DOI: 10.7589/JWD-D-20-00011

Journal of Wildlife Diseases, 57(1), 2021, pp. 60–70

ANTIBODY PREVALENCE TO AFRICAN SWINE FEVER VIRUS, *MYCOBACTERIUM BOVIS*, FOOT-AND-MOUTH DISEASE VIRUS, RIFT VALLEY FEVER VIRUS, INFLUENZA A VIRUS, AND *BRUCELLA* AND *LEPTOSPIRA* SPP. IN FREE-RANGING WARTHOG (*PHACOCHOERUS AFRICANUS*) POPULATIONS IN SOUTH AFRICA

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ABSTRACT: The warthog (*Phacochoerus africanus*) can be used as a model for investigating disease transmission at the human, wildlife, and livestock interface. An omnivore and scavenger, a warthog moves freely between natural ecotypes, farmland, and human communities and is susceptible to diseases of zoonotic, agricultural, and conservation concern. A retrospective study using 100 individual serum samples collected from May 1999 to August 2016 was performed to determine antibody prevalence to seven pathogens in warthogs from five locations in northeastern South Africa. Higher prevalence of antibodies to African swine fever virus and *Mycobacterium bovis* were detected in warthogs from the Greater Kruger National Park ecosystem in comparison to lower prevalence of antibodies to *M. bovis* and no antibodies to African swine fever virus in warthogs from uMhkuze Game Reserve. Low prevalence of antibodies to foot-and-mouth disease virus, Rift Valley fever virus, and influenza A virus was detected in all locations, and no antibodies against *Brucella* and *Leptospira* spp. were detected. No statistically significant difference in antibody prevalence was found between sexes for any disease. At the univariate analysis, *M. bovis* seropositivity was significantly different among age categories, with 49% (35/71) of adults found positive versus 29% (4/14) of juveniles and 9% (1/11) of sub-adults (Fisher's exact test, P=0.020), and between the sampling locations (Fisher's exact test, P=0.001). The multivariate model results indicated that juvenile warthogs had lower odds of testing positive to *M. bovis* antibodies than adults (juveniles' odds ratio [OR]=0.17, 95% confidence interval [CI]: 0.02–1.0), although this result was not statistically significant at the 5% level (P=0.052). For warthogs sampled at Satara Buffalo Camp, the odds (OR=0.22, 95% CI: 0.035–0.96) of being *M. bovis* antibody positive were significantly lower (P=0.043) than for warthogs sampled at Skukuza. Of particular interest in this study was the detection of warthogs seropositive for influenza A virus.

Key words: African swine fever virus, avian influenza virus, *Brucella* spp., foot-and-mouth disease virus, *Leptospira* spp., *Mycobacterium bovis*, Rift Valley fever virus, warthog.

INTRODUCTION

Human population growth and socioeconomic demands have increased the rate of land development for agriculture and settlement, with progressive loss of wilderness and buffer zones. Also, the trend is to develop wild and adjacent land for activities such as ecotourism, game ranching, trophy hunting, and forest harvesting (Weaver and Skyer 2003; Ogutu et al. 2009; Kleinschroth et al. 2017). The result is increased contact between humans, wildlife, and livestock, with subsequent increased potential for disease transmission (Siembieda et al. 2011; Hassell et al. 2017).

A significant factor in disease transmission involving wildlife is direct or indirect contact between infected individuals and susceptible populations where competition for resources occurs (Bengis et al. 2002). Both wildlife and domestic species that move between natural and developed landscapes pose risks of pathogen transfer. In sub-Saharan Africa, the warthog (*Phacochoerus africanus*) can be used for investigating disease transmission at the human, wildlife, and livestock interface. Warthogs are omnivores and scavengers with burrowing capabilities, and they readily move between natural ecotypes, farmland, and human communities (Michel et al. 2006; Jori et al. 2011). Warthogs can be found grazing or drinking with wild and domestic ungulates and are associated with other peridomestic species in areas of human development. Wild carnivores and dogs (*Canis lupus familiaris*) prey on and scavenge warthogs, and humans contact warthog tissues through bushmeat harvest, game ranching, pest management, and sport hunting (Jori et al. 2011; Hoffman et al. 2017).

