Journal of Wildlife Diseases, 38(3), 2002, pp. 649–652 © Wildlife Disease Association 2002

Antibodies to Selected Viral and Bacterial Pathogens in European Wild Boars from Southcentral Spain

Joaquín Vicente,¹ Luís León-Vizcaíno,² Christian Gortázar,¹.³ María José Cubero,² Mónica González,² and Pablo Martín-Atance²¹ Instituto de Investigación en Recursos Cinegéticos (CSIC/UCLM), P. O. Box 535, E-13080 Ciudad Real, Spain; ² Infectious Diseases, Department of Animal Pathology, Faculty of Veterinary Science, 30071 Murcia, Spain; ³ Corresponding author (email: gortazar@irec.uclm.es)

ABSTRACT: Serum samples from 78 European wild boars (Sus scrofa) harvested during the 1999-2000 hunting season were tested for antibodies to Brucella spp., classical swine fever virus, Erysipelothrix rhusiopathiae, Haemophilus parasuis, Leptospira interrogans serovar pomona, Mycoplasma hyopneumoniae, pseudorabies virus (PRV), porcine parvovirus (PPV), porcine reproductive and respiratory syndrome virus, Salmonella serogroups B, C, and D, Streptococcus suis, and swine influenza virus (SIV) serotypes H1N1 and H3N2. Samples were collected from Sierra Morena and Montes de Toledo in southcentral Spain. Antibodies were detected to PRV (36%), L. interrogans serovar pomona (12%), PPV (10%), E. rhusiopathiae (5%), SIV serotype H1N1 (4%), Salmonella serogroup B (4%), and Salmonella serogroup C (3%). Our results suggest that more research is needed to describe the epidemiology of infectious diseases of Spanish wild boars.

Key words: European wild boar, serosurvey, Spain, Sus scrofa.

The European wild boar (Sus scrofa) is Spain's most important big game species. As in other European countries, wild boar populations in Spain have increased in the last two decades (Gortázar et al., 2000). Fencing and feeding is common in southcentral Spain, which may have contributed to the boar population increase. Free-living domestic, feral, and wild suids have been implicated in the epidemiology of diseases affecting humans, livestock, and wildlife. However, little information on prevalence and distribution of the most important infectious agents among wild boar populations in Spain is available, with the exception of one report from Sierra Morena (León et al., 1980). The objective of this serosurvey was to determine exposure of two wild boar populations from southcentral Spain to selected infectious agents.

The study areas are located in two

mountain chains which extend from east to west in south central Spain, Montes de Toledo (39°05′-39°30′N, 4°05′-4°45′W) and Sierra Morena (38°10′-38°45′N, 3°30′-6°15′W). Altitude ranges from 600 to 1,500 m above sea level in both areas. The climate is Mediterranean and the habitat is characterized by scrubland and forests, where evergreen oaks (Quercus ilex) are the dominant species. The principal wild ungulates are the Iberian red deer (Cervus elaphus hispanicus) and the European wild boar. Domestic livestock, mainly cattle but also goats and free-roaming domestic pigs, occasionally share grazing and watering places with wild boars, especially in eastern Sierra Morena.

From December 1999 to February 2000, blood samples from 78 shot wild boars were immediately collected from the heart during field necropsies (Table 1). Serum was obtained after centrifugation and stored at -20 C until used. Age was determined based on tooth eruption pattern. Individuals <12 mo old were classified as yearlings; those from 13–24 mo old were considered subadults, and those >24 mo old were classified as adults.

Pathogens, serologic tests employed, threshold antibody titers and techniques are reported in Table 2. No antibodies were detected to *Brucella* spp., classical swine fever virus (CSFV), *Haemophilus parasuis*, *Mycoplasma hyopneumoniae*, porcine reproductive and respiratory syndrome virus (PRRSV), *Salmonella* serotype D, *Streptococcus suis* serotypes 1 and 2, and swine influenza virus (SIV) serotype H3N2. Antibodies were detected against pseudorabies virus (PRV; 36%), *Leptospira interrogans* serovar pomona (12%), por-

TABLE 1. Age class, sex, and location of animals serologically tested.

| Location | $\begin{array}{c} \text{Yearlings} \\ (\text{M/F})^{\text{b}} \end{array}$ | Subadults (M/F) | Adults (M/F) | Total ^a (M/F) |
|---------------|--|--------------------|-----------------|-----------------------------|
| Sierra Morena | 5 (3/2) | 11 (4/7) | 28 (11/14) | 45 (18/23) |
| Montes Toledo | 4 (2/1) | 5 (2/1) | 20 (5/15) | 33 (9/20) |
| Total | 9 (5/3) | 16 (6/8) | 48 (16/29) | 78 (27/43) |

