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Evaluation of a Commercial ELISA for the Detection of Antibodies to *Sarcoptes scabiei* in Wild Boar (*Sus scrofa*)

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ABSTRACT: Sarcoptic mange occurs in free-ranging wild boar (*Sus scrofa*) but has been poorly described in this species. We evaluated the performance of a commercial indirect enzyme-linked immunosorbent assay (ELISA) for serodiagnosis of sarcoptic mange in domestic swine when applied to wild boar sera. We tested 96 sera from wild boar in populations without mange history ("truly noninfected") collected in Switzerland between December 2012 and February 2014, and 141 sera from free-ranging wild boar presenting mange-like lesions, including 50 live animals captured and sampled multiple times in France between May and August 2006 and three cases submitted to necropsy in Switzerland between April 2010 and February 2014. Mite infestation was confirmed by skin scraping in 20 of them ("truly infected"). We defined sensitivity of the test as the proportion of truly infected that were found ELISA-positive, and specificity as the proportion of truly non-infected that were found negative. Sensitivity and specificity were 75% and 80%, respectively. Success of antibody detection increased with the chronicity of lesions, and seroconversion was documented in 19 of 27 wild boar sampled multiple times that were initially negative or doubtful. In conclusion, the evaluated ELISA has been successfully applied to wild boar sera. It appears to be unreliable for early detection in individual animals but may represent a useful tool for population surveys.

Key words: ELISA, evaluation, *Sarcoptes scabiei*, sarcoptic mange, seroconversion, wild boar.

Sarcoptic mange is a parasitic skin disease affecting numerous mammal species worldwide and causing high mortality in free-ranging wildlife (Alasaad et al. 2011). However, in wild boar, mange is apparently associated with low mortality despite high morbidity (Haas et al. 2015) and so far the disease has been poorly investigated in this species.

In recent years, sarcoptic mange has been newly detected in the free-ranging wild boar population in Switzerland and the question was raised as whether the disease has been endemic but previously undetected or has truly emerged in the population (Haas et al. 2015). A serosurvey on new and archived samples may contribute to assessing the current and former spread of the infection in the population. We evaluated the sensitivity and specificity of a commercial enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to *Sarcoptes scabiei* in domestic pigs when applied to wild boar sera.

As negative controls, we used blood samples from 96 wild boars from populations without mange history. They included four captive animals from a healthy zoo collection (Basel Zoo, Basel, Switzerland) and 92 free-ranging boars from the region of Geneva (46°6′00″ to 46°18′0″N; 5°54′00″ to 6°18′00″E), Switzerland, where ecological studies including phototrapping and animal captures have been performed (Hebeisen et al. 2008) and where sarcoptic mange has not been reported in wild boar. Samples from zoo animals were collected at slaughter on 14 February 2014 and immediately centrifuged; serum was aliquoted before shipping to the laboratory. Samples from free-ranging wild boars were collected from hunted animals between December 2012 and April 2013 by professional game wardens and consisted of blood or serosanguinous fluid retrieved from the heart or thoracic cavity. They were sent to the laboratory immediately after collection and centrifuged at 2,000 × G for 20 min. Zoo animals were juvenile females 6–8 mo old. Age of the free-ranging animals was determined in the field based on body

weight and coat color (Hebeisen et al. 2008). There were 55 females and 37 males, including 39 juveniles (6–12 mo), 25 subadults (12–24 mo), and 28 adults (≥2 yr).

We also tested blood samples from 141 free-ranging wild boars with mange-like lesions (MLLs). Three animals were shot by hunters in the region of Solothurn (47°12′00″ to 47°24′00″N; 7°24′00″ to 7°30′00″E), Switzerland, and submitted to necropsy; *S. scabiei* was detected in skin samples of all three cases (Haas et al. 2015). Additionally, 138 samples were collected from 50 live wild boars with MLLs that were captured, marked, and recaptured several times in the Petite Pierre National Reserve (48°30′0″N, 7°0′0″E), Bas-Rhin region, France, where mange is common in wild boar (S.R. pers. obs.). Captures were carried out weekly from 10 May to 23 August 2006 for another study (Rossi et al. 2011). Each animal was marked with ear-tags to allow identification and blood was taken from the jugular vein. Animals were venipunctured one to eight times each, but at a maximum of once a week for welfare consideration. Age was determined based on tooth wear of the inferior jaw (Matschke 1967). There were piglets (2–6 mo; 20 males and 23 females), four subadult males, and three adult females. Blood was immediately centrifuged at 1,500 × G for 15 min. Mange-like lesions were classified in four categories: 1) mild (erythema, alopecia without skin thickening), 2) localized and moderate (erythema, alopecia with local skin thickening and crusts), 3) generalized and severe (erythema, alopecia with skin thickening and crusts on more than two-thirds of the body surface), and 4) healing (thickened skin, hair regrowth, absence of erythema; only animals >12 mo). A skin scraping was made on 21 animals. Mites were detected by light microscopy in 17 boars and identified as *S. scabiei* based on morphologic characteristics (Sloss and Kemp 1978).

Serum samples were stored at −20 C until analysis. The commercial indirect ELISA SARCOPTES-ELISA 2001® Pig (AFOSA GmbH, Blankenfelde-Mahlow, Germany) was used according to the manufacturer's instructions to detect anti-*Sarcoptes* antibodies. Product specification indicates a sensitivity of 94% and a specificity of 97% in domestic pigs. It uses *Sarcoptes* mites from pigs as antigen and was the most sensitive (88.6%) of four commercially available indirect ELISAs for the detection of *S. scabiei* antigens applied on pig samples (Löwenstein et al. 2004). Twenty samples with doubtful results were tested a second time.

