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Serosurvey of Selected Viral and Bacterial Diseases in Wild Swine from Oklahoma

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ABSTRACT: Blood samples collected from 120 wild swine (*Sus scrofa*) in thirteen Oklahoma (USA) counties during 1996 were tested for antibodies against six viral and two bacterial diseases. No antibodies to swine brucellosis, pseudorabies, transmissible gastroenteritis, and vesicular stomatitis were detected. Antibody titers to one or more leptospiral serovars were found in 44% of the samples, the two most frequent serovars being *Leptospira interrogans* serovars *bratislava* (29%) and *pomona* (27%). Antibody against porcine parvovirus and swine influenza virus was detected in 17% and 11% of the swine, respectively. Two samples (2%) were positive for antibody to the recently emerged porcine reproductive and respiratory syndrome virus.

Key words: Leptospirosis, porcine parvovirus, porcine reproductive respiratory syndrome, serosurvey, *Sus scrofa*, swine influenza, vesicular stomatitis.

Feral swine (*Sus scrofa*) are widely distributed in the southern one-half of Oklahoma (USA), with two high-density areas in the southcentral and southeastern regions (Stevens, 1996). Some wild swine populations have been exposed to pseudorabies virus (PRV), *Brucella suis* (*B. suis*), porcine parvovirus (PPV), *Leptospira interrogans* (*L. interrogans*), and vesicular stomatitis virus (VSV) New Jersey serotype in various parts of the United States (Clark et al., 1983; Corn et al., 1986; New et al., 1994; Pirtle et al., 1989; Stallknecht et al., 1993; van der Leek et al., 1993a, b; Zygmont et al., 1982). The domestic swine population in Oklahoma has increased by 595% between 1991 and 1996 (Bloyd and Cole, 1997). While most of this increase has been due to large-scale commercial operations, Oklahoma still harbors a large number of small-scale "backyard" swine operations that could potentially get in contact with feral swine. A two-way transmission of infectious diseases might potentially occur between feral and domestic swine populations. Although the size of the Oklahoma feral swine population is still unknown, contacts between feral and domestic swine occur frequently (Stevens, 1996). Indeed, such contacts and, presumably, the potential for disease transmission from wild to domestic swine have been used as part of the justification for destroying feral swine in Oklahoma (Stevens, 1996).

Although the occurrence of swine disease agents in feral swine in various parts of the United states is well documented, it is important that this information be updated from time to time to expand the geographic and etiologic base and, most importantly, to include newly emerged diseases. The objective of this study was to survey feral swine in thirteen Oklahoma counties (Bryan, Caddo, Choctaw, Coal, Custer, Grady, Hughes, Jefferson, Kiowa, Logan, Oklahoma, Pontotoc, and Roger Mills) (Fig. 1) for evidence of exposure to eight swine diseases, including the recently emerged porcine reproductive and respiratory syndrome (PRRS). The study area is located between 33°48′ to 36°05′N and 94°58′ to 100°00′W. This area was chosen because most of the recent expansion in swine operations occurred there and because of the possibility of obtaining sera from feral swine in that region.

Between January and December 1996, blood was collected in clot tubes from 120 apparently adult (≥7-mo-old) feral swine. No data was collected regarding the sex or distribution per county of these swine. The serum was separated and stored at −20 C until used. The sera were tested for

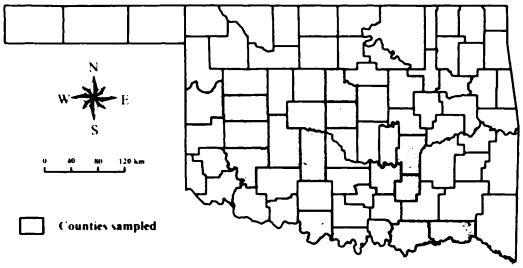

FIGURE 1. Oklahoma counties from which feral swine sera were collected.

antibodies against *B. suis,* PPV, porcine reproductive respiratory syndrome virus (PRRSV), PRV, swine influenza virus (SIV), transmissible gastroenteritis virus (TGEV), VSV New Jersey and Indiana serotypes, and *L. interrogans* serovars *bratislava, canicola, grippotyphosa, hardjo, icterohemorrhagiae,* and *pomona.* Seven of the disease agents were chosen because of their importance in domestic swine populations in Oklahoma as indicated by the frequency of requests for diagnostic service, and VSV was included to determine if feral swine might be a reservoir for a disease that is not present in domestic swine.

