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## Persistence of Pseudorabies Virus in Feral Swine Populations

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**ABSTRACT:** Serologic surveys for evidence of exposure to pseudorabies virus (PRV) in feral swine were conducted from November 2001 to April 2002 at 10 sites in the southeastern United States, where evidence of previous PRV exposure had been documented during 1979–89. Sera were tested in the field on the day of collection by latex agglutination. Maximum sample size per site was to be 30 animals, but sampling was discontinued before reaching this number when positive results were obtained. Positive results were obtained at all of the study sites, demonstrating long-term persistence of PRV in feral swine populations. Overall, 38 of 100 (38%) animals were positive for antibodies. Consistent results from latex agglutination tests conducted in the field and laboratory demonstrated that this test was useful as a rapid and reliable diagnostic tool when used in the field.

**Key words:** Feral swine, latex agglutination, PRV, pseudorabies, *Sus scrofa*.

The US Department of Agriculture began a national pseudorabies eradication program in 1989, and as of 2001, domestic swine in 41 states and territories were considered free of pseudorabies virus (PRV; Marsh and Leafstedt, 2001). However, PRV is well established in feral swine populations in the United States, and feral swine represent a potential reservoir of this virus for infection of domestic swine and native wildlife. From 1978 to the mid-1980s, feral swine from 37 populations were surveyed for PRV antibodies in the southeastern United States, and evidence of PRV was detected in 23 populations (Nettles and Erickson, 1984; Corn et al., 1986). In Florida, 11 of 13 populations were positive (van der Leek et al., 1993). Nettles (V. F. Nettles, pers. comm.) summarized data presented in various published and unpublished studies and found that of over 15,000 feral swine tested in the United States through 1995, 28% were seropositive.

The epizootiology of PRV among feral

swine includes venereal transmission (Romero et al., 2001) and acquisition of infection as adults (Pirtle et al., 1989; van der Leek et al., 1993). Infections in adult feral swine do not cause morbidity or mortality (Tozzini et al., 1982; Romero et al., 2001) but can result in latent infections, and it is possible that the virus can persist indefinitely once a population has become infected. Conversely, because of marked changes in population density, this virus potentially could be eliminated naturally from feral swine populations. This question has important implications for PRV surveillance, control, and eradication measures directed at feral swine. The objectives of this study were to determine whether PRV persists in feral swine populations over long time spans and, secondarily, to determine whether latex agglutination (LA) can be used in the field as a rapid diagnostic tool.

Selection of study sites was based on evidence of PRV infection or exposure in feral swine at specific sites before 1989. The sites selected and results of previous surveys at these sites are given in Table 1. Maximum sample size per study site was set at 30 animals if all results at the given site were negative, but sampling was discontinued when positive results were obtained with fewer animals. On the basis of rules of probability, negative results in tests from 29 animals from a large (>400) homogeneous population would indicate a maximum prevalence of infection of 10% ( $P=0.05$ ). We selected this as a conservative cutoff point because the prevalence of seropositive feral swine in infected populations averages 28%. Ages of feral swine were determined on the basis of tooth replacement, with a modification of the technique described by Matschke (1967),

TABLE 1. Results of surveys for evidence of pseudorabies virus in feral swine at 10 sites in the southeastern United States.

Site <sup>a</sup>	Oldest record		Recent record		Current study, 2001–02	
	Year	Prevalence (%)	Year	Prevalence (%)	No. tested/ no. positive	Prevalence (%)
1	—	—	1985 <sup>b</sup>	30	6/10	60
2	—	—	1980 <sup>c</sup>	25	2/2	100
3	—	—	1988 <sup>d</sup>	10	2/7	29
4	—	—	1985 <sup>b</sup>	70	2/14	14
5	—	—	1989 <sup>e</sup>	69	9/16	56
6	1979 <sup>c</sup>	20	1986 <sup>c</sup>	8	5/18	28
7	1980 <sup>c</sup>	40	1988 <sup>e</sup>	33	3/7	43
8	—	—	1981 <sup>c</sup>	17	3/10	30
9	—	—	1981 <sup>c</sup>	17	1/4	25
10	1980 <sup>c</sup>	60	1988 <sup>e</sup>	11	5/12	42

