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Exposure of feral swine (*Sus scrofa*) in the United States to selected pathogens

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Exposure of feral swine *(Sus scrofa)* in the United States to selected pathogens

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Abstract

Feral swine *(Sus scrofa)* are widely distributed in the United States. In 2011 and 2012, serum samples and tonsils were recovered from 162 and 37 feral swine, respectively, in the US to evaluate exposure to important swine endemic pathogens. Antibodies against porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2) were found in 2.5% and 25.3% of tested sera, respectively. Positive serological reactions against *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae* have been detected in 19.7% and 69.7% of animals. More than 15% of animals presented antibodies against these 2 pathogens simultaneously. Most animals were also seropositive for *Lawsonia intracellularis*. Feral swine can also be involved in transmission of zoonotic agents. Almost 50% of animals possessed antibodies against *Salmonella*. In addition, 94.4% of animals were carriers of *Streptococcus suis* in their tonsils. In conclusion, feral swine may be considered as a potential reservoir for different endemic diseases in domestic pigs, as well as for important zoonotic agents.

Résumé

Les porcs sauvages (Sus scrofa) *sont largement distribués aux États-Unis. En 2011 et 2012, aux États-Unis des échantillons de sérum et d'amygdales furent obtenus de 162 et 37 porcs sauvages, respectivement, afin d'évaluer l'exposition à d'importants agents pathogènes porcins endémiques. Des anticorps contre le virus du syndrome reproducteur et respiratoire porcin (VSRRP) et le circovirus porcin de type 2 (CVP2) furent détectés chez 2,5 % et 25,3 % des sérums testés, respectivement. Des réactions sérologiques positives envers* Mycoplasma hyopneumoniae *et* Actinobacillus pleuropneumoniae *ont été détectées chez 19,7 % et 69,7 % des animaux. Plus de 15 % des animaux avaient des anticorps contre ces deux agents pathogènes simultanément. La plupart des animaux étaient également séropositifs pour* Lawsonia intracellularis. *Les porcs sauvages peuvent également être impliqués dans la transmission d'agents zoonotiques. Près de 50 % des animaux avaient des anticorps contre* Salmonella. *De plus, 94,4 % des animaux étaient porteurs de* Streptococcus suis *dans leurs amygdales. En conclusion, les porcs sauvages peuvent être considérés comme des réservoirs potentiels de différentes maladies endémiques des porcs domestiques, aussi bien que d'agents zoonotiques importants.*

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Feral swine *(Sus scrofa)* are considered resident in at least 38 of the 50 states in the US and in at least 1 Canadian province (1,2) and populations appear to be expanding. Their range is also expanding due to both natural fecundity and transplantation by hunters. Feral swine not only damage natural and agricultural resources, but may also be reservoirs for important diseases. There is increased interaction and greater potential for disease in both domestic pigs and humans (zoonosis) (3). In the US, surveillance has focused on foreign animal diseases, specifically classical swine fever, and regulatory diseases, such as Aujeszky's disease and Brucellosis. There are reports, however, that suggest that economically important infectious diseases that endemically affect domestic pigs are present in the feral herd. For example, exposure to the porcine circovirus type 2 (PCV2), swine influenza virus, porcine respiratory coronavirus, porcine parvovirus, *Mycoplasma hyopneumoniae, Actinobacillus pleuropneumoniae* (App), Salmonella, and *Haemophilus parasuis* has been reported (4–7). Carrier animals of *Streptococcus suis* have also been reported in Germany (8). Since the risk of contact between feral swine and domestic pigs is likely to increase in North America, we evaluated the exposure of feral swine to selected common infections observed in domestic pigs. Such exposure may result from recent contact between feral swine and domestic pigs (8,9) and some diseases may have become endemic in feral swine populations.

Serum and other tissues are routinely collected from feral swine in the US by biologists with the National Wildlife Disease Program (National Wildlife Research Center, Fort Collins, Colorado). In 2011 and 2012, 162 serum samples were obtained from feral swine in 15 states and 37 tonsils were obtained from 6 states. The states from which the serum samples were obtained are listed in Table I. Tonsil samples came from the following states (number of tonsils): Alabama (2), Arkansas (9), Florida (5), Georgia (6), Hawaii (10), and Louisiana (5). Samples were collected opportunistically from animals taken for the purpose of managing wildlife damage. Blood was obtained from euthanized animals via cardiac puncture or