^a Sex unknown for three European wild boars from Sierra Morena and three from Montes de Toledo. Age and sex unknown for one animal from Sierra Morena and four from Montes de Toledo.

cine parvovirus (PPV; 10%), Erysipelothrix rhusiopathiae (5%), Salmonella serotype B (4%), swine influenza virus (SIV) serotype H1N1 (4%), and Salmonella serotype C (3%). Antibodies to PRV were detected in all age groups and both sexes. Prevalence of antibodies to PRV in Montes de Toledo was 52% and in Sierra Morena 23%.

The prevalence of PRV antibodies (36%) found in our study is comparable to those reported in European wild boars from other Mediterranean areas such as the islands of Corsica, France (32%; Albina et al., 2000), Sardinia, Italy (28%; Oggiano et al., 1991), and Tunisia (54%; Iridi

et al., 1996). Prevalences of PRV antibodies in feral pigs from Florida (Corn et al., 1986) and Texas (Van der Leek et al., 1993) were 35% and 36% respectively, and are similar to the overall prevalence found in our study. The prevalence of PRV in Sierra Morena (23%) was higher than the 3.0% reported by León et al. (1980) for the same area in an earlier study.

Antibodies to PPV (10%) were present in both Spanish populations. Seroprevalence was lower than that reported in Italy (99%; Mignone et al., 1995) and Germany (77%; Lutz and Wurm, 1996). Our results are more like those reported for feral pig

TABLE 2. Pathogens, serologic tests employed, threshold antibody for a positive test, and techniques used for serol assay of European wild boars in central Spain.

| Agrant | Tout | Positive threshold | Reference |
|--|------------------|-----------------------|---------------------------|
| Agent | Test | threshold | Reference |
| Pseudorabies virus gII | ELISAa | 0.093 | Qvist et al., 1989 |
| Actinobacillus pleuroneumoniae 1, 2, 4, and 7 | CFT^b | 1:20 | Gutiérrez et al., 1991 |
| Brucella abortus S 99 | CFT, RBc | | Jones et al., 1983 |
| | ELISA | | |
| Classic swine fever virus pg55 | ELISA | 0.049 | Muller et al., 1996 |
| Erysipelothrix rhusiopathiae serotype 2b | $\mathrm{TAT^d}$ | 1:80 | Ikram, 1977 |
| Haemophilus parasuis 1 and 2 | CFT | 1:20 | Rapp et al., 1985 |
| Leptospira interrogans serovar pomona | MAT^e | 1:400 | Faine, 1982 |
| Mycoplasma hyopneumoniae | ELISA | 0.089 | Fald et al., 1992 |
| Porcine parvovirus | $\mathrm{HIT^f}$ | 1:320 | Joo et al., 1976 |
| Porcine reproductive and respiratory syndrome virus, | | | |
| ORF7 antigen | ELISA | 0.09 | Ingenasa® (Madrid, Spain) |
| Salmonella B, C, and D | TAT | 1:160 | Duval, 1977 |
| Streptococcus suis 1, and 2 | AIT^g | +/- | Euzéby, 1999 |
| Swine influenza virus H1N1, and H3N2 | HIT | 1:20 | Schmidt, 1964 |

a Enzyme-linked immunosorbent assay.

 $^{^{}b}$ M = males, F = females.

^b Complement fixation test.

c Rose Bengal.

^d Tube agglutination test.

^e Microscopic agglutination test.

 $^{^{\}mathrm{f}}$ Hemagglutination inhibition test.

g Agar immunodiffusion test.

populations in the USA (New et al., 1994; Saliki et al., 1998). Antibodies to SIV serotype H1N1 were found at a low prevalence (4%) as compared to the 24% reported in Poland (Markowska-Daniel and Pejsak, 1999). Antibodies to SIV H1N1 serotype have also been reported in feral swine in the USA (Saliki et al., 1998; Gipson et al., 1999). No antibodies against CSFV were detected in the present study. This finding contrasts with a previous report in which wild pigs from Sierra Morena were found to carry CSFV specific antibodies (León et al., 1980).

The prevalences of antibodies to *Leptospira interrogans* serovar pomona, *E. rhusiopathiae*, and *Salmonella* serogroup B and C, and absence of antibodies against *Brucella* spp. (or to cross-reacting *Yersinia* spp.), and *Streptococcus suis* Type 1 and 2, are similar to the only previous report from Sierra Morena (León et al., 1980). In contrast, prevalence of brucellosis in wild pig populations may be high in other European countries (Hars et al., 2000).