Although warthogs are susceptible to several diseases of zoonotic, agricultural, and conservation concern, investigations have focused on the epidemiology of African swine fever (ASF) and the prevalence of African swine fever virus (ASFV), for which the warthog is the natural host (Gallardo et al. 2011). More recently, studies have investigated the role that warthogs play in the maintenance of *Mycobacterium bovis*, the causative agent of bovine tuberculosis (BTB; Miller et al. 2016; Roos et al. 2018), but there has been limited screening for other infectious diseases. The objective of this study was to analyze serum samples collected from May 1999 to August 2016 to determine the prevalence of antibodies to selected pathogens in warthog populations in northeastern South Africa. Serologic screening was performed for foot-and-mouth disease virus (FMDV); Rift Valley fever virus (RVFV); influenza A virus (IAV); ASFV; and the causative agents of BTB, leptospirosis, and brucellosis.

MATERIALS AND METHODS

Study areas and sample collection

Banked serum samples were obtained from Veterinary Wildlife Services, Kruger National Park (KNP), and included sera from 100 warthogs from five locations in South Africa (Fig. 1). As is common practice in South Africa, sampling occurred primarily during the cooler austral winter between May 1999 and August 2016, with 87% of samples collected after 2012. Samples were collected opportunistically during management procedures such as removal of warthogs from tourist areas and limited culling or as part of South African National Parks Animal Use and Care Committee–approved research investigating immobilization protocols. Blood samples were collected from the medial saphenous vein of immobilized warthogs into vacuum tubes (VACUETTE®, Greiner Bio-One GmbH, Frickenhausen, Germany) containing no anticoagulant and placed in a cooler containing ice packs. Within 6 h of collection, samples were centrifuged for 10 min at 1,300 × G. Harvested sera were placed in cryotubes (Cryo.s™, Greiner Bio-One GmbH) and stored at –80 C until analyzed.

The majority of samples were collected within KNP in and around three tourist camps; Satara (SC; 24°23′52″S, 31°46′40″E; n=17) in the center of KNP, Skukuza (SZ; 24°59′43″S, 31°35′34″E; n=45) on the banks of the Sabie River, and Crocodile Bridge (CB; 25°21′30″S, 31°53′32″E; n=1) near the southern border of KNP. These areas permitted contact of warthogs with other wildlife and also with humans through shared recreational and residential spaces. In particular, SZ has a large human population living in the staff village.

The fourth sampling location, Marloth Park (MP; 25°20′36″S, 31°46′58″E; n=23), is a private residential and wildlife reserve along the southern bank of the Crocodile River. Although bordered by

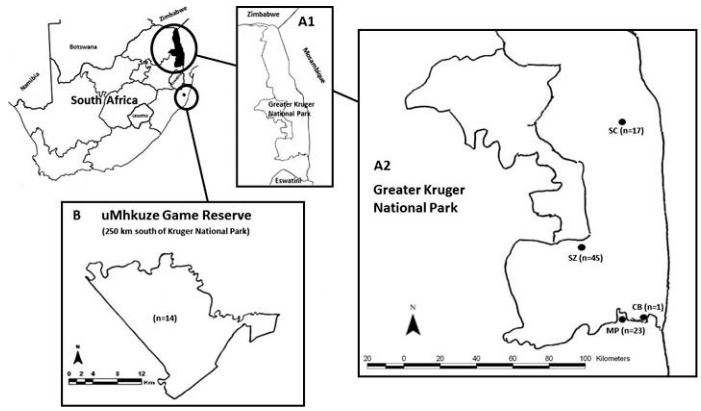

FIGURE 1. Five warthog (*Phacochoerus africanus*) serum sample collection sites in northeastern South Africa. A1) Greater Kruger National Park. A2) Greater Kruger National Park sample collection sites: SC=Satara; SZ=Skukuza; CB=Crocodile Bridge; MP=Marloth Park. B) uMkuze Game Reserve. Number (*n*) of warthogs sampled at each site is included.