<sup>a</sup> Site 1 = Callaghan Ranch, Webb County, Texas (27°52'N, 99°24'W); 2 = Hal's Lake Hunt Club, Clarke County, Alabama (31°18'N, 87°51'W); 3 = Hobcaw Barony, Georgetown County, South Carolina (33°20'N, 79°13'W); 4 = King Ranch, Kleberg County, Texas (27°30'N, 97°57'W); 5 = Myakka State Park, Sarasota County, Florida (27°14'N, 82°18'W); 6 = Ossabaw Island State Heritage Preserve, Chatham County, Georgia (31°46'N, 81°5'W); 7 = Prairie Lakes Wildlife Management Area, Osceola County, Florida (27°57'N, 81°8'W); 8 = Rhett's Island Wildlife Management Area, McIntosh County, Georgia (31°20'N, 81°23'W); 9 = St. Marks National Wildlife Refuge, Wakulla County, Florida (30°7'N, 83°59'W); 10 = Tosohatchie Wildlife Management Area, Orange County, Florida (28°30'N, 80°59'W).

<sup>b</sup> Corn et al. (1986).

<sup>c</sup> Southeastern Cooperative Wildlife Disease Study (unpubl. data).

<sup>d</sup> Wood et al. (1992).

<sup>e</sup> Van der Leek et al. (1993).

and only adults (>8 mo of age) were sampled. Data on sex were collected for all feral swine.

Blood samples were obtained via cardiac puncture or venipuncture during November 2001 through April 2002 from live-trapped and hunter-killed feral swine and from feral swine collected for the purpose of our study at 10 study sites in the southeastern United States. Serum samples were obtained by centrifugation and tested by LA with a commercial kit (Viral Antigens Inc., Memphis, Tennessee, USA) on the day of collection. The kit protocol states that sera initially should be screened at a 1:4 dilution. Samples testing negative at this dilution are considered negative, but samples testing positive are to be tested further at a 1:40 dilution. Those also positive at the higher dilution are then considered positive. This procedure was followed in the field. However, the test protocol also specifies that sera negative at 1:40 be retested at 1:4 with the use of heat-inactivated sera. To confirm results

and evaluate reliability of the field tests, all samples were retested in a laboratory at the Southeastern Cooperative Wildlife Disease Study (The University of Georgia, Athens, Georgia, USA) with heat-inactivated sera at 1:4 dilutions.

Serologic evidence of exposure to PRV was found in all 10 feral swine populations sampled (Table 1). Final results based on laboratory tests revealed that 38 of 100 animals (38.0%) were positive by LA testing. Fifteen of 40 (37.5%) males and 23 of 60 (38.3%) females were positive.

Seven samples were tested only in the laboratory because of limited reagent supplies in the field; therefore, a comparison of field and laboratory results was made for 93 samples. Of these, 83 (89%) showed complete agreement. Four feral swine seropositive at 1:4 when tested in the field but negative at 1:40 were later confirmed as negative after heat inactivation. According to the test protocol, these samples could be considered false positives that were detected by the recommended re-

testing. Also, six serum samples that initially tested negative in the field later tested positive in the laboratory after heat inactivation. The discrepancies in these results could be a result of subjective differences in test analysis, lack of technical experience among the field personnel, or less than optimal conditions that can be encountered in field situations. In some cases, agglutination particles in positive reactions were very fine and thus difficult to interpret without magnification. Adherence to the recommended testing protocol eliminated the initial false positive tests, and small numbers of false negatives should not be a problem in evaluating antibody responses in a feral swine population, as long as sample sizes are adequate.

The LA test has been used to test specimens from feral swine in previous studies (Pirtle et al., 1989; van der Leek et al., 1993; Gresham et al., 2002). When compared with other serologic methods for detecting PRV antibodies in domestic swine, LA has been shown to be highly sensitive and accurate (Schoenbaum et al., 1990). Because of its simplicity and accuracy, the LA test therefore is useful as a rapid diagnostic tool in field surveillance for PRV in feral swine populations. Use of the test in the field during collection allows for termination of collections when positive results are found. This allows for a reduced number of samples and increased efficiency.

The time elapsed since the last known survey at the 10 study sites ranged from 13 to 22 yr. Pseudorabies has been present in at least six of the 10 sites for 20 yr or more. We conclude that PRV persists in feral swine populations and that there is little or no need for routine surveillance for PRV in feral swine populations with a history of exposure to PRV. The emphasis for surveillance should be on populations of unknown status.

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