The standard card agglutination test (V-tech, Inc., Pomona, California, USA) was used for *B. suis,* with sera being scored as positive or negative. An indirect fluorescent antibody test (IFAT) was used for PPV and TGEV at a screening dilution of 1:5. The PPV reagent slides were prepared using PPV-infected porcine kidney (PK-15) cells spotted onto teflon-coated slides (Cel-Line associates, Newfield, New Jersey, USA) following a method previously described for bovine viral diarrhea IFAT staining (Corapi et al., 1990). Porcine parvovirus seed stock and PK-15 cells were obtained from the National Veterinary Services Laboratories (NVSL) (Ames, Iowa, USA). The TGEV slides were obtained from a commercial source (VMRD, Inc., Pullman, Washington, USA). Sera showing a distinct intracellular fluorescence at 1:5 dilution were scored as positive. *Leptospira interrogans* testing was done using the microscopic agglutination-lysis test (MALT) (Cole et al., 1973). Bacterial seed stocks for the six *L. interrogans* serovars were obtained from the NVSL (Ames, Iowa, USA) and cultured in our laboratory prior to use in the MALT. Samples giving titers of ≥100 against one or more serovars were considered positive for leptospiral antibody.

Pseudorabies antibody was detected using the commercial semi-automated latex agglutination test (Viral Antigens, Inc., Memphis, Tennessee, USA) (Rodgers et al., 1996) and sera were scored as positive or negative based on a manufacturer-recommended cutoff index value of 8. A hemagglutination-inhibition (HI) test (Chernesky, 1996) utilizing SIV A/swine/Iowa/31 serotype H1N1 (obtained from the NVSL, Ames, Iowa, USA) was used to detect SIV antibody at a screening dilution of 1:5. Detection of PRRSV antibody was done using a commercial ELISA kit (Idexx Laboratories, Westbrook, Maine, USA) according to the manufacturer's instructions, with results being expressed as a sample/positive (S/P) ratio. All ELISA-positive (S/P ratio ≥0.4) as well borderline reaction samples (SP ratio 0.2–0.39) were re-tested by IFAT (Yoon et al., 1992), in a titration format starting at 1:20 dilution, using spot slides (Corapi et al., 1990) containing MARC-145 cells (NVSL, Ames, Iowa, USA) infected with the VR-2332 strain of PRRSV (ATCC, Manassas, Virginia, USA). The IFAT result was considered definitive in determining the antibody status of each animal. Testing for VSV NJ and IN antibody was performed at the NVSL (Ames, Iowa, USA) using a competitive ELISA (Katz et al., 1995).

Table 1 summarizes the results obtained for the eight disease agents. All 120 samples could not be tested for antibodies against all eight infectious agents because of insufficient quantities of sera for a few samples. Evidence of exposure to four of the eight infectious agents was detected; the most prevalent agent was *L. interrogans* (44%) followed by PPV (17%), SIV

TABLE 1. Serologic test results for antibodies against selected diseases in Oklahoma feral swine.

Disease agent	Test method(s)[a]	Sera tested	Positive	
			Number	%
Brucella sp.	Card agglutination test	120	0	0
Leptospira interrogans	MALT	117	52	44
Porcine parvovirus	IFAT	117	20	17
PRRS virus	ELISA, IFAT	117	2	1.7
Pseudorabies virus	Autolex	120	0	0
Swine influenza virus	HI	117	13	11
TGE virus	IFAT	117	0	0
VSV	c-ELISA	109	0	0

[a] See text for abbreviated names of tests.

(11%) and PRRSV (2%). The frequencies of occurrence of antibodies to the six *L. interrogans* serovars tested were, in descending order: *bratislava,* 29%; *pomona,* 23%; *hardjo,* 17%; *grippotyphosa,* 7%; *icterohemorrhagiae,* 3% and *canicola,* 1%. Positive leptospiral antibody titers ranged from 100 to 6,400.