KNP to the north, the eastern and western borders are adjacent to crop farms and limited human dwellings. To the south is a private game reserve covering roughly the same area as the residential units. Game roam freely between the housing units, and breeching of the fences along the river by animals living inside KNP sometimes occurs.

The fifth sampling location, uMhkuze Game Reserve (MZ; 27°39′0″S, 32°15′0″E; *n*=14), is located 250 km south of KNP and 40 km inland from the east coast of South Africa. The reserve is surrounded largely by rural communities with domestic stock and contains most native large mammals.

Serologic assays

Serologic assays for *M. bovis* had been previously performed at Stellenbosh University (ethical approval SU-ACUD15-00029; section 20 approval 12/11/1/7/2). Additional sera were heat treated at 56 C for 30 min and then transported on ice blocks at approximately 4 C for testing at the Agricultural Research Council-Onderstepoort Veterinary Institute, Pretoria, South Africa.

Serologic assay for M. bovis *antibodies:* Sera were screened for antibodies to *M. bovis* by using an indirect purified protein derivative enzyme-linked immunosorbent assay (ELISA) and TB ELISA-VK® kit (Vacunek, Bizkaia, Spain; Roos et al. 2016). Cut-off values for the assays were set according to Roos et al. (2016) for the indirect purified protein derivative ELISA (optical density [OD]≥1.04) and to the manufacturer's cut-off for the commercial TB ELISA-VK (ELISA-Index≥0.2).

Serologic assay for antibodies to ASFV: Sera were tested using a blocking ELISA (Ingezim PPA CROM antibody detection, Eurofins Ingenasa, Madrid, Spain) for detection of antibodies against the VP72 protein of ASFV. Testing was according to the manufacturer's specifications with sera diluted 1:1 before testing, and all samples were tested in duplicate. The test was considered valid if the assay OD of the negative control was at least four times higher than the OD of the manufacturer's positive control sera. Sera were considered positive for ASFV antibodies if the sample OD value was lower than the positive cut-off value.

Serologic assay for antibodies to FMDV: Sera were analyzed for FMDV-specific antibodies by using a liquid-phase blocking ELISA (Hamblin et al. 1986). Assays were performed using an in-house ELISA for South African Territories serotypes (SAT 1, SAT 2, SAT 3) that are maintained by African buffaloes (*Syncerus caffer*) in some regions of sub-Saharan Africa (Siembieda et al. 2011). Briefly, ELISA plates were coated with rabbit anti-FMDV antibody. Serum premixed with FMDV antigen was then added to the coated plates. Antibody titers were expressed as the 50% endpoint titers, and sera with titers ≥1.6 $\log_{10}$ were classified as positive.

Serologic assay for antibodies to RVFV: Sera were screened for the presence of immunoglobulin M and immunoglobulin G antibodies against RVFV by using a competitive ELISA (ID Screen Rift Valley Fever Competition Multispecies ELISA, ID-Vet, Montpellier, France; Lubisi et al. 2019). In brief, test and control sera were diluted 1:1 in dilution buffer in recombinant RVFV nucleoprotein pre-coated ELISA plate wells. After incubation at 37 C for 1 h, the plates were washed three times, anti-nucleoprotein

peroxidase conjugate was added, and the plates were incubated at room temperature for 30 min. After additional washes, substrate solution was added followed by incubation at room temperature for 15 min before addition of stop solution. The presence of antibodies to RVFV was shown by lack of a color change, whereas absence of antibodies to RVFV was shown by a change in substrate color to blue, measured as OD at a wavelength of 450 nm by using an ELX808 microplate absorbance reader (BioTek, Winooski, Vermont, USA). Results were calculated as sample OD/negative control OD (S/N) as a percentage, where S/N% $\leq$40% was positive, $>$40% but $\leq$50% was doubtful, and $>$50% was negative.

