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PREVALENCE OF PSEUDORABIES (AUJESZKY'S DISEASE) VIRUS ANTIBODIES IN FERAL SWINE IN FLORIDA

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ABSTRACT: Serum samples collected from feral swine (*Sus scrofa*) throughout Florida (USA) from 1980 to 1989 were tested for antibodies to pseudorabies virus (PRV) by the serum neutralization test, the latex agglutination test, or by the enzyme-linked immunosorbent assay. Seropositive swine were detected at 11 of 13 sites with a composite seroprevalence of 34.8% (579 of 1,662 samples; range = 5.9% to 58.2%) for sites with seropositive swine. Data on age and sex of the swine were available from three sites. Seroprevalence in males and females did not differ significantly (P = 0.62 for the combined data). Seroprevalence in adult (≥8 mo) and juvenile swine (<8 mo) was significantly different at all sites ($P < 0.05$ for the combined data). From these data, PRV infections appear to occur widely in populations of Florida feral swine and may seriously undermine efforts to eradicate this virus from the domestic swine population of the USA.

Key words: Feral swine, wild swine, *Sus scrofa*, pseudorabies virus, Aujeszky's disease virus, seroprevalence.

INTRODUCTION

Florida (USA) has a population of approximately 500,000 feral swine (*Sus scrofa*) with an estimated commercial value exceeding $8 million (US$) annually (Degner et al., 1983). Feral swine are present in all 67 counties (Frankenberger and Belden, unpubl.), with the highest population densities in south central Florida and along the northern Gulf coast (Fig. 1). Although they are infected with several viral and bacterial disease agents, and play host to numerous parasites, two diseases are of particular importance: pseudorabies and swine brucellosis. Observations on the prevalence of antibodies to *Brucella* sp. in Florida feral swine are presented in an accompanying paper (van der Leek et al., 1992).

Pseudorabies virus (PRV) seropositive wild swine, including European wild boar and wild boar/feral swine hybrids, have been detected among free-living populations in 11 states (Alabama, Arkansas, California, Florida, Georgia, Hawaii, Louisiana, Mississippi, Oklahoma, South Carolina, Texas; USA) (Clark et al., 1983; Nettles and Erickson, 1984; Corn et al., 1986; Nettles, 1989), with a reported overall seroprevalence of 19% (236 of 1,221 samples) for the southeastern states (Nettles, 1989). Infections in domestic swine traditionally have been associated with high piglet mortality, following involvement of the central nervous and respiratory systems, and fever, anorexia and abortion in adults (Pensaert and Kluge, 1989). The extent of clinical disease and prevalence of latent infections in feral swine is unknown. Experimentally infected adult

