squared and two-sample difference of proportions tests confirmed significant differences between samples with and without associated physical evidence and the presence and depth of human blood proteins. While DNA has largely replaced immunological analysis in forensic analyses, our results suggest that in particular situations, CIEP may still be a valuable tool in criminology.

KEYWORDS: forensic science, forensic archeology, blood protein, soil, immunology, electrophoresis, electrochemistry, Kosovo

Those who commit murder often remove the bodies from the murder scene in an effort to conceal their crime. This activity is evident on a grand scale in the former Yugoslavia, where there was an evolution of concealment methods used by the perpetrators of human rights abuses. As investigations by the International Criminal Tribunal for the Former Yugoslavia (ICTY) progressed, forensic experts began to find that some mass grave sites were dug up and the previously buried remains were removed to other locations, creating what Jessee and Skinner (1) refer to as secondary inhumation sites. Similarly, locations described by witnesses as execution or ambush scenes were curiously devoid of evidence. Bodies, shell casings, and other physical evidence of the executions had been removed. Indeed, some scenes were so clean of physical evidence that, despite eyewitness accounts, investigators were unable to correlate the sites. However, the absence of a body, while an obstacle to investigators, is not necessarily an end to the investigation. This paper represents an initial effort to develop a method to reveal the hidden evidence at allegedly sanitized sites.

This study was designed to determine whether or not human blood proteins can be detected in the soils of a crime scene, and, in this case, in a scene more than a year old. Specifically, an immunological test, crossover immunoelectrophoresis (CIEP), was employed to determine the presence of human blood protein in the soil of an ambush site in central Kosovo associated with evidence of bloody injury and death. If the method proved valid in this instance, it could be extended to sites that had been more thoroughly cleaned of evidence.

Immunology as Evidence

Immunological tests have been used regularly in both the medical and legal professions to identify diseases, blood, and bloodstains (2–5). CIEP specifically tests for immunoglobulins, a group of glycoproteins present in the serum and tissue fluids of all mammals, by bringing a blood protein (antigen) into contact with a suitable antiserum (antibodies raised against specific antigens) (6). With CIEP, the interaction between antigen and antisera is enhanced through the use of an electrophoretic force. If a precipitate reaction forms between the two agents, then the antigen and antiserum are from the same animal family. Thus, positive identification to the taxonomic family level of the antigen origin can be established (7–9). Cross-reactivity can occur with related species. For example, deer antisera will also react to elk and moose blood. In the case of humans, other primate blood can cross-react. The method is very sensitive, detecting up to 10^{-8} g of protein, it is relatively inexpensive, and it lends itself well to multiple examinations (2,10). Only 1 g of soil is needed to run a single suite of CIEP tests.

Prior to the introduction of cost-effective DNA testing, the immunological analysis of suspected blood deposits at crime scenes for human protein was regularly performed. However, soil at older crimes scenes were not sampled because it was assumed that blood proteins could not survive in the hostile microbiological environment for any length of time. In archeological contexts, only limited studies of the survivability of blood protein in soil has been conducted. Newman et al. (11) found bison blood proteins in the soil from a buffalo kill site in Alberta, and, although few blood proteins were found at another site, she concluded that "useful" information could be provided by such soils analyses. In a study conducted by

Nolin et al. (12), sediment samples from two suspected food storage pit features were analyzed and found to contain cervidae and bovine proteins. Aside from these two limited studies, as far as we know, no other CIEP tests of sediments have been carried out, in either an archeological or a forensic context. No study on the immunological analysis of older crime scene soils appears in the literature. DNA testing has now virtually replaced CIEP at contemporary crime scenes. After all, why bother testing blood to determine whether it is human or not when you can examine the blood to identify the exact person through DNA analysis? However, DNA analysis may be a victim of its own success. The DNA molecule is relatively fragile, while proteins are known to be more resistant to environmental change (4,13). Although immunological testing has taken a back seat to DNA analysis in forensic laboratories, it should be noted that archeologists continue to use it successfully to answer questions of past human activity, particularly with respect to diet and stone-tool usage (7,8,10,14–20). In addition, blind immunological tests have recently been conducted on six known bone fragments (three human, to include one archeological sample, and three nonhuman) in which all were successfully identified (21,22).