Of the 120 sera tested for PRV and *B. suis,* none were positive for any of the two agents. Similarly, none of the 117 and 109 tested for TGEV and VSV, respectively, were positive. These results are surprising, especially for *B. suis,* and PRV for which antibody prevalence rates varying from 3% to 23% and from 8% to 36%, respectively, have been found in some feral swine populations in other parts of the United States (Corn et al., 1986; Pirtle et al., 1989; van der Leek et al., 1993a, b; Zygmont et al., 1982). Our failure to detect any PRV- or *Brucella*-positive wild swine may result from (1) efforts to eradicate pseudorabies and brucellosis in Oklahoma are succeeding, as evidenced by the state's recent reclassification from stage IV to stage V in the national PRV eradication campaign; (2) the sera tested were not from a random sample and may thus not be representative of the feral swine population, or (3) a corollary to the above is that both diseases might currently be so rare in the population that a sample size much larger than that used in this study is required to be sure to detect at least one positive animal. Indeed, in the past we have detected PRV-positive feral pigs in follow-up studies when PRV has been detected in domestic swine from the same study area (J.T. Saliki, unpubl. data). In regard to *B. suis,* it also is important to note that the card agglutination test used in this study was designed for *B. abortus* and might be problematic for *B. suis* antibody detection.

Regarding TGEV, no published information on its seroprevalence in wild swine in the USA was available. However, the same caveats listed above apply to TGEV and its possible occurrence in feral swine in Oklahoma may not be excluded on the basis of this study. The situation with VSV is different since VSV has not been observed in Oklahoma for over 20 yr. Hence, its absence in the feral swine population is not surprising. The recent recrudescence in VSV has included two states bordering Oklahoma (New Mexico and Colorado) but results of intensive monitoring of the domestic swine, horse and cattle populations indicate that this latest flare-up does not involve Oklahoma (G. Eskew, unpubl. data).

Previous studies have shown that various serovars of *L. interrogans* are widely distributed among feral swine in different parts of the USA, with antibody prevalence rates ranging from 22–87% of animals sampled (Clark et al., 1983; Corn et al., 1986; New et al., 1994). Our data confirm those results by finding that 44% of the 117 feral hogs tested had antibodies against at least one serovar of *L. interro-*

gans. Interestingly, *L. interrogans* serovars *bratislava* and *pomona* are also the most frequently detected serovars among domestic swine samples tested in our laboratory (J.T. Saliki, unpubl. data). Of the 52 *L. interrogans*-positive samples, 56% had positive titers against a single serovar while 44% had positive titers against 2 or more (≤5) serovars. A majority (61%) of samples in the latter group had a very high titer (≥4-fold) against one serovar than the others, indicating that most of the positive swine might have been infected with only one serovar but the test was detecting cross-reactive antibodies.

Serological evidence for PPV infection has recently been reported in wild swine populations in the Great Smoky Mountains National Park (USA), with a seroprevalence rate of 14% (New et al., 1994). Our seroprevalence of 17% is comparable to and confirms those findings. There is no published information on the seroprevalence of SIV in wild swine in the USA. The antibody prevalence level of 11% in this study indicates that SIV infects wild swine in Oklahoma. However, its importance to wild swine health remains to be determined. In domestic swine, both clinical and subclinical SIV infections are known to occur (Easterday and Hinshaw, 1992); it is not known if a similar situation exists in wild swine.

In this study, two wild swine (2%) were positive for PRRSV antibody by both the ELISA and IFAT, with IFAT titers of 80 and 160. This is an interesting finding because of the immense economic importance of PRRSV in the domestic population. Indeed, evidence of PRRS infection has been found wherever there is a sizeable swine population. However, this is the first report of evidence of PRRSV infection in wild swine. Although two tests were used for PRRSV antibody detection in this study, there still might be a slim chance that these could be false positive reactions. Only the isolation of PRRSV from wild swine specimens can definitively confirm the occurrence of PRRS among feral swine populations.

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