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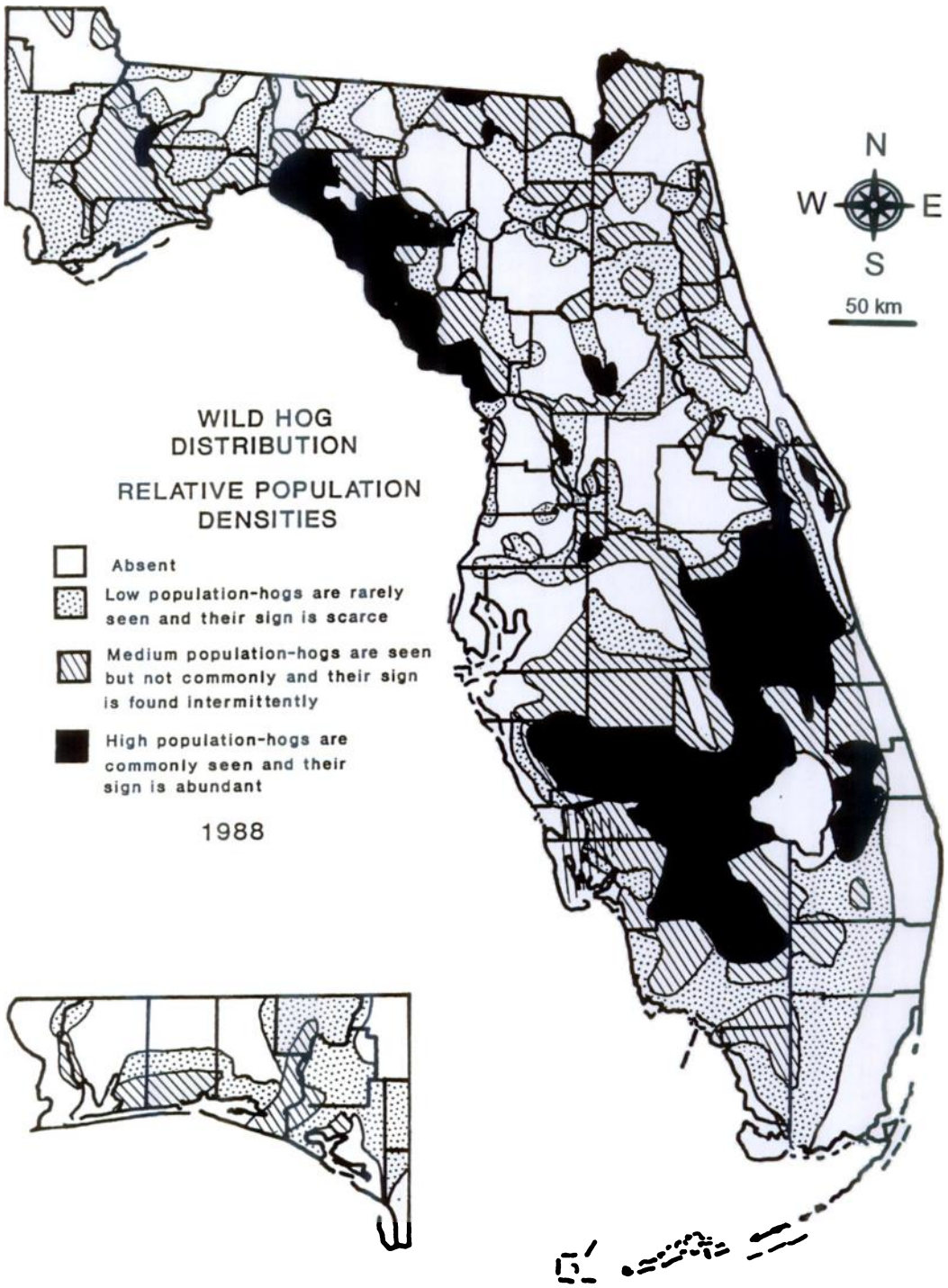


FIGURE 1. Distribution and abundance of feral swine in Florida (Frankenberger and Belden, unpubl.).

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TABLE 1. Prevalence of pseudorabies antibodies in feral swine in Florida.

County[a]	Site	Year sampled	Test used	Number tested	Number positive	Prevalence (%)
1) Jefferson, Taylor, Wakulla	Aucilla WMA[b] (30°10'N, 84°04'W)	1988	LAT[c]	17	5	29
2) Taylor	Tide Swamp WMA (29°50'N, 83°30'W)	1988	LAT	2	1	50
3) St. Johns	Guana River WMA (30°05'N, 81°20'W)	1988	LAT	4	1	25
4) Pasco	Larkin Ranch (28°20'N, 81°07'W)	1986, 1987	LAT	21	0	0
5) Sumter, Lake, Polk	Green Swamp WMA (28°20'N, 81°00'W)	1988	LAT	9	0	0
6) Orange	Tosohatchee WMA (28°30'N, 81°00'W)	1986, 1987	LAT	71	16	22
		1988	LAT	9	1	11
7) Osceola	Bull Creek WMA (27°55'N, 81°00'W)	1988	LAT	12	5	41
8) Osceola	Three Lakes WMA (28°00'N, 81°15'W)	1988	LAT	3	1	33
9) Sarasota	Myakka River State Park (27°15'N, 82°15'W)	1986, 1987	LAT	134	73	54
		1989	SN[d]	43	30	69
10) Charlotte	Cecil M. Webb WMA (26°50'N, 81°55'W)	1988	LAT	58	23	39
11) Glades	Fisheating Creek Wildlife Refuge (26°50'N, 81°55'W)	1980	SN	637	138	21
		1983	SN	108	29	26
		1983	ELISA[e]	476	223	46
		1986, 1987	LAT	51	26	51
12) Palm Beach	J.W. Corbett WMA (26°50'N, 81°10'W)	1988	LAT	17	1	5
13) Collier	Buck Island Ranch (27°10'N, 81°15'W)	1986, 1987	LAT	20	6	30