Serologic assay for antibodies to IAV: Sera were assayed using a competitive ELISA according to the manufacturer's protocol for detection of antibodies against a highly conserved epitope of IAV nucleoprotein (Influenza A Virus Antibody Test kit, IDEXX, Hoofddorp, the Netherlands). Sera were diluted 1:10 in dilution buffer and added to virus-coated wells in microtiter plates; the plates were then incubated for 1 h at room temperature. Next, wells were washed three to five times with wash solution, conjugate was dispensed, and the plates were incubated for 30 min at room temperature. After washing, tetramethylbenzidine substrate was added, and the plates were incubated for 15 min at room temperature. Stop solution was added, and absorbance was recorded at 650 nm by using an ELISA microplate reader (BioTek). Results were calculated as S/N, and for non-avian species the cut-off values were as follows: negative, S/N$\geq$0.6 and positive, $<$0.6.

Serologic assay for Brucella *spp. antibodies:* Sera were assayed for antibodies against *Brucella* spp. by using the rose Bengal rapid agglutination test (Nielsen 2002). Visual readings were performed after mixing equal volumes of warthog serum and controls with *Brucella abortus* antigen, with any visible agglutination considered a positive result.

Serologic assay for Leptospira *spp. antibodies:* Sera were assayed for antibodies against eight *Leptospira* spp. serovars (Bratislava, Canicola, Pomona, Icterohaemorrhagiae, Tarassovi, Szwajizak, Grippotyphosa, and Hardjo) by using a microscopic slide agglutination test. Sera were incubated with antigen suspensions of serovars, and darkfield microscopy was used to determine whether the sera reacted to the leptospiral antigens; agglutination of 80–100% was classified as a positive reaction. For samples where a reaction was observed, titrations were made to determine whether sera were positive at a dilution of 1:50. For positive samples, darkfield microscopy was used to read the results, with the endpoint being the highest twofold dilution of serum at which 50% of the leptospires were agglutinated (Goris and Hartskeerl 2014).

Data management and statistical analysis

Descriptive analysis was performed to evaluate data distribution of the outcome of interest (seven pathogens under investigation, separately) and potential risk factors including location, age category, and sex. The prevalence of antibody to each pathogen was calculated based on test results. As an initial screening procedure, a univariate analysis was conducted using standard 2×2 contingency tables to evaluate and compare prevalences of antibodies to each of the pathogens (independently) among warthogs from different locations, age categories, and sexes, by using Fisher's exact text (FET), due to sample size and data distribution. Only for *M. bovis* and due to the number of positive and negative results in each category for sex, age, and location, factors with an initial $P<0.25$ in the univariate analysis were used to build a multivariate model. Because of the relatively small sample size, an exact multivariate logistic regression analysis was performed to evaluate associations between the risk of being found positive while accounting (adjusting) for the combined effect of factors that showed an initial association with the *M. bovis* antibody prevalence in the univariate analysis. Adjusted odds ratios ([ORs] with their corresponding 95% confidence intervals [CIs]) were obtained for each risk factor, and statistical significance was defined as $P<0.05$. We used STATA 12 (StataCorp LLC, College Station, Texas, USA) for the statistical analysis.

RESULTS

Descriptive results

Data on location, sex, and age category were available for each warthog (distribution of samples by location, date, number, sex, and age category) and are summarized in Supplementary Material Table S1. Apparent antibody prevalences to the seven pathogens and prevalences based on location, sex, and age category are summarized in Tables 1 and 2. All locations, except MZ, are contained within the Greater Kruger National Park (GKNP), a region including KNP and adjoining private game reserves. *Mycobacterium bovis*, ASFV, and FMDV are endemic in GKNP. *Myco-*

TABLE 1. Antibody prevalence data for seven pathogens in 100 free-ranging warthogs (*Phacochoerus africanus*) from five locations in northeastern South Africa.