The present study attempts to fill the gap between the criminologist's understanding of immunological analysis and the archeologist's knowledge that blood proteins can survive in a buried context for thousands of years. However, instead of ancient artifacts or bones, the soil from an ambush scene in central Kosovo, sampled one and a half years after the event occurred, is tested for the presence of human blood protein.

The passage of time between the ambush and CIEP analysis of the soil is an important element in this study. As forensic investigators rarely test soils if the event in question is more than a few weeks old, let alone months or years, positive results would suggest that investigators should reevaluate their assumptions that microorganisms rapidly destroy biological evidence. The presence of human blood proteins in murder and burial/dump site soils, combined with witness statements and other possible physical evidence, could provide powerful documentation to support an investigator's evaluation of an older crime scene.

Materials and Methods

According to investigations performed by the ICTY, in April 1999, a Serbian paramilitary group surprised a number of unarmed Kosovar Albanian civilians who had been hiding on a wooded hillside near the village of Stutiča in central Kosovo (Fig. 1). The Serb gunmen opened fire, killing six to seven of the ethnic Albanians and wounding an unknown number. The surviving Albanians were forced to strip off their outer layer of clothes and turn over all their valuables to the Serb gunmen. The survivors were then marched out of the area; the dead remained where they had fallen. Later (the exact time is unknown), local ethnic Albanian villagers visited the ambush site and hastily buried the bodies in shallow graves nearby. After Serbian forces were driven out of Kosovo by the NATO intervention, returning Albanian families exhumed the bodies from their temporary graves and relocated them to formal cemeteries.

The ICTY investigated the Stutiča site in July 2000, more than a year after the ambush. Clothing, hair, bone fragments, shell casings, and projectiles were found on the surface of the area. These were flagged and recorded using a Sokkia Set 600 Total Station (Topcon Corp., Tokyo, Japan) (Fig. 2) and then collected. The scene was heavily wooded and would have provided cover for the Albanians, but dense undergrowth limited the space that could be physically occupied. A trail through the dense undergrowth became wide enough at points to allow several people to gather together to sit, eat, and sleep. One of these areas, a small depression c. 3 × 3 m, appeared to be a spot where at least one body had fallen. Within this depression, two human skull fragments, an intact first cervical vertebra, and two projectiles were recovered. On the path adjacent to the depression, an additional scatter of skull fragments and hair was discovered, possibly representing a second person.

Soils from the scene were sampled in October 2000, more than 2 months after the initial investigation at the scene and a full year and a half after the ambush. The evidence flags that had been placed in July were still in their original locations. These flags were used to guide the sampling process. A total of 11 small test pits were excavated in the depression and adjacent trail (Test Pits 1–11). A 12th test pit (Test Pit 12), intended to provide a control sample, was excavated c. 5 m southeast of the center of the depression along the trail leading toward the village. Within the depression, five test pits were excavated directly beneath locations where either a bone or a projectile was found. Along the adjacent path, two additional test pits were excavated at the location where the additional skull fragments were discovered. The remaining four test pits (Test Pits 8–11) excavated in the depression were not associated with any physical evidence. These four additional pit locations were dug in locations to provide even distribution of test pits over the depression. Table 1 provides a list of the test pits and associated evidence.

Each test pit was c. 5 × 10 cm wide and 9 cm deep. The pits were excavated in six separate levels, each level c. 1.5 cm deep. Soil samples from each level were placed in sealed plastic evidence bags. The shovel and trowel used to remove the samples were washed with water after each sample was taken. A total of 72 samples were taken from the 12 test pits. The samples were later air dried, repackaged, and stored out of direct sunlight for 6 months prior to processing.

