

SEROPREVALENCE OF *BRUCELLA* SPP, *LEPTOSPIRA* SPP AND *TOXOPLASMA GONDII* IN WILD BOAR (*SUS SCROFA*) FROM SOUTHERN BRAZIL

Iara Maria Trevisol¹, Beatris Kramer¹, Arlei Coldebella¹, Virginia Santiago Silva^{1*}

¹Embrapa Suínos e Aves, Concórdia, SC, Brazil

*virginia.silva@embrapa.br

Abstract

The wild boar (*Sus scrofa*), exotic invasive specie, is currently distributed in many Brazilian states, including Santa Catarina (SC) and Rio Grande do Sul (RS). Since the wild pigs are susceptible to zoonotic pathogens as *Leptospira* spp, *Toxoplasma gondii* and *Brucella* spp, their large populations, movements and dispersion can spread diseases, being a potential transmission source to humans, livestock and other sylvatic sympatric species. Despite its importance are there few information about sanitary status of this wild populations and their impact for human and livestock health in Brazil. Objective this work was to investigate the presence of antibodies to *Brucella* spp, *Toxoplasma gondii* and *Leptospira* spp in blood samples of wild boar legally hunted for population control in SC and RS states. From January 2014 to July 2016, 193 samples were tested to antibodies against *Brucella* spp (buffered acidified plate agglutination test-BAPA) and *Leptospira* spp (micro agglutination test-MAT). Of these 193, 149 were tested to *Toxoplasma gondii* antibodies (HAI test). Overall, serological results showed negativity to *Brucella* spp and low prevalence for *Toxoplasma gondii* (2,7%). On the other hand, the seroprevalence of *Leptospira* spp was 6,74% with significantly greater percentage of positivity in RS (12,5%) compared to SC (3,88%). The most prevalent serovars were Icterohaemorrhagiae and Pomona, with titers ranging from 1:400 to 1:12,800 in RS. Icterohaemorrhagiae was the most prevalent serovar in SC but with lowest titres. Seroprevalence of these pathogens in wild population indicate the environmental health, including sympatric livestock and wildlife. Ecological factors should be considered to understand the role of wild boars in the cycle of these diseases. Our results indicate that hunters and consumers of wild boar meat of must be aware about zoonotic risk in carcass handling and meat intake.

Introduction

The European wild boar (*Sus scrofa*) is an exotic animal to the Brazilian fauna. Currently the invasive specie is distributed in many Brazilian states, including Santa Catarina (SC) and Rio Grande do Sul (RS). Since the wild pigs are susceptible to zoonotic pathogens as *Leptospira* spp, *Toxoplasma gondii* and *Brucella* spp, their large populations, movements and dispersion can spread diseases, being a potential transmission source to humans, livestock and other sylvatic sympatric species. Despite the importance of the subject there is little information about sanitary status of this wild population and their impact for human and livestock health in Brazil.

Brucellosis is a zoonotic infection caused by the bacterial genus *Brucella*. It affects domestic and wild mammal species and it is usually transmitted from animals to humans by ingestion through infected food products, direct contact with infected animal or contaminated environment.

Leptospirosis is a zoonosis caused by pathogenic spirochetes of the genus *Leptospira*. The bacteria can be found worldwide in soil and water. Leptospirosis is commonly associated with rodents in settings of poor sanitation, agricultural occupations and activities involving fresh water, mud, or soil exposure. Human infection can occur through direct contact with infected animals or by exposure to water or soil contaminated by urine of infected animals.

Toxoplasmosis is a zoonotic infection caused by *Toxoplasma gondii* protozoan capable of infecting an unusually wide range of hosts. Domestic and wild felidae are considered the definitive hosts, while the other species are considered intermediate hosts as people and other warm-blooded animals. The main routes of transmission, both in the definitive and intermediates hosts, are the ingestion of tissues of infected animals or ingestion of food contaminated with infective oocysts or by transplacental route.

Objective of this work was to investigate the presence of antibodies against to *Brucella* spp, *Toxoplasma gondii* and *Leptospira* spp in blood/serum samples of wild boar legally hunted for population control in SC and RS states.