Pathogen	No. seropositive warthogs (%)						
	Satara (n=17)	Skukuza (n=45, 43[a])	Crocodile Bridge (n=1)	Marloth Park (n=23)	uMkhuze Game Reserve (n=14, 13[a])	Total (n=100, 97[a])	P[b]
Mycobacterium bovis	3 (18)	21 (49)	0	15 (65)	1 (8)	40	0.001
African swine fever virus	17 (100)	43 (96)	1 (100)	23 (100)	0	84	<0.001
Foot-and-mouth disease virus	3 (18)	1 (2)	0	0	0	4	0.088
Rift Valley fever virus	1 (6)	1 (2)	0	1 (4)	0	3	0.859
Influenza A virus	0	5 (11)	0	4 (17)	0	9	0.244
Brucella abortus	0	0	0	0	0	0	—
Leptospira spp.	0	0	0	0	0	0	—

[a] Number of animals tested for *M. bovis*.

[b] Univariate analysis: Fisher's exact test, P value. — = data not calculable.

bacterium bovis is endemic in MZ that, like GKNP, lies within the control zone for ASFV.

Reactive antibodies to ASFV were detected in the majority of samples (84%; 84/100), with a significant difference (P<0.001) in prevalence between GKNP (98%; 84/86) and MZ (0%; 0/14). *Mycobacterium bovis* reactivity was found in 42% (40/97) of warthogs tested. Antibody prevalence to IAV was 9% (9/100), although positive animals were only found in two GKNP locations. Prevalences of reactive antibodies were also low for FMDV (4%; 4/100) and RVFV (3%; 3/100), with positive animals occurring in two and three GKNP locations, respectively. Antibodies to *Brucella* spp. and *Leptospira* spp. were not detected in any warthog.

Univariate analysis for age, sex, and location

No significant differences were observed between females and males for any of the seven pathogens under investigation (Table

TABLE 2. Antibody prevalence data for seven pathogens in 100 free-ranging warthogs (*Phacochoerus africanus*) from five locations in northeastern South Africa by sex and age category.

Pathogen	No. seropositive warthogs (%)								
	Sex			Age category				Total	
	Female (n=54, 53[a])	Male (n=46, 44[a])	P[b]	Adult (n=71)	Sub-adult (n=12, 1[a])	Juvenile (n=16, 14[a])	Unknown[c] (n=1)	(n=100, 97[a])	P[b]
Mycobacterium bovis	23 (43)	17 (37)	0.682	35 (49)	1 (9)	4 (29)	0	40	0.020
African swine fever virus	44 (82)	40 (87)	0.587	65 (92)	3 (25)	15 (94)	1	84	<0.001
Foot-and-mouth disease virus	1 (2)	3 (7)	0.331	4 (6)	0	0	0	4	1.000
Rift Valley fever virus	2 (4)	1 (2)	1.000	2 (3)	1 (8)	0	0	3	0.382
Influenza A virus	5 (9)	4 (9)	1.000	6 (9)	0	2 (13)	1	9	0.606
Brucella abortus	0	0	—	0	0	0	0	0	—
Leptospira spp.	0	0	—	0	0	0	0	0	—

[a] Number of animals tested for *M. bovis*.

[b] Univariate analysis: Fisher's exact test, P value. — = data not calculable.

[c] Unknown age category.

2). For ASFV, statistically significant differences were observed at the initial univariate analysis between age groups, with 25% (3/12) prevalence in sub-adults compared with 92% (65/71) and 94% (15/16) in adults and juveniles, respectively (FET, $P<0.001$; Table 2). Also, significant differences in ASFV antibody reactivity (FET, $P<0.001$; Table 1) were found between locations, with 0% (0/14) in MZ, 96% (43/45) in SK, and 100% in SC (17/17), MP (23/23), and CB (1/1).