Four grams of soil were removed from each of the 72 Stutiča soil samples. The CIEP test was conducted by Margaret Newman at the University of Calgary in the following manner. The antigen and antisera were placed into paired wells, punctured in agarose gel, c. 1.5 mm in diameter and 5 mm apart. The gel had a pH of 8.5. The gel platform was placed over an electrophoresis tank containing two basins of a barbital buffer with a pH of 8.6. Each basin was supplied with electric current set at a constant 100 V. The basin under the antigen received a positive charge, while the basin under the antisera received a negative charge. One end of a paper wick was placed in each basin with the other end of the wicks touching the sides of the agarose gel. Thus, the wicks acted as electric contacts between the buffer within the basins and the gel, supplying the antigen side with positive current and the antisera side with negative. The electric current enhances and speeds up any precipitin reaction between the antigen and antisera that would naturally occur in the absence of electricity. A reaction will occur only when the known antisera, raised against a specific type of animal, is tested against an antigen from that type of animal.

Only 1 g of soil from each sample was used for each test. One milliliter of 5% ammonium hydroxide was added to each 1 g of soil. The mixture was then vortexed and placed in a rotating mixer for 24 h at 4°C. Next, the samples were centrifuged, and the resultant solution placed in sterile Eppendorf tubes. Initial screening of the samples for nonspecific protein reactions not based on the immunological specificity of the antibody was carried out against preimmune serum (i.e., serum from a nonimmunized animal). The preimmune serum was developed at the University of Calgary while all the antisera were sourced from Cappel in Aurora, Ohio,

FIG. 1—*Map of Kosovo displaying approximate location of ambush (circle).*

and rigorously tested for purity and specificity for forensic laboratories. All samples were negative against the preimmune serum. Positive and negative controls, also prepared in 5% ammonia hydroxide, were run with each gel to ensure procedure quality. Positive controls are antigens that react with the specific antisera tested against, while negative controls are antigens that will not react with the antisera. No opposite reactions were observed during testing. Duplicate testing was carried out on all positive samples.

Usually, a whole suite of antisera would be used to test suspected blood stains on an archeological artifact in an effort to determine what kind of animal was processed. This would be an unnecessary exercise at most crime scenes where an investigator would usually be interested only in knowing whether human blood was present. In the case of the Stutiča scene samples, analysis focused only on blood from animals known to be in the area: bovine, sheep, and human antisera were used. Cow, bison, and musk-ox antigen would react to bovine antiserum, while goat would also react to sheep antiserum. Herding of both sheep and

cows are common in rural Kosovo and both types of animals were observed near Stutiča. Game animals in the region are rare. While all primate antigens would react to human antiserum, the only primates in the region are, of course, human.

Results

Only human antiserum produced positive reactions. The bovine and sheep antisera returned negative results for all 72 samples. Table 2 lists the positive and negative reactions to human antiserum. The negative reactions to preimmune serum, explained earlier, eliminate the possibility that contaminants may have caused false positive reactions. Duplicate testing on all positive reactions confirms the presence of human blood proteins within the soil samples.

In Table 2, each sample reaction result is shown in a column to the right of the sample depth. Positive reactions to human antiserum were obtained in 44 of the 72 samples (61%). Three of the positive results were weak-positive reactions (Level 5 in Pit 7, and
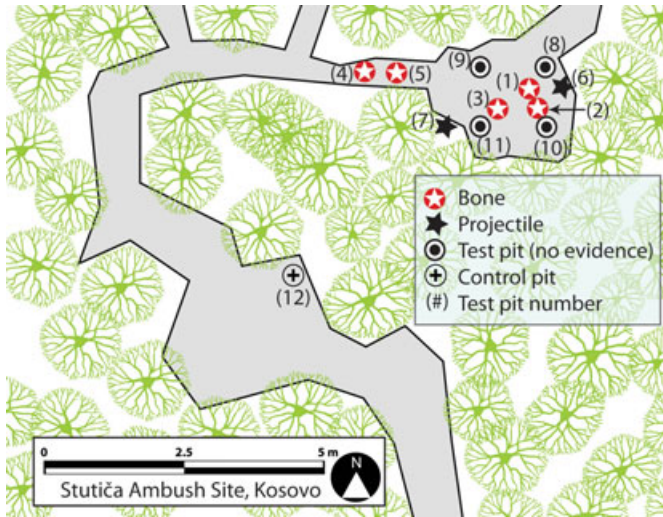
FIG. 2—*Stutiča Ambush Site. Shaded area is the preexisting path through dense undergrowth cleared by Explosive Ordinance Disposal technicians.*