[a] Counties are listed north to south starting in the Florida Panhandle.
[b] WMA, Wildlife management area.
[c] LAT, Latex agglutination test.
[d] SN, Serum neutralization test.
[e] ELISA, Enzyme-linked immunosorbent assay.

wild swine have little or no clinical disease, but do transmit the virus (Tozzini et al., 1982; Mengeling and Pirtle, 1989). Swine are considered to be the reservoir host for PRV (Pensaert and Kluge, 1989); PRV also infects a wide range of secondary hosts, including cats, dogs, cattle, sheep and goats (Fenner et al., 1987). In secondary hosts, infections are characterized by intense pruritus and central nervous system dysfunction with signs such as frenzy, paralysis and drooling; hence the commonly used terms "pseudorabies" and "mad itch." Infections, which are invariably fatal, usually are contracted by ingestion, but also may be acquired by aerosol inhalation (Fenner et al., 1987).

Based on serologic surveys for PRV antibodies in domestic swine of slaughter age in the USA, there has been a steady increase in prevalence in PRV infections from 0.56% in 1974 to 8.78% in 1984 (Thawley et al., 1987). Following completion of several pilot projects during the 1980's, eradication of PRV was recommended as an industry goal (Bradshaw,

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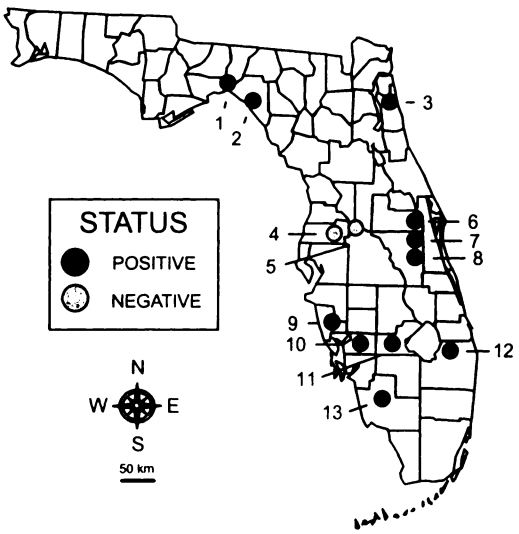


FIGURE 2. Distribution of sites with feral swine in Florida positive for pseudorabies virus antibodies (numbers are keyed to Table 1).

1986). The State-Federal-Industry Pseudorabies Eradication Program began in January 1989 with a goal of eradicating PRV by the year 2000 at an estimated cost of $250 million (U.S. Animal Health Association Committee on Pseudorabies, 1986). Circumstantial evidence has since been presented from several states, including Tennessee and Florida, incriminating PRV-infected feral swine as a source of infection for domestic swine (Creswell, 1989; Ormiston, 1989). The risk associated with PRV infections is appreciably greater than the risk associated with brucellosis since PRV is transmitted more readily, including by aerosol over several miles (Christensen et al., 1990).

Feral swine are an important prey item for the Florida panther (*Felis concolor coryi*) (Maehr et al., 1990). Since PRV infections are fatal in felines, infected feral swine may be a cause of mortality for this endangered species. Presently, the impact of this disease on the panther population as well as on other indigenous wildlife species is largely unknown. Pseudorabies virus has been isolated from raccoons (*Procyon lotor*) (Goyal et al., 1986) and a black bear (*Ursus americanus*) (Schultze et al., 1986), and PRV antibodies have been detected in a Florida black bear cub (Pirtle et al., 1986).

In view of the potential importance of this reservoir of PRV as a cause of disease in domestic swine, other livestock and indigenous wildlife, we determined the seroprevalence of PRV in feral swine in Florida as an aid to their future management.