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Table I. Seroprevalence against different pathogens in 162 serum samples from feral swine from various states in the United States[a]

Antibodies against	Number of positive sera	Percentage of positive samples
M. hyopneumoniae[b]	32	19.7
PRRSV	4	2.5
PCV2	41	25.3
L. intracellularis	130	80
Salmonella[b]	80	49.4
A. pleuropneumoniae[c]	113	69.7
Both *M. hyopneumoniae*[b] and *A. pleuropneumoniae*[b]	25	15.4

[a] Samples came from the following states (number of samples): Alabama (n = 23); Arkansas (n = 6); California (n = 3); Florida (n = 21); Georgia (n = 9); Hawaii (n = 26); Kansas (n = 10); Louisiana (n = 8); Michigan (n = 1); Missouri (n = 1); Mississippi (n = 14); North Carolina (n = 9); New Mexico (n = 6); Oklahoma (n = 22). Three samples from another state were kept confidential by request.

[b] Suspicious samples were considered to be positive.

[c] Animals tested positive to any serotype of *A. pleuropneumoniae* (multi-App test).

orbital draw. Tonsils were removed and placed in Ziplok bags. Serum samples and tonsils were stored at −80°C. These were convenience samples, selected simply to represent feral swine from a broad geographic area.

Using the respective diagnostic tests, sera were tested for the presence of antibodies against the following pathogens: porcine reproductive and respiratory syndrome virus (PRRSV) (PRRS X3 Ab Test; IDEXX Laboratories, Westbrook, Maine, USA); PCV2 [immunofluorescence test (IFAT)] (10); *M. hyopneumoniae* (IDEXX HerdChek); *Lawsonia intracellularis (L. intracellularis)* (IFAT) (11); *Salmonella* (Diakit Salmonella Swine; Maxivet Laboratories, St. Hyacinthe, Quebec); and *Actinobacillus pleuropneumoniae* (App). For the latter pathogen, a mix-enzyme-linked immunosorbent assay (ELISA) test detecting all serotypes was used first [long-chain lipopolysaccharide ELISA (LC-LPS ELISA multi-App)] (12) and then, serotype /serogroup-specific LC-LPS ELISA was subsequently used for serotypes 1/9/11, 2, 3/6/8/15, 4/7, 5, 10, 12, 13, and 14 (13). To simplify the analysis, suspicious samples were considered to be positive in those tests where this classification exists (*M. hyopneumoniae*, *Salmonella*, and serotype/serogroup specific App tests).

Tonsils were processed to detect the presence of *S. suis* (14). A piece of tonsil weighing 0.5 g was taken and reduced to small pieces with a scalpel and then added to 5 mL of phosphate buffered saline (PBS) containing 0.1% bovine serum albumin (BSA). After vortex mixing for 2 min and filtering through filter paper, 100 μL of filtrate was plated onto blood-agar plates at 37°C (5% CO_2) for 18 h. Bacterial growth was harvested by washing the agar plates with 3 mL of sterile Tris-ethylenediamine tetra-acetic acid (EDTA) buffer (pH 8). Template deoxyribonucleic acid (DNA) from mixed cultures was prepared using the QiAMp DNA Mini Kit (Qiagen; Valencia, California, USA). Three polymerase chain reaction (PCR) tests were carried out: a first PCR based on the amplification of the gene coding for 16S ribosomal ribonucleic acid (rRNA) of *S. suis* (species-specific) (15); a second PCR based on the amplification of the *cps2J* gene (involved in capsule synthesis of serotype 2, detecting also serotype 1/2); and a third PCR also based on a capsule gene, *cps1I,* which detects serotypes 1 and 14 (16).

General results of serological analyses are presented in Table I. No clear association of results for any test with a specific state could be observed, probably due to the relatively low number of samples per state. Few animals (2.5%) presented a positive reaction for PRRSV, which is in agreement with what was previously reported in the US and Europe (4,6,7). Although it was hypothesized that a possible emergence of PRRSV in feral swine populations may eventually pose a threat to disease-free domestic swine (9), data obtained during the last 15 y seem to indicate that such a risk is still low. Persistence of PRRSV plays a significant role in transmission and dissemination of the virus in domestic pig populations. Persistently infected pigs can harbor the virus for up to 250 days (17). Indirect routes of infection, such as contaminated boots and coveralls, needles, mosquitoes, houseflies, transport vehicles, and aerosol (up to 9.2 km), also play an important role (17). Although some hypothesis may be raised, there is no clear explanation for the low prevalence of antibodies against PRRSV in feral swine.