As in former studies of red fox (*Vulpes vulpes*; Nimmervoll et al. 2013) we defined sensitivity of the test as the proportion of ELISA-positive samples from animals with macroscopically visible MLLs and demonstrated mite infestation at the time of sample collection (truly infected, $n=20$), and specificity as the proportion of samples from animals without MLLs (truly noninfected, $n=96$) that were found negative.

Considering the 116 samples from animals with either absence of MLLs or confirmed mite infestation, we obtained a sensitivity of 75% and a specificity of 80% (Table 1). Of the 50 wild boars with MLLs that were sampled several times (over 5–90 d), 41 (82%) were antibody-positive at least once. Of the remaining animals, one had a doubtful result and eight (sampled one to five times) were seronegative. Seven of these eight wild boars presented MLLs in stages 1–2, including a piglet with a positive skin scraping, and there was one animal (of the 8) with healing skin lesions. Considering all analyzed samples, the percentage of antibody-positive samples increased with the chronicity of lesions (Table 2).

Overall, 23 wild boars were antibody-positive at the first sampling, and seroconversion was observed in 19 of 27 (70%) individuals with an initially antibody-negative or doubtful result. In these animals, the first antibody-positive result was detected on average 27.5 d (range: 6–63 d)

TABLE 1. Estimation of the sensitivity and specificity of a commercial indirect enzyme-linked immunosorbent assay (ELISA; SARCOPTES-ELISA 2001® Pig) when applied to wild boar (*Sus scrofa*) sera. "Truly infected" animals were free-ranging wild boars presenting mange-like lesions (MLLs) and confirmed infestation with *Sarcoptes scabiei*. "Truly noninfected" animals were wild boars from populations without mange history. Results are expressed as the number of animals and as percentage (in parentheses).

ELISA results	MLLs and mites (n=20)	No MLLs (n=96)
Positive (%)	15[a] (75)	12 (13)
Negative (%)	5 (25)	77[b] (80)
Doubtful (%)	0	7 (7)

[a] Including sera of three necropsied mangy wild boars from Switzerland.

[b] Including four sera obtained from mange-free captive wild boars from the Basel Zoo.

TABLE 2. Antibody-positive results obtained with the SARCOPTES-ELISA 2001® Pig for free-ranging wild boars (*Sus scrofa*) presenting mange-like lesion. Results are classified according to the stage of mange lesions (1: mild, 2: localized and moderate, 3: generalized and severe, 4: healing). If not specified, samples originated from piglets (<6 mo old). No statistical analysis was performed due to sample composition (paired and unpaired samples).

Stage	No. tested	No. seropositive	% Seropositive
1	44[a]	20[a]	45
2	75[b]	45[b]	60
3	15	11	73
4	7[c]	5	71
Total	141	81	57

[a] Including one adult.

[b] Including one adult and two juveniles.

[c] All animals ≥1 yr (four subadults and three adults).

after the first sampling (capture intervals: 5–35 d). False negative results were obtained in two animals with repeated positive results.

The sensitivity (75%) and specificity (80%) of the SARCOPTES-ELISA 2001 Pig for diagnosis of sarcoptic mange in wild boar were lower than indicated by the manufacturer for domestic pigs (sensitivity, 94%; specificity, 97%). They were also lower than data obtained for pigs (sensitivity, 88.6%) with the same test (Löwenstein et al. 2004) and data obtained with the SARCOPTES-ELISA 2001 Dog applied on red fox sera (sensitivity, 98.2%; specificity, 91.9%; Nimmervoll et al. 2013), but they were comparable to results obtained with an in-house ELISA applied on sera of free-ranging Iberian wolf (*Canis lupus*; sensitivity, 75%; specificity, 87.5%; Oleaga et al. 2011).

Positive results for 12 "truly noninfected" free-ranging animals may be due to cross-reactions with other mites (Dockmann 2004), subclinical *S. scabiei* infestations (Ippen et al. 1995), or a lack of detection of mild skin lesions by game wardens. Hemolysis is not expected to have affected our results (Casaubon et al. 2013).

Negative results obtained for "truly positive" animals may be due to differences between the antigen used in the ELISA and the mites infesting the host. Changes in antigen conformation may lead to variable epitope exposure to antibodies and lower test sensitivity (Casais et al. 2013). Wild boar can be repeatedly infested by mites from different subpopulations (Rasero et al. 2010), and each mite strain produces some unique antigen (Arlian et al. 1996), which may involve differences in the circulating antibodies. Furthermore, repeated freeze-thaw cycles (as occurred for the used samples from captured wild boar) may cause a decrease in optical density values (Boadella and Gortázar 2011), a factor possibly contributing to the doubtful or negative results in a few wild boars expected to be antibody positive. However, when results are considered at an individual scale including repeated sampling over time, sensitivity increases (82%), indicating a role of the dynamics of the immune response in the success of antibody detection. The date of infection of the wild boars was unknown but, in some cases, the clinical picture evolved and seroconversion was documented 6–63 d after the first sampling. This is in agreement with the observations of Dockmann (2004) in domestic pigs (seroconversion 5–7 wk post-infection or 3–4 wk after appearance of the

first clinical signs) and of Arlian et al. (1994) in domestic rabbits (highest antibody titer 64 d postinfection). Thus, samples from piglets in an initial stage of the disease (this study) are more likely to be antibody-negative than severely diseased animals such as foxes with advanced mange lesions (Nimmervoll et al. 2013), indicating an influence of sample composition when evaluating the performance of a serologic test.

The evaluated commercial ELISA for the detection of *Sarcoptes scabiei* var. *suis* in domestic pigs was successfully applied to wild boar sera. However, given the delay of the antibody response after infection and potential cross-reactions, it is not reliable for early detection in individual animals but it may represent a useful tool for serosurveys.

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