Several pathogens for which antibodies were detected in this survey could affect the dynamics of European wild boar populations. For example, *Salmonella choleraesuis* caused clinical septicemia in farmed wild boars from Sierra Morena (Pérez et al., 1999).

Extensive production of domestic pigs is economically important in farms within the study areas and elsewhere in southcentral Spain, and the high prevalence of PRV antibodies in wild boars indicates that these animals may pose a threat to the success of an eradication program currently underway in domestic swine in Spain. Wild boars may also be a reservoir for pathogens that could affect endangered species; feral swine were thought to be the source of PRV for a Florida panther (Felis concolor) in the USA (Glass et al., 1994). Sierra Morena is one of the last strongholds of the Iberian lynx (*Lynx pardinus*), the most endangered feline species according to the International Union for the

Conservation of Nature (Nowell and Jakson, 1996).

The authors thank FEDER/CICYT projects 1FD97-0164 for partial support. This is a contribution to AGL2001-3947. J. Vicente had a half time grant from the Universidad de Castilla-la Mancha. We also thank A. Peña and landowners for allowing access to their properties and assisting with the sample collection.

LITERATURE CITED

- ALBINA, E., A. MESPLEDE, G. CHENUT, M. F. LE POTIER, G. BOURBAO, S. LE GAL, AND Y. LEFORBAN. 2000. A serological survey on classical swine fever (CSF), Aujeszky's disease (AD) and porcine reproductive and respiratory syndrome (PRRS) virus infections in French wild boars from 1991 to 1998. Veterinary Microbiology 15: 43–57.
- CORN, J. L., P. K. SWIDEREK, B. O. BLACKBURN, G. A. ERIKSON, A. B. THIERMANN, AND V. F. NET-TLES. 1986. Survey of selected diseases in wild swine in Texas. Journal of the American Veterinary Medical Association 189: 1029–1032.
- DUVAL, J. 1977. Infecciones por salmonelas. In Examenes de laboratorio. Técnicas en bacteriología 3. Serología bacteriana, Dague y Chabbert (eds.). Barcelona, Spain, pp. 125–167.
- EUZÉBY, J. P. 1999. Streptococcus suis: Une brève revue. Revue du Médecine Véterinaire 150: 981–988.
- FAINE, S. 1982. Guidelines for the control of leptospirosis. Organisation Mondiale de la Santé. Geneve, Switzerland, pp. 171.
- FALD, N. C., P. QVIST, N. F. FRIIS, AND A. MEILING. 1992. A monoclonal blocking ELISA detecting serum antibodies to *Mycoplasma hyopneumoniae*. Veterinary Microbiology 30: 35–46.
- GIPSON, P. S., J. K. VEATCH, R. S. MATLACK, AND D. P. JONES. 1999. Health status of a recently discovered population of feral swine in Kansas. Journal of Wildlife Diseases 35: 624–627.
- GLASS, C. M., R. G. MCLEAN, J. B. KATZ, D. S. MAH-ER, C. B. CROPP, L. J. KIRK, A. J. MCKEIRNAN, AND J. F. EVERMANN. 1994. Isolation of pseudorabies (Aujeszky's disease) virus from a Florida panther. Journal of Wildlife Diseases 30: 180– 184.
- GORTÁZAR, C., J. HERRERO, R. VILLAFUERTE, AND J. MARCO. 2000. Historical examination of the status of large mammals in Aragon, Spain. Mammalia 64: 411–422.
- GUTIERREZ, C. B., R. I. TASCON, J. A. VAZQUEZ, AND E. F. FERRI. 1991. Cross-reactivity between *Actinobacillus pleuropneumoniae* serotypes com-