On the other hand, 25% of serum samples were positive for antibodies against PCV2, with a titer range from 1/64 to 1/4096. The higher seropositivity for PCV2 might indicate more efficient transmission ability, either from commercial to feral swine or within feral populations. More studies are needed to clarify this difference. Although the percentage of antibodies to PCV2 positive samples was higher than that of PRRSV, it was considerably lower than previously reported data in the US (6,7,18). There is no clear explanation for such differences. The higher prevalence reported by Corn et al (72%) (7) and Sandfoss et al (59%) (19) might be explained, at least in part, by the fact that samples for these studies were taken in North Carolina, which has high densities of commercial pigs (usually positive for PCV2) and feral swine nearby. Another difference is the test used, since most previous studies were done by ELISA, whereas sera were tested by IFAT in the present study, although both tests present good sensitivity/specificity (18). Finally, results obtained in the present study are similar to those from 2027 serum samples tested by ELISA in 2011 from all over the US (26% of seropositivity, J. Baroch, unpublished data.). Taken together, the results of these studies suggest that, while 25% seroprevalence may be an average level nationwide, much higher levels can be found in certain areas.

Respiratory diseases are an important cause of economic losses in domestic pigs. Among respiratory pathogens, *M. hyopneumoniae* and App are frequently associated with disease. There are very few data on the seroprevalence against *M. hyopneumoniae* of feral swine in the US. Almost 20% of the samples herein were positive (Table I), which confirms data from Baker et al (6). In Europe, similar results were obtained in Spain and Slovenia (4,20). Moreover, Sibila et al (20) reported that *M. hyopneumoniae* DNA was detected in nasal swabs and lung samples. Although it has been previously reported that infections in feral swine due to *M. hyopneumoniae* are mainly subclinical, a potential risk for transmission to domestic pigs should be considered as significant.

Interestingly, there are no data about the infection by App of feral swine in North America. In Europe, there are only 2 reports, one of which shows a 35.8% prevalence observed by DNA detection in tonsils and lungs of feral swine by species-specific PCR in Germany (21). The real prevalence of exposure is probably underestimated in this study since DNA detection usually presents lower sensitivity than antibody detection (22). The second study reported a seroprevalence of 52% using an App species-specific ELISA (ApxIV ELISA) in Slovenia (4). In the present study, almost 70% of samples were positive to App, using a serological test that detects antibodies against all known serotypes of this pathogen (12). The higher prevalence of seropositivity may be due to higher exposure of US feral swine to the pathogen and/or to a higher sensitivity of the serological test used. In fact, the LC-LPS ELISA test was shown to present a higher sensitivity than the ApxIV ELISA test (23). Interestingly, 70% of Canadian domestic pig farms were also shown to be positive by serology for App in a previous study (24), which indicates that the level of infections seems to be similar in wild and domestic pigs.

We further analyzed, for the first time, the serotype/serogroup of App present in the feral swine population studied. Positive results have been obtained for all serotypes/serogroups present in domestic pigs in North America (Table II). Negative results were obtained only for serotype 14, which has only been reported in Hungary (22). Results showed a higher prevalence (more than 30% each) of serotypes 4/7, 3/6/8/15, and 12 compared to the remaining serotypes (Table II). It is important to note that these serotypes are also easily transmitted and the seroprevalence in domestic pigs within infected farms is usually high (22). In fact, the same serotypes were predominant in the Canadian study using sera from domestic pigs (24). Although the ELISA test may detect cross-reacting antibodies against both serotypes 4 and 7, seropositive results in North America are usually associated with serotype 7, which is presently the most frequent serotype isolated from diseased domestic pigs in Canada (M. Gottschalk, unpublished observations). Only 2 strains of serotype 4 have been isolated in Canada (from healthy domestic pigs) and no isolation of this serotype has been reported in the US (22).

The long-chain lipopolysaccharide ELISA (LC-LPS ELISA) for serotype 1 used in the present study detects cross-reacting antibodies against serotypes 1, 9, and 11. Since serotypes 9 and 11 have never been isolated in North America, however, positive samples are considered to be against serotype 1. In this regard, approximately 7% of tested sera were positive to serotype 1, which is considered a highly virulent serotype. Many efforts have been made to eradicate this serotype from most genetic nucleus farms in North America. Indeed, feral swine might act as a reservoir for this important serotype. As happens in domestic pigs, animals may be colonized by more than 1 serotype of App. In fact, 33.6%, 13.3%, and 3.5% of sera were simultaneously positive to 2, 3, and 4 serotypes/serogroups, respectively. Finally, more than 15% of animals presented simultaneous antibodies against both *M. hyopneumoniae* and App (Table I). These pathogens have already been shown to synergistically cause serious respiratory disease in dually infected domestic pigs (22).