TABLE 1—*Test pit evidence association.*

Test Pit No.	Associated Evidence
1	Cranial fragment
2	Cranial fragment
3	Vertebra (C1)
4	Cranial fragment
5	Cranial fragment
6	Projectile
7	Projectile
8	None
9	None
10	None
11	None
12 (control)	None

Levels 5 and 6 in pit 8). A weak positive is when a reaction between the antiserum and antigen is present, but its manifestation is slight. Weak-positive reactions are common in archeological samples and should be considered a positive reaction regardless of its strength. The remaining 28 samples returned negative results.

Further examination of Table 2 reveals that all but one of the 24 top two levels (Levels 1 and 2) tested positive for the presence of human blood. That is a 96% positive finding. It may be assumed that there was surficial blood throughout the area, although in varying quantities. Larger quantities of blood may be a factor in assisting with the preservation and discovery of blood proteins on ancient stone artifacts (23). Thus, it is possible that those tests with the deepest positives had the greatest quantity of surficial blood.

This is borne out to some extent by the fact that the test pits associated with evidence, bone or bullet projectiles (Test pits 1–7), had the greatest number (the deepest) of positive reactions. A total of 31 samples of 42 (74%) from test pits with evidentiary association tested positive for blood protein reaction. In pits with no associated evidence, only 13 samples of 30 (43%) tested positive. A chi-squared test to compare the results from test pits with associated evidence to pits without associated evidence indicates that this difference is statistically significant ($p < 0.0089$) (Table 3). Thus, those locations where there was clear evidence of violence and, in the case of bone, clear evidence of contact with the ground surface, appear to have had the greatest quantity of blood absorbed into the ground.

Another way to look at these data is to test the reactions for the upper three levels against the lower three levels. A glance at Table 2 reveals a clear difference in the rate of positive returns above and below 4.5 cm (between Levels 3 and 4). There were 29 positive reactions above 4.5 cm, and 15 below. This, too, was a significant difference ($p < 0.0007$) (Table 4).

As the chi-squared tests indicate, significantly more positive results occur in samples from test pits associated with physical evidence, and within samples taken from the upper three levels (0.0–4.5 cm below ground surface) of each pit. Combining these findings, two additional statistical tests were conducted comparing depth of positive reactions with the presence or absence of physical evidence. A two sample difference of proportions tests were employed, which test the significant proportional (percentile) differences between groups.

In the levels above 4.5 cm (Levels 1–3), a total of 18 positive reactions were observed in the 21 samples from test pits associated with evidence (Test Pits 1–7), while a total of 11 positive of 15 were observed in samples from test pits with no associated evidence (Test Pits 8–12). The calculated difference of proportion has an associated *p*-value of 0.3524 (Table 5). The proportions test was then run against the same groups, but within levels below 4.5 cm (Levels 4–6). Here, 13 of 21 samples from test pits associated with physical evidence were contrasted against two positive of 15 total observations from test pits not associated with any physical evidence; $p < 0.0001$ (Table 6).

These test results demonstrate that while there is no significant difference between samples in the levels above 4.5 cm, in the lower levels, the samples with associated physical evidence produced significantly more positive results than the ones lacking an association. It is probable that those areas associated with physical evidence contained more blood, which could filter more deeply into the soil. Other factors, however, could also be operating. There might have been more ground surface disturbance where physical evidence was found, which also might allow for better blood penetration. Differential preservation might also be a factor. A slight difference in soil composition with depth in the area tested is noted. The upper levels (Level 3 was characterized) have a slightly higher

TABLE 2—*Results of human antiserum reactions using crossover immunoelectrophoresis analysis.*

Level	Depth (cm)	Pit 1*	Pit 2*	Pit 3*	Pit 4*	Pit 5*	Pit 6[†]	Pit 7[†]	Pit 8	Pit 9	Pit 10	Pit 11	Pit 12
1	0.0–1.5	+	+	+	+	+	+	+	+	+	+	+	+
2	1.5–3.0	+	+	+	+	+	−	+	+	+	+	+	+
3	3.0–4.5	−	+	+	+	+	−	+	−	−	−	−	+
4	4.5–6.0	−	−	+	+	+	+	−	−	−	−	−	−
5	6.0–7.5	+	−	+	−	+	+	+(w)	+(w)	−	−	−	−
6	7.5–9.0	+	−	+	−	+	+	−	+(w)	−	−	−	−

w, weak reaction.
*Associated with human bone.
[†]Associated with a bullet projectile.

TABLE 3—*Chi-squared test of evidence association.*

	Positive	Negative	Total
Associated evidence	31	11	42
Nonassociated evidence	13	17	30
Total	44	28	72
Chi-square	6.839		
p-value	0.0089		

TABLE 4—*Chi-squared test of all samples above and below sectioning line.*

	Positive	Negative	Total
Above 4.5 cm	39	7	36
Below 4.5 cm	15	21	36
Total	44	28	72
Chi-square	11.455		
p-value	0.0007		

TABLE 5—*Difference of proportions test of upper levels for samples with and without associated evidence.*

	Positive	Total	Proportion
Evidence	18	21	0.8571
No evidence	11	15	0.7333
Distribution	0.3238		
p-value	0.3524		

TABLE 6—*Difference of proportions test of lower levels for samples with and without associated evidence.*

	Positive	Total	Proportion
Evidence	13	15	0.8666
No evidence	2	15	0.1333
Distribution	0.4999		
p-value	0.0001		

clay and phosphorus content than Level 6, which had slightly more silt and sand and less phosphorus.

Although it seems clear that blood proteins were indeed preserved in soil for up to 2 years, two results are problematic. First, all 12 test pits produced positive results somewhere within their six levels, including the control. In addition, there are levels with negative results sandwiched between levels that produced positive results. With respect to the latter, postdepositional processes like bioturbation could eradicate some evidence. In addition, some sampling error may be involved. The small amount of soil withdrawn from the larger sample may not directly underlie/overlie the positive sample above or below it. In either case, it is clear that multiple samples should be taken when conducting such analysis.

The fact that Test 12, the control sample, also produced evidence of human blood proteins is an unfortunate result. This is most likely due to the aforementioned large amount of bloodshed and the proximity of the control test to the killing zone. The location of Test Pit 12, 5 m distant from the depression where the majority of the samples were taken, was chosen for a number of reasons, the principal one being safety. At the time that the samples were collected, Kosovo had *c.* 50,000 land mines, 30,000 unexploded cluster bombs, and thousands of other unexploded ordinances scattered around the countryside. All ICTY sites were surveyed for landmines by military Explosive Ordinance Disposal (EOD) technicians; however, at the Stutiča site, the cleared area was limited to the path through the dense undergrowth leading to and running through the site (the shaded area in Fig. 2). Terrain farther away from the site (south of Test Pit 12) that had been cleared by EOD was where the victims were temporarily buried prior to their move to the village cemetery, so that area was avoided. It was unknown whether the area beyond the temporary graves had been surveyed. Subsurface testing, then, had to be confined to places along the site pathway itself.

Test Pit 12 was placed where a high concentration of shell casings was found. The rationale behind this placement was that the ejected casings marked the location where the Serbian gunmen took up position to fire upon their victims. It was assumed no victims would be present where the shooters stood. However, it is possible that a victim may indeed have been killed or wounded at the place where the shooters stood, or a wounded, bleeding victim may have passed over the area and deposited blood into the soil as he was being led along the trail away from the scene. Another possibility is that the control samples were contaminated by either the shovel or the trowel used to take them. As discussed in the methods section, both the shovel and trowel were washed between each sample. However, contamination cannot be ruled out. Nevertheless, with the possibility that human blood was indeed present in the control pit location, the control samples must be viewed as additional samples of the execution site.