For *M. bovis*, the initial univariate analysis showed that reactive antibody prevalence was highest in warthogs from MP (65%; 15/23), followed by SZ (49%; 21/43) and then SC (18%; 3/17); the sample from CB was negative, and only 8% (1/13) of the samples were positive in MZ, and these results were statistically significant (FET, $P=0.001$; Table 1). There were also significant differences between age categories, with 49 (35/71) of *M. bovis*–positive samples from adult warthogs versus 29% (4/14) among juveniles and 9% (1/11) in sub-adults (FET, $P=0.020$).

Multivariate analysis for *M. bovis*

In the final multivariate analysis for *M. bovis*, and after adjusting for the effect of age in the multivariate model, samples obtained from SC had lower odds of testing positive to *M. bovis* (OR=0.22, 95% CI: 0.35–0.96) than samples obtained from SZ, and this difference was statistically significant ($P=0.043$). Samples obtained from MP were 3.6 times more likely to be positive (OR=3.66, 95% CI: 0.86–22.6) compared with samples obtained from SZ; however, this difference was not statistically significant at the 5% level ($P=0.090$). After adjusting for the effect of location, samples from juvenile warthogs showed lower odds of testing positive to *M. bovis* antibodies than adults (juveniles OR=0.17, 95% CI: 0.02–1.0), although this result was not statistically significant at the 5% level ($P=0.052$).

DISCUSSION

Apparent prevalences of selected pathogens were determined in several warthog populations in South Africa. Relatively high ASFV and *M. bovis* antibody prevalences were detected in warthogs in the GKNP, with lower levels found for IAV, RVFV, and FMDV. For warthogs from MZ, no antibodies to ASFV or FMDV were found, and reactive antibodies to *M. bovis* were low (8%; 1/13).

In South Africa, ASF is confined to the northern regions. Based on studies in endemic areas, the high prevalence (98%; 84/86) of ASFV antibody–positive warthogs in the GKNP was expected (Quembo et al. 2016). Although MZ is located within the ASF control zone in northeastern KwaZulu-Natal province, a 1978 study found low antibody prevalence in warthogs (2%) and a 23-fold lower ASFV infection rate (0.06%) in *Ornithodorus* spp. ticks (vector) compared with KNP (Thomson et al. 1983). More recently (2002), a survey in MZ that used DNA PCR did not detect the virus in ticks despite an increase in the warthog population and burrow infestation rate (Arnot et al. 2009). Consequently, the absence of antibodies to ASFV in MZ warthogs in this study was also expected. In 2012, an outbreak of ASF in pigs occurred outside of the control zone, raising concerns about the accepted line between endemic ASF areas and the southern ASF-free zone (Magadla et al. 2016). Subsequent studies in the ASF-free zone near the line of demarcation failed to identify virus in the warthogs or ticks. However, intensification of surveillance programs of warthogs and ticks for virus has been recommended due to changing farming practices and the occurrence of multiple ASF outbreaks in South Africa in 2019 (South African Government 2019). Because MZ is located close to the ASF-control boundary, it would be prudent to include the reserve's warthog population in future surveillance efforts.

Bovine tuberculosis is endemic in GKNP, with multiple wildlife species affected (Hlokwe et al. 2014; Brüns et al. 2017), and the high prevalence of antibodies to *M. bovis* observed in warthogs from GKNP in this study has been reported previously (Roos et al. 2018). Disease transmission to warthogs likely occurs primarily through ingestion at