- paring different antigens and serological tests. Research in Veterinary Science 50: 308–310.
- HARS, J., M. VALERY, F. CHADUC, B. GARIN-BASTUJI, O. PINGUET, AND S. ROSSI. 2000. Surveillance de la brucellose du sanglier et du lievre dans le Departement de l'Allier. Resultats de l'enquête menée en 1999/2000. Bulletin d'Information sur la pathologie des Animaux Sauvages en France 23: 121–138.
- IKRAM, M. 1977. Diagnostic microbiology. Laboratory procedures for veterinary technicians, P. Pratt (ed.). Mosby Year Book, Inc., St. Louis, Missouri, pp. 119–259.
- JONES, R., R. TAMAYO, W. PORAT, N. GIESSMAN, L. SELBY, AND G. BUENING. 1983. A serological survey of brucellosis in white-tailed deer (*Odo-coileus virginianus*) in Missouri. Journal of Wildlife Diseases 19: 321–323.
- JOO, H. S., C. R. DONALDSON-WOOD, AND R. H. JOHNSON. 1976. A standardised haemaglutination inhiition test for porcine antibody. Australian Veterinary Journal 52: 422–424.
- JRIDI, M., H. BOUZGHAIA, AND B. TOMA. 1996. Maladie d'Aujeszky chez le sanglier en Tunisie. Epidemioligie et Santé Animal 30: 99–105.
- LEÓN, L., A. MIRANDA, A. PEREA, J. CARRANZA, AND M. HERMOSO DE MENDOZA. 1980. Actas de la II Reunión Iberoamericana de Conservación y Zoología de Vertebrados, Cáceres, Spain, pp. 490–502.
- LUTZ, W., AND R. WURM. 1996. Serologische untersuchungen zum nachweis von antikörpern gegen viren des seuchenhaften spätaborts, der Aujeszkyshen krankheit, der europäischen schweinepest und porzine parvoviren beim wildschwein (Sus scrofa, L., 1758) in Nordrhein-Westfalen. Zeitschrift für Jagdwissenschaft 42: 123–133.
- MARKOWSKA-DANIEL, I., AND Z. PEJSAK. 1999. Serological prevalence of influenza virus in pigs and wild boar in Poland. Medycyna Weterynaryjna 55: 302–305.
- MIGNONE, W., C. ERCOLINI, M. PERRUCHON, AND M. POGGI. 1995. Stato sanitario dei cinghiali selvatici (Sus Scrofa) in Liguria: Indagini sierologiche. BIPAS 12: 68–71.
- MULLER, A., K. R. DEPNER, AND B. LIESS. 1996. Evaluation of a gp 55 (E2) recombinant-based ELISA for the detection of antibodies induced by classical swine fever virus. Deutsche Tierärztliche Wochenschrift 103: 451–453.

- NEW, J. C., JR., K. DELOZIER, C. E. BARTON, P. J. MORRIS, AND L. N. D. POTGIETER. 1994. A serologic survey of selected viral and bacterial diseases of European wild hogs, Great Smoky Mountains National Park, USA. Journal of Wildlife Diseases 30: 103–106.
- NOWELL, K., AND P. JAKSON. 1996. Wild cats. Status survey and conservation action plans. International Union for the Conservation of Nature, Gland, Switzerland, 382 pp.
- OGGIANO, A., C. PATTA, A. LADDOMADA, AND A. CACCIA. 1991. Indagine sieroepidemiologica sulla diffusione della malattia di Aujeszky nei cinghiali della Sardegna. Atti della Societá Italiana delle Scienze Veterinarie 45: 1157–1161.
- PÉREZ, J., R. ASTORGA, L. CARRASCO, A. MÉNDEZ, A. PEREA, AND M. A. SIERRA. 1999. Outbreak of salmonellosis in farmed European wild boars (Sus scrofa ferus). The Veterinary Record 145: 464–465.
- QVIST, P., K. J. SORENSEN, AND A. MEYLING. 1989. Monoclonal blocking ELISA detecting serum antibodies to the glycoprotein gII of Aujeszky's disease virus. Journal of Virological Methods 24: 169–179.
- RAPP, V. J., R. F. ROSS, AND T. F. YOUNG. 1985. Characterization of *Haemophilus* spp. isolated from healthy swine and evaluation of cross-reactivity of complement-fixing antibodies to *Haemophilus* pleuropneumoniae and *Haemophilus* taxon minor group. Journal of Clinical Microbiology 22: 945–950.
- SALIKI, J. T., S. J. RODGERS, AND G. ESKEW. 1998. Serosurvey of selected viral and bacterial diseases in wild swine from Oklahoma. Journal of Wildlife Diseases 34: 834–838.
- SCHMIDT, N. 1964. Tissue culture methods and procedures for diagnostic virology. *In* Diagnostic procedures for viral and rickettsial disease, E. Lenette and N. Schmidt (eds.). American Public Health Association, Inc., New York, pp. 78–176.
- VAN DER LEEK, M. L., H. N. BECKER, E. C. PIRTLE, P. HUMPHREY, C. L. ADAMS, B. P. ALL, G. A. ERICKSON, R. C. BELDEN, W. B. FRANKENBER-GER, AND E. P. GIBBS. 1993. Prevalence of pseudorabies (Aujeszky's disease) virus antibodies in feral swine in Florida. Journal of Wildlife Diseases 29: 403–409.

Received for publication 12 April 2001.