Discussion

The analysis of the Stutiča soils demonstrates that enough protein microstructure can remain intact in some soils for at least a year and a half (2 years if the 6 months the soil samples were stored in a dried state prior to analysis are included), and that these proteins can be reliably identified using immunological testing techniques. Why blood proteins survived in the Stutiča samples is not fully understood but it may have to do with the particular characteristics of the soil matrix, the microstructure of the proteins themselves, or the biological processes involved in protein degradation. In thermal experiments with amino acids, the base elements that make up proteins, rapid degradation was seen followed by stability, suggesting that only the most stable components survive the hostile environment outside the body (24). Similarly, it has been suggested that degradation of protein occurs rapidly as blood dries but then becomes more stable, degrading at a substantially slower rate from then on (25). In a study of surgical tools from the American Civil War, Newman et al. (26) demonstrated that biologically active protein and DNA are still detectable after more than 130 years of exposure to the atmosphere. Tests on denatured protein and extensively washed bloodstains provide evidence of the resilience of blood proteins, even when attempts are made to conceal them (3). With respect to their study of buried stone tools, Cattaneo et al. (22) postulated that the amount of blood on the artifacts, good drying condition prior to burial, and the type of matrix in which the artifacts are buried are all critical elements in preservation.

Soils high in sand and clay appear to help preserve protein from microbial attack better than other matrixes (27–30). Loy (28) suggested that positively charged blood proteins bind to negatively charged silica particles in clay and that this action helps to protect proteins from micro-organisms that would feed upon them. In support of this, Wiechmann et al. (29) report that highly negatively charged protein components preserve better for longer periods of time in buried contexts. Other studies indicate that the types of clays that provide the best protection for proteins are clays with a high-base exchange, such as illites and smectites (27,30,31). In addition, clay morphology may protect protein molecules upon

absorption by orienting the molecules in a manner that makes them inaccessible to micro-organisms (27).

The Louisiana State University Soil Characterization Laboratory carried out soil profiles of Test Pit 1, Levels 3 and 6, from the Stutiča site. The mineralogy of the soils was characterized by approximately equal proportions of smectite and chlorite clays with a small amount of mica. Table 7 indicates that the phosphorus levels of the samples are low. Such low levels are typical of forest soils such as the Stutiča site. Soil class for Levels 3 and 6 are clay and silty clay loam, respectively. In addition, Newman et al. (11) suggested that blood proteins may not survive in highly acid soils (pH < 4); the pH at Stutiča was around 6.8–6.9 (Table 7).

As blood proteins are water soluble, proteins in soil are potentially susceptible to dissolution and degradation by groundwater. However, evidence exists that protein aggregation may be taking place, helping proteins survive. By binding together, proteins form higher molecular weight and are therefore less soluble (4,32–34). Proteins may also bind with insoluble fatty acids, thus protecting them against groundwater dissolution (7). As bodies left at a scene decompose, insoluble fatty acids will be released providing a chance for them to interact with blood proteins present.

All of the possible preservation mechanisms mentioned—a large quantity of blood, insoluble fatty acids, and high clay soil content—were either present or were potentially present at the Stutiča site. Six to seven individuals were reportedly killed and an unknown number wounded at the site. Areas sampled contained human skull fragments indicating that the victims sustained massive trauma to their heads. A lot of blood would have been deposited in the sampled areas as a result of these wounds. It is also suspected that the dead remained where they had fallen for a period of time before their initial, temporary burial near the site. The exact amount of time the dead lay at the site prior to burial is unknown, however; an undamaged first cervical vertebra was located at the site, suggesting that it became disarticulated from the body, not through the trauma of a gunshot or carnivore activity, but by the process of decomposition. If decomposition of the bodies began before they were removed, insoluble fatty acids as well as blood would have been deposited in the soil, perhaps allowing the two to bind. A search for insoluble fatty acids was not conducted with the Stutiča samples, although these findings suggest that such a search may yield interesting results. Furthermore, the Stutiča soils have high clay content. It is possible that all of these preservation factors, or a combination, helped the Stutiča blood protein survive over a long period of time. Investigators should take note of the positive results found at Stutiča and be encouraged to test soils from suspected murder/execution sites for blood evidence regardless of the amount of time between the murder event and the sampling.

A final consideration regarding detection of blood proteins in soil at a possible crime scene is the potential of inadvertently finding proteins from an event other than the one under investigation. As indicated, archeology has demonstrated that blood proteins can survive on stone tools and other artifacts for thousands of years. Therefore, if an event occurred in the past (perhaps centuries before) where an amount of blood was spilled on the ground, could that blood protein be detected during the analysis of soil samples taken during the course of a contemporary investigation? Perhaps. There has been very limited archeological research on the survivability of blood proteins extracted from soil, and those results have been mixed (11). Other than this current study, the authors are not aware of any investigation conducted on the detection of blood proteins deposited in soil at crime scenes. We therefore cannot attest to protein survivability in soil beyond the 2-year time frame of this study. Still, considering the archeological research conducted on recovered artifacts, we may assume that detecting blood proteins from a past unrelated event is a possibility. Nevertheless, it should be noted that the same concerns also apply to all crime scene detection techniques. For example, the use of luminol to detect hidden stains on a wall or floor does not differentiate between possible past and current crime scenes, or even if the stain is related to a crime at all. DNA analysis of the stain may indicate that it is organic material from a resident of the household, but those results cannot determine whether that material was deposited during a criminal act or by accident days (or weeks) prior to the crime being investigated. The context of the crime scene and investigation must be taken into account along with the strengths and weaknesses of any given detection techniques. While CIEP may be an appropriate test of soils sampled from a witness identified murder scene located in someone's yard, an unlikely location for blood had been spilled in the past, it would be unwise to use it at a contemporary murder scene located in say the Coliseum in Rome or at the killing fields of Cambodia.

Like other detection techniques, CIEP testing of soil should be used in conjunction with other evidence given the context of the investigation. The aim is to corroborate existing evidence, and not to be a stand-alone, all-inclusive technique. The ambush scene at Stutiča is a good example of employing CIEP in the proper context. This scene was chosen as a model because the investigators had both witness accounts of the ambush and physical evidence (bone, shell cases, etc.) present at the site. The CIEP results support these two forms of evidence, and a reasonable conclusion can be made that these three forms of evidence are strongly related. Could the witnesses be lying? Yes. Could the physical evidence have been planted? Yes. Could the CIEP analysis have detected blood deposited at that location a century before? Yes (we assume). However, it is more reasonable to conclude that, as the witnesses indicated, a number of men were caught unaware at the Stutiča site, several killed and wounded, and the rest taken prisoner. CIEP cannot confirm that the blood detected belonged exclusively to the men killed and wounded during the ambush, but the corroborating evidence strongly indicates that it is likely the case.

Conclusion

Soil samples collected at a known ambush scene where a number of people were killed and wounded were examined using CIEP. The soils were sampled a year and a half after the murders, dried out, and stored for an additional 6 months prior to testing. Positive immunological reactions occurred in the majority of samples collected. The probable large amount of blood spilled, deposition of insoluble fatty acids, and the soil matrix, singularly or in combination, may have contributed to the large number of positive reactions. Samples taken between the depths of 0 and 3 cm were most likely to produce positive results, and more samples returned positive reactions if the test pits from which the samples were taken were associated with additional physical evidence recovered at the scene. Furthermore, in test pits excavated with no additional

TABLE 7—*Stutiča site soil profile.*

Test Pit 2 Sample	pH	Phosphorus mg/kg Soil	Clay (%)	Silt (%)	Sand (%)
Level 3 (3.0–4.5 cm)	6.9	3.7	46.5	34.5	19.0
Level 6 (7.5–9.0 cm)	6.8	1.6	39.0	40.8	20.2

physical evidence, soils sampled below 4.5 cm produced very little positive reactions and contrasted sharply with results from samples tested from pits associated with physical evidence recovered at the same levels.

CIEP testing is not new to forensic investigations. However, investigators often overlook or ignore soils at murder scenes, especially if the suspected deposits of blood are a few months or even a few weeks old. The successful findings of the CIEP analysis of the Stutiča soils should encourage investigators to examine crime scene soils regardless of the time between deposits and sampling. Future testing of crime scene soils for older deposits of blood proteins, with an eye toward developing information on the variables responsible for preservation or degradation (e.g., soil types, climate, weather conditions, and other variables), eventually could provide us with a practical, working knowledge of where and when to test blood protein evidence.

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