Serology has also been done against 2 intestinal pathogens, *L. intracellularis* and *Salmonella*. There is only 1 recent report on seroprevalence of wild pigs against *L. intracellularis*, which showed approximately 25% of animals were positive in South Korea (25).

Table II. Seroprevalence against different serotypes of *A. pleuropneumoniae* in 162 serum samples from feral swine from various states in United States

Serotype	Number of positive sera[a]	Percentage of total samples	Percentage of *A. pleuropneumoniae* positive samples
1[b]	12	7.4	10.6
2	10	6.2	8.8
3/6/8/15	50	30.9	44.2
4/7	56	34.6	49.6
5	3	1.9	2.7
10	9	5.6	8.0
12	49	30.2	43.4
13	10	6.2	8.8
14	0	0	0

[a] Suspicious samples were considered to be positive.
[b] The ELISA test used detects antibodies that cross-react against serotypes 1, 9, and 11. However, only serotype 1 has been isolated in North America (22).

The few other available studies used detection of DNA in intestine mucosa and/or feces. For example, 9.1% and 29.6% of tested wild pigs were positive when tested by PCR in the Czech Republic and Australia, respectively (26,27). There are no available data on the presence of this infection in feral swine in the US and Canada. By serology, 80% of animals tested positive, which represents a similar distribution to that observed for domestic pigs in North America (11,28). The lower prevalence observed in the few European studies is probably due to the lower sensitivity of the PCR techniques compared to serological testing used in the present study. Results suggest that feral swine may act as a reservoir for this pathogen. It has been shown that pigs can shed *L. intracellularis* intermittently for a period of 12 wk after infection (28), indicating the capability for long-term colonization and survival of this pathogen in subclinically infected animals. It is important to note that *L. intracellularis* has been identified in wild animals such as wolves, foxes, and a red deer (28). Further studies are needed to evaluate the role of feral swine in the epidemiology of the infection.

Interestingly, almost half of animals tested presented antibodies against *Salmonella*. Similar results were obtained by Vengust et al (4), with 49% of positive animals, and higher than that reported in Spain (5). In a previous study, no positive samples could be identified in Texas using fecal specimens (7), although this method is less sensitive than serology. This is the first serological study on *Salmonella* carried out in feral swine in the US. The indirect ELISA test kit used in the present study for *Salmonella* in swine contains 12 different inactivated antigens extracted from the most predominant serogroups of *Salmonella* (B, C, N, and E) found in North America. *Salmonella* is considered a zoonotic agent and may be transmitted to humans via infected carcasses. In fact, *Salmonella* was isolated from feral swine carcasses processed for human consumption in Australia (29).

Since there is no validated serological test for *S. suis*, the presence of this pathogen in tonsils was detected by PCR. To the best of our knowledge, this is the first study of this pathogen in feral swine in North America. A total of 34 tonsils (91.2%) were colonized by *S. suis*.

Similar results were obtained in a survey carried out in farms with domestic pigs in Canada (24). Only 4 tonsils (10.8%) were positive for serotypes 2 or 1/2, however, and all were negative for serotype 1/14. *Streptococcus suis* is a normal inhabitant of domestic pigs and an emerging zoonotic agent. Serotypes 2 and 14 are the most important types isolated from diseased humans (30). Data are almost identical to another study carried out in Germany, where *S. suis* was isolated from 92% of animals and approximately 10% of animals were positive for the *cps2* gene. The putative zoonotic potential of the *cps2* positive strains in German wild boars was highly suspected since they were very similar to a strain recovered from a meningitis case in a hunter infected with *S. suis* after butchering a wild boar (8). In fact, many cases of *S. suis* human disease have been described in boar hunters in Europe (30).

In conclusion, feral swine in the US may be considered as a potential reservoir for different endemic diseases described in domestic pigs. In fact, feral pigs have been shown to come in contact with domestic pigs and potentially transmit pathogens (3,7,9). These animals may also be a reservoir for important zoonotic agents.

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