MATERIALS AND METHODS

Sera were collected from 1,692 feral swine at thirteen sites from 1980 to 1989 (Table 1) and stored at −20 C prior to examination. Live swine were sampled from 1980 through 1987, and in 1989. During 1988, only hunter-killed feral swine, presented at check stations in selected wildlife management areas (WMA's), were available for sampling. Since serum quality was a concern, these sera were screened for the presence of immunoglobulin G (IgG) by double immunodiffusion (van der Leek, 1990) before examination for specific antibody to PRV.

At three sites, most feral swine were categorized as juveniles (<8 mo) or adults (≥8 mo) using tooth eruption patterns (Matschke, 1967). At the Fisheating Creek Wildlife Refuge a more detailed age structure of the 1983 feral swine sampling was established using the same method.

Sera were tested using a latex agglutination test (LAT), an enzyme-linked immunosorbent assay (ELISA) or a serum neutralization (SN) test. The LAT was performed using a commercial kit (Viral Antigens, Inc., Memphis, Tennessee, USA) and results were interpreted as positive or negative at a 1:4 dilution. The serum neutralization test was performed as described by Hill et al. (1977) with titers ≥1:4 called positive. The ELISA was performed as described by Snyder and Erickson (1981); results were interpreted as positive or negative.

The Chi-square or Fisher's Exact test (dependant on sample size) were used to evaluate differences in seroprevalence between adults and juveniles, and males and females, using a proprietary computer software program (EPI-STAT©, Round Rock, Texas). The significance level for both these tests was set at $P = 0.05$.

RESULTS

Swine with antibodies to PRV were detected at 11 of 13 sites (Table 1, Fig. 2). The composite seroprevalence was 34.8% (579 of 1,662 samples; range = 5.9% to 58.2%) for the 11 sites with seropositive swine.

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TABLE 2. Prevalence of pseudorabies antibodies by age and sex in three populations of feral swine in Florida.

Age	Sex	Fisheating Creek Wildlife Refuge[a]	Myakka River State Park[b]	Tosohatchee WMA[b]	All three sites
Adult	Male	91/132 (69%)[c]	24/34 (71%)	3/5 (60%)	118/171 (69%)
	Female	84/118 (71%)	23/31 (74%)	6/9 (67%)	113/158 (72%)
	Total	175/250 (70%)	47/65 (72%)	9/14 (64%)	231/329 (70%)
Juvenile	Male	18/108 (3%)	7/27 (26%)	0/15 (0%)	25/150 (17%)
	Female	29/116 (25%)	6/25 (24%)	0/13 (0%)	35/154 (23%)
	Total	47/224 (21%)	13/52 (25%)	0/28 (0%)	60/304 (20%)
All ages	Male	109/240 (45%)	31/61 (51%)	3/20 (15%)	142/321 (45%)
	Female	111/234 (47%)	29/56 (52%)	6/22 (27%)	146/312 (47%)

[a] ELISA, Enzyme-linked immunosorbent assay used.
[b] LAT, Latex agglutination test used.
[c] Number of swine with PRV antibodies ÷ number of swine tested (% positive).

Seroprevalence in males and females did not differ significantly at any of the sites studied. The seroprevalence in adults (≥8 mo) was significantly higher ($P < 0.05$) than that in juveniles (<8 mo) at all three sites tested, with values of 70.0% and 21.0%, respectively, for the Fisheating Creek Wildlife Refuge; 72.3% and 25.0%, respectively, for the Myakka River State Park; and 64.3% and 0.0%, respectively, for the Tosohatchee WMA (Table 2). Seropositive swine were present in all nine age categories considered at Fisheating Creek Wildlife Refuge (Table 2, Fig. 3).