Material and methods

From January to July 2016 legally authorized hunters harvested blood samples from 193 wild boars. Immediately after the death, blood were collected from the heart or thoracic cavity, and transported to the Embrapa laboratory. Serum was recovered by centrifugation and frozen at -20°C until analysis. The 193 samples were tested to detect antibodies against *Brucella* spp with buffered acidified plate agglutination test (BAPA), and with micro agglutination test (MAT) to detect antibodies against *Leptospira* serovars: Autumnalis, Ballum, Bratislava, Grippotyphosa, Hardjo, Icterohemorrhagiae, Pomona, Tarassovi and Wolffi. Titres $\geq 1:100$ were considered reagent. Of those 193 sera, 149 was assayed using qualitative and semi-quantitative indirect hemagglutination assay (IHA) to *Toxoplasma gondii* antibodies. A titre 1:64 is considered positive. Fisher Exact Test was used to evaluate the effect of Federative Unit, gender and age on the seroprevalence. The age was based on the live weight, juvenile with less than 40kg and adult with 40kg or more. Results were evaluated by the FREQ procedure of the SAS software.

Results and discussion

All 193 samples tested for antibodies to *Brucella* spp were negative. Little studies have been devoted to the investigation about wild boar brucellosis in Brazil. According a study in 2010, samples from captive wild boar from the same area and others regions of the country, also not presented positivity to antibodies to *Brucella* spp. The low prevalence also occurs in domestic pigs in Brazil 0.0% to 0.34%.

The examination of 149 wild boars serum samples for *Toxoplasma gondii* revealed only four seropositive individuals, representing the prevalence of 2.7% (Table 1). The results show a low prevalence of the parasite, compared with, Central Italy (8.1% to 14%), Austria (19%) and USA (18%). The same occurs when compared to the seroprevalence of *T. gondii* in Brazilian domestic pigs from 4% to 54.1% in different regions or compared with European data that range from 8.1% to 38.4%. Our results suggest low or rare contact of wild boar populations with environments and infected animals.

In this study, overall the 6.7% prevalence of *Leptospira* spp antibodies in wild boars were detected. An expressive percentage of positivity (12.5%) for *Leptospira* spp in RS samples were detected comparing to SC (3.88%) (Table 1). Five samples from SC were positive for *Leptospira* spp (Table 2), from these, four were positive for the serovar Icterohaemorrhagiae, one reacted to serovar Grippotyphosa and one to Pomona serovar, being the Suidae the main reservoir of these last serovars. The low seroprevalence to *Leptospira* spp found in SC wild boar (3,88%) may be associated the region where the samples of SC were collected, that is characterized by forested areas with abundant and diverse fauna and flora, as well as crops and pig production in the vicinity of forest areas. These conditions can reduce exposure to the agent since wild boars would feed on seeds, roots and crops, decreasing contact with potentially infected animals and carcasses.

From the RS samples were found 12.5% reagents to *Leptospira* spp (Table 2), with higher titres than those found in SC wild boars. These titres indicate late infection and/or that the wild boar population has been persistently exposed to the pathogens. The most prevalent serovar was Icterohaemorrhagiae, but the titres for the Pomona serovar were the highest, followed by the three reagents to the Autumnalis serovar. There was only one reagent to the serovar Grippotyphosa and one to Hardjo in RS samples.

The RS samples were collected in a region of in Pampa biome, characterized by fields with grasses great diversity of sylvatic animals, few crops and abundance of livestock, especially sheep and bovine creations. The wild boars are omnivorous and scarcity of vegetation favors carnivorous habits, increasing the chance of contact and ingestion of carcasses and potentially infected animals. Thus, in addition to possible contaminated environmental sources, the highest seroprevalence for *Leptospira* spp found in RS may be related to a scarce supply of food by local vegetation favoring them contact and feeding of infected sympatric dead animals, as sheeps, capybaras (*Hydrochoerus hydrochaeris*) or other abundant species in that region.

Serovar Icterohaemorrhagiae is generally associated with direct or indirect contact with rodents and Suidae are natural reservoirs of Pomona while Autumnalis serovar reagents probably were results of the contact with canines, goats and sheep that are the natural reