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A Comparison of Immunodiffusion and Latex Agglutination Tests for the Identification of White-Tailed Deer Tissues

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ABSTRACT: Latex agglutination and agar gel immunodiffusion were used to identify muscle tissue extracts. Both tests reacted with white-tailed deer extracts at a higher dilution than extracts from other cervids and both detected cervid antigens in sausage extracts. Latex agglutination was consistently more sensitive than agar gel diffusion. Sausage spices at five times normal concentrations and ascorbic acid did not produce false positive reactions.

KEYWORDS: pathology and biology, deer, chemical analyses, immunodiffusion, agglutination, cervid, antigens, sausage

Immunological methods have been used for many years to determine if a bloodstain is of human or animal origin [*1*]. Adaptation of these methods to the field of wildlife forensic science has been a relatively recent development.

Agar gel immunodiffusion tests are frequently utilized in species identification of animal blood or tissues [*2–4*]. The U.S. Department of Agriculture (USDA) has designated an agar gel immunodiffusion test as their official method for identification of animal tissues. The agglutination of latex particles which have been coated with immunoglobulins has been used to identify human and animal bloodstains [*5,6*]. A rapid field technique has been described for the identification of deer blood by latex agglutination [*7*]. No information is available on the use of this technique in the identification of cervid muscle tissues.

Species identification of tissue antigens in partially cooked sausages is complicated by heat denaturation of proteins [*8*] and by the presence of additives which in some instances have been reported to cause false-positive reactions [*9*].

This study compared the specificity and sensitivity of the two methods for the identification of frozen white-tailed deer muscle. The two methods were also utilized to identify white-tailed deer tissue in sausages of known composition. The effects of sausage spices and sodium ascorbate on the specificity of the tests was investigated.

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Materials and Methods

Anti-deer serum (Lot 24320, Cappel Division, Cooper Biomedical, Malvern, PA) produced in goats and solid phase adsorbed to eliminate species cross-reactivity was used with both methods. The titer of the antiserum was 1/960 when tested against deer serum by immunodiffusion. The antiserum which contained 0.05% sodium azide as a preservative and 0.02*M* phosphate buffered saline at pH 7.3 was reconstituted with distilled water as recommended by the manufacturer for performance of the agar gel immunodiffusion tests. The method of Itoh [*10*] was utilized to coat latex particles with anti-deer serum to produce a preparation referred to as sensitized latex.

Extracts of muscle tissues and sausages were prepared as described by Fugate and Penn [*2*]. Only crystal clear tissue extracts were used for testing. Serial dilutions (1 : 2 to 1 : 256) of extracts were prepared with phosphate buffered saline at pH 7.2.

Summer sausage and ring bologna were prepared from ground venison, beef, pork, spices, and water (Table 1). Heated and unheated sausages were prepared. The heated sausages were smoked at 125°F (51.6°C) for 10 h followed by heating at 170°F (76.6°C) until the core temperature reached 145°F (62.7°C) (approximately 2 h).

Agar gel immunodiffusion tests were performed by the method of Fugate and Penn [*2*] modified to use a pattern of wells in agar described by Garvey et al. [*11*]. Wells were cut in agar coated microscope slides with a stainless steel gel punch which produced a pattern of six wells each 4 mm in diameter around a central well. Each well was 4 mm from the adjacent well and 4 mm from the central well. Anti-deer serum, 20 μL, was pipetted into the central well and known deer extract, 20 μL, was placed into each well at the 12 and 6 o'clock positions. Unknown extracts (20 μL) were placed in each of the remaining four wells. After loading the wells, slides were incubated 24 h in a humidity chamber at room temperature. The slides were examined for lines of precipitation and the results recorded. Unknown samples with lines of precipitation which formed a continuous band or line of identity with that produced by the known deer extract were considered to contain cervid tissues.

The sensitized latex agglutination test was performed as described by Oates et al. [*7*]. Twenty microlitres of meat extract was placed on a glass plate and one drop of sensitized latex was added with a capillary pipette. The drops were mixed with a clean applicator stick over a circular area with a diameter of 15 mm. The plate was rotated gently and readings taken at 2 and 4 min over a lighted black background. Positive and negative controls were included each time the test was performed.

The effect of sausage additives and spices on the tests was determined by adding spices to individual 10-g samples of finely minced venison, beef, and pork. Each sample was placed in 30 mL of distilled water. One tube of meat from each animal species received the concentration of spices normally used in sausage manufacturing, one received two times that amount, and the third received five times the normal amount (Table 2). Meat samples with spices

TABLE 1—*The composition of sausages used for comparison of methods for the identification of deer tissue.*

	Percent by Weight			
Type of Sausage	Venison	Pork	Beef	Added Water
Summer A	52	35	0	13
Summer B	70	17	0	13
Summer C	46	29	12	13
Ring bologna	43.5	43.5	0	13
Ring bologna	65	22	0	13

TABLE 2—*The amount of sausage additives and spices added to 10-g samples of minced meats.*

Ingredient	Weight in Grams		
	Normal Amount	2 × Normal	5 × Normal
Witt's cure salt	0.25	0.50	1.25
Soyle Royle	0.2	0.40	1.0
O.V.A. (nonfat dry milk)	0.15	0.30	1.75
Dextrose	0.1	0.2	0.5
Witt's prepared summer sausage seasoning	0.09	0.18	0.45
Black pepper	0.006	0.012	0.03
Garlic	0.006	0.012	0.03
Monosodium glutamate	0.006	0.012	0.03

were mixed for 5 min and samples taken at 0 h, 3 h, 6 h, 30 days, and 60 days. Preparations were held at 40°F (4.4°C) and agitated daily.

Sodium ascorbate was added to three 5.0-mL aliquots of beef and pork extracts to give final concentrations of 0.05, 0.1, and 0.5%. Aliquots were prepared in triplicate and examined for reaction with anti-deer serum by latex agglutination and agar gel diffusion.

Results

The specificities of latex agglutination and agar gel diffusion tests were compared on extracts of white-tailed deer *(Odocoileus virginianus),* mule deer *(Odocoileus hemionus),* moose *(Alces alces),* elk *(Cervus elaphus),* pronghorn antelope *(Antilocapra americana),* beef, horse, sheep, goat, pig, rabbit, and chicken. Both tests reacted with muscle tissues from animals in the family Cervidae (deer, moose, and elk), but not with tissues from the domestic animals or pronghorn antelope. As illustrated in Table 3, the tests reacted with white-tailed deer tissue at a higher dilution than with tissues of the other cervids. The latex agglutination test was consistently more sensitive than the agar-gel diffusion procedure.

The maximum dilution of sausage tissues which were positive in each test is illustrated in Table 4. The latex agglutination test was more sensitive than the agar gel diffusion test in detecting deer tissues in sausages. In most cases, the tests were less sensitive on heated sausages than on unheated sausages.

TABLE 3—*The relative sensitivity of agar gel diffusion and latex agglutination tests in the identification of cervid muscle antigens.*

Sample	Highest Dilution at Which the Test Was Positive	
	Latex Agglutination	Agar Gel Diffusion
White-tailed deer *(Odocoileus virginianus)*	1:256	1:32
Mule deer *(Odocoileus hemionus)*	1:128	1:8
Moose *(Alces alces)*	1:64	1:8
Elk *(Cervus elaphus)*	1:128	1:4

TABLE 4—*The relative sensitivity of agar gel diffusion and latex agglutination tests in the detection of venison in heated and unheated sausages.*

Type of Sausage	Highest Dilution at Which the Test Was Positive	
	Latex Agglutination	Agar Gel Diffusion
Summer A (unheated)	1:32	1:16
Summer A (heated)	1:16	1:8
Summer B (unheated)	1:32	1:16
Summer B (heated)	1:16	1:8
Summer C (unheated)	1:64	1:16
Summer C (heated)	1:16	1:4
Ring bologna A (unheated)	1:16	1:8
Ring bologna A (heated)	1:16	1:4
Ring bologna B (unheated)	1:32	1:16
Ring bologna B (heated)	1:16	1:4

Sausage spices added to venison, beef, and pork at concentrations normally used in sausage manufacturing, at two times and five times normal concentrations, had no effect on the tests. Sodium ascorbate at concentrations of 0.05, 0.1, and 0.5%, failed to produce false positive reactions when added to beef and pork extracts.

Discussion

Commercial anti-deer serum was specific for cervid tissue in both the latex agglutination and agar gel diffusion tests. The tests were more sensitive for white-tailed deer; however, differences were not of sufficient magnitude to differentiate between cervid species.

The latex agglutination test was consistently more sensitive than the agar gel diffusion method. However, latex agglutination has the disadvantage of requiring some degree of interpretation, of being time dependent, and of not providing permanent visual records of the results.

A comparison of the sensitivities of the two procedures in detecting deer antigens in sausage demonstrated results consistent with those obtained by examining cervid muscles tissues. The somewhat less sensitive agar gel diffusion method would be less likely to detect trace amounts of cervid antigens which could be present in meat mixtures as a result of incomplete cleaning of equipment.

The specificity and sensitivity of the procedures are dependent upon the quality of the antiserum. Each new lot of antiserum should be tested for both specificity and sensitivity. The titer against deer serum of the commercial antiserum used in our tests was lower than that reported for unabsorbed serum; however, the specificity was greatly improved [*6*].

The sensitivity of the two procedures for the detection of deer antigens in sausage was reduced by commercial smoking followed by gradual heating to an internal temperature of 145°F (62.7°C). However, both procedures readily detected deer tissues in the experimental sausages. The proportion of venison in the sausages was relatively high (43.5 to 70%), but in a range consistent with commercial practices.

The addition of sausage spices and additives to beef and pork at levels up to five times normal amounts did not cause false positive reactions in samples taken repeatedly up to sixty days.

Sodium ascorbate added to beef and pork at levels of 0.05, 0.1, and 0.5% had no effect on the latex agglutination and agar gel diffusion tests. A previous report indicated that ground beef extracted with a 0.05% solution of sodium ascorbate gave a false-positive ring test [*9*]. The problem with false-positive reactions as a result of ascorbate appears to be limited to the ring precipitin reaction.

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