DISCUSSION

Most sera were collected during the early 1980's in association with other projects conducted by staff of the Florida Game and Fresh Water Fish Commission. Because this agency undertook fewer projects relating to feral swine in the late 1980's, we also used sera collected from hunter-killed swine during 1988. Only two (1.5%) of 136 sera collected produced no lines of precipitation, indicating insufficient IgG content (data not shown); thus, lacking other opportunities to collect large numbers of samples from several sites, we believe collection of sera at hunter-check stations is an expedient approach. Hemolysis and dilution of the samples, however, are potential disadvantages of this sampling method (van der Leek, 1990).

The SN and LAT tests, and ELISA all are considered official pseudorabies tests by the United States Department of Agriculture. We did not compare the sensitivity and specificity of these tests. As simpler, more sensitive and more specific tests emerged during this study, they were incorporated; thus, differences between the tests used must be considered when evaluating these data. Schoenbaum et al. (1990) compared the SN, LAT, and ELISA in unvaccinated, experimentally infected domestic swine and found specificities approaching 100% for each. These authors found that the LAT detected infections

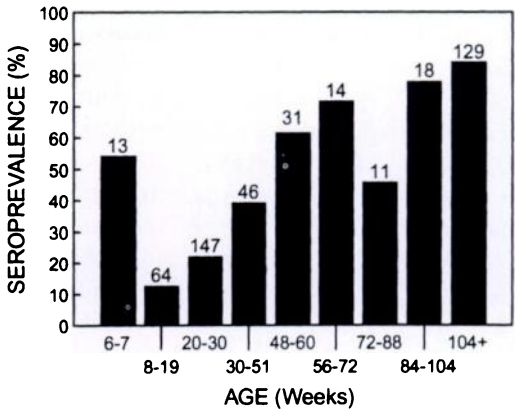


FIGURE 3. Prevalence (%) of pseudorabies antibody by age as determined by enzyme-linked immunosorbent assay in feral swine from Fisheating Creek Wildlife Refuge, Glades County (the sample size per age group is shown above each bar).

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earlier than ELISA, which in turn detected infections earlier than SN, confirming earlier work showing that SN is less sensitive than the ELISA (Martin et al., 1983). The LAT therefore provides high sensitivity and specificity, is easy to perform and can be used in the field.

Pseudorabies virus-seropositive feral swine were distributed throughout Florida at >80% of the sites examined. Most feral swine populations in Florida probably are infected with PRV. The widespread distribution of PRV infections in feral swine will become increasingly important as the eradication of the disease in domestic swine progresses. Management practices for feral swine will need to be examined. The illegal interstate shipment of feral swine to hunting preserves in other states is of particular concern. Such relocations may impact the domestic swine industry and undermine the national pseudorabies eradication program currently underway. Although the impact of aerosol shedding by feral swine in transport has not been studied, this could pose a risk to domestic swine operations situated en route. Spread of PRV from an infected site to surrounding uninfected areas is well recognized in the domestic swine industry. It also is known that feral swine enter domestic swine marketing channels (Degner et al, 1983; Alshouse, 1989). Since domestic swine often are raised throughout the southeastern USA in dirt lots with minimal barriers, the opportunity for contact between feral and domestic swine is great. The subsequent marketing of recently infected domestic swine therefore must also be considered.

In an earlier study (Pirtle et al., 1989) conducted on Ossabaw Island, Georgia, seroprevalence in males and females did not differ significantly in any age group; our data are in agreement. In the Ossabaw study, adults had a significantly higher seroprevalence than juveniles with seroprevalences of 28.5% and 1.2%, respectively. In contrast to the data of Pirtle et al. (1989), we found a higher prevalence of PRV antibodies in juvenile feral swine from Myakka River State Park and Fisheating Creek Wildlife Refuge. At Fisheating Creek Wildlife Refuge there was a gradual increase in seroprevalence (Fig. 3) after the disappearance of maternal antibodies, which may persist for up to 15 wk (Pensaert and Kluge, 1989). This indicates exposure of juvenile swine to infectious virus. We concur, however, with Pirtle et al. (1989) that the presence of PRV infections in a feral swine population is best determined by testing adult swine.

There is an urgent need for a risk assessment study to elucidate the importance of PRV infected feral swine in light of the national eradication program currently underway. Also the potential impact of PRV on indigenous wildlife, particularly the endangered Florida panther, deserves further study.

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