shared food and water resources, similar to BTB in wild boars (*Sus scrofa*; Naranjo et al. 2008; Vicente et al. 2013). In this study, antibody prevalence was highest in MP and SZ, locations with the highest human populations. A possible explanation is that warthogs, drawn by reliable food sources (e.g., gardens and human food waste) and a reduction in predators, occur at concentrations higher than normal around human settlements. This may allow for increased transmission of BTB between warthogs. Also, given the increased food availability, infected individuals may survive longer with a prolonged period of bacterial shedding into the environment. Interestingly, the distribution of antibody-positive warthogs from GKNP in this study was similar to that reported for African buffaloes and lions (*Panthera leo*; Michel et al. 2006; Sylvester et al. 2017), with the highest percentage of *M. bovis* antibody–positive warthogs reported in southern sampling sites, compared with SC that is >50 km north of the Sabie River. The lower antibody prevalence in MZ may be due to absence of BTB reservoirs in this reserve outside GKNP. A lower prevalence of *M. bovis* antibody was found in juvenile than in adult warthogs in this study overall. This may reflect the time dependence of exposure to a contaminated landscape, or, as reported in wild boar, the chronic nature of BTB (Santos et al. 2009). Given the findings of this study, inclusion of warthogs in surveillance programs may assist in detecting *M. bovis* in ecosystems and documenting expansion into previously BTB-free areas. Furthermore, controlling movement of warthogs out of endemic BTB areas may play an integral part in preventing pathogen spread.

Foot-and-mouth disease virus is an important transboundary pathogen in Africa. Although disease outbreaks occur primarily in domestic livestock, susceptibility has been reported in >50 wild artiodactylid species (Weaver et al. 2013). In our study, antibody to FMDV was detected in three warthogs from SC in 2015 (SAT 1) and one warthog from SZ in 2013 (SAT 2). Although SAT serotypes circulate in buffalo continuously, the carrier state for most infected artiodactylids lasts 14–45 days (Weaver et al. 2013; Tekleghiorghis et al. 2016). As only 10 warthog samples (one antibody positive) were collected during reported outbreaks (2000–13; Brahmbhatt et al. 2012; Tekleghiorghis et al. 2016), this may explain the low overall antibody prevalence of 5% (4/86) in GKNP. It is interesting that, in SC, where warthogs comingle with buffalo, a prevalence of 18% (3/17) was identified, compared with 2% (1/45) in SZ, where buffaloes are not common.

The role of warthogs in FMDV transmission is unknown. Unlike domestic swine (*Sus scrofa domesticus*), which excrete greater amounts of aerosolized virus than cattle, warthogs and the sympatric bushpig (*Potamochoerus larvatus*) do not excrete FMDV heavily after experimental infection (Weaver et al. 2013) and may represent less risk during an outbreak than pigs. Because warthogs move between wild and developed habitats, and share resources with buffaloes, it is important to consider including warthogs in foot-and-mouth disease surveillance programs.

Rift Valley fever is a health concern for humans and livestock, and wildlife are believed to play a role in RVFV maintenance and transmission (Evans et al. 2008; Lwande et al. 2015). Prevalence of antibody to RVFV in warthogs has been reported as 0% to >75% in active epizootic zones and <25% during interepizootic periods (Anderson and Rowe 1998; Evans et al. 2008; Britch et al. 2013). In our study, antibodies were identified in three warthogs from GNKP (3% overall prevalence; 3/84). Evidence of RVFV activity in GKNP during the period of sample collection is limited to one outbreak reported in 1999 (Pienaar and Thompson 2013), although a survey of white rhinoceros (*Ceratotherium simum*) in 2007 revealed a high antibody prevalence (49%; Miller et al. 2011). During a 2006 outbreak in Kenya, antibody prevalence among seven wildlife species was 8.4%, but it was 14% in warthogs (Evans et al. 2008). Given the potential for more frequent or severe outbreaks related to climatic changes,

warthogs may be useful as a sentinel species during periods of heightened RVFV activity.

Despite the 2009 pandemic H1N1 in domestic swine in Africa and a marked increase in pig production, surveillance for influenza in swine is limited (Meseko et al. 2014; Adeola et al. 2015; Snoeck et al. 2015). Our study is the first report of exposure to IAV in warthogs. The overall antibody prevalence was low at 9% (9/100), with four warthogs testing positive from MP and five from SZ, the two study locations with the highest concentration of humans and year-round residents. Because it is unknown whether warthogs serve as mixing vessels for IAV similar to domestic swine, further studies are warranted, including virus subtype determination and investigation of the epidemiologic role of warthogs.

The lack of warthogs with antibodies to brucellosis in this study was expected. In sub-Saharan Africa, *B. abortus* has been detected in buffaloes, the only wildlife reservoir in Africa (Godfroid 2002). Although brucellosis has been identified in other African artiodactylids, antibody-positive warthogs have not been reported (Madsen and Anderson 1995; Alexander et al. 2012; Assenga et al. 2015a). This suggests that, similar to domestic pigs, African suids are resistant to infection with *B. abortus* and presumably are more sensitive to infection with *Brucella suis*. Given the lack of *B. suis* activity in the study areas, including warthogs in future surveillance programs is not indicated unless wild suids are found with signs consistent with brucellosis.

Leptospirosis is recognized as an emerging zoonosis worldwide (Siembieda et al. 2011; Assenga et al. 2015b). Studies in Africa have identified antibodies against *Leptospira interrogans* across most mammalian groups, suggesting wildlife influences leptospirosis epidemiology (Jobbins et al. 2013; Assenga et al. 2015b; Jobbins and Alexander 2015). Both buffalo in KNP and cattle living adjacent to the park have tested positive for *L. interrogans* (Myburgh et al. 1990). Because warthogs share water sources with these species, the absence of antibodies to all eight serovars is surprising. Although studies in Zimbabwe and South Africa suggest that warthogs are not appropriate reservoirs (Anderson and Rowe 1998; Hunter et al. 1988), a recent study in Botswana confirmed the leptospire renal carrier state of warthogs (Jobbins and Alexander 2015). Given the distribution of leptospirosis throughout Africa, warthogs should be included in surveillance programs, particularly where ecotourism facilities and communities adjacent to natural areas exist.

Although serosurveys provide evidence of specific antibodies to pathogens in a population, there are limitations with regard to interpretation of the results. Reactive antibody prevalence is a measure of immune sensitization and not necessarily disease or infection pressure within the screened population. Therefore, disease prevalence is difficult to predict with this type of screening. A drawback in our study was the use of tests that were not validated for use in suids and, in the absence of diagnostic sensitivity and specificity values, test results could not be corrected for estimation of true prevalence rates (Lewis and Torgerson 2012). Other limitations in this study are related to sample size and sampling period. The 100 samples evaluated in this study were collected over a 17-yr period, thus affecting the ability to make any inference about persistence versus current or recent status of the selected diseases in the geographical locations studied. Future investigations should include targeted sampling of warthogs in different locations over a shorter study period as well as obtaining a representative sample size of different ages and sexes for each location. We consider these data valuable for future studies, despite the limitations inherent in this retrospective study using banked serum samples.

In conclusion, this study provides retrospective information on the apparent prevalence of antibodies to seven pathogens of zoonotic, agricultural, and/or conservation concern in selected warthog populations in South Africa. Understanding pathogen exposure will assist wildlife health officials in managing this species, which plays an important role in ecosystem health as well as

providing ecotourism and other economic value. These data suggest that warthogs may be a useful sentinel for disease surveillance in the event of a future outbreak of disease in livestock or humans living within or adjacent to the sampling locations.

ACKNOWLEDGMENTS

We acknowledge the contributions of Alicia McCall, Eduard Goosen, Marius Kruger, the Veterinary Wildlife Services capture team, and the State Veterinary field personnel from KNP for assistance with sample collection from the warthogs and Brittany Grenus for assistance with manuscript preparation. This work was supported by KNP Veterinary Wildlife Services, South African Medical Research Council, National Research Foundation of South Africa (grant 86949), and Smithsonian Institution National Zoological Park. The content is the sole responsibility of the authors and does not necessarily represent the official views of South African National Parks, South African Medical Research Council, National Research Foundation, or Smithsonian Institution National Zoological Park.

SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/JWD-D-20-00011.

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Submitted for publication 16 January 2020.
Accepted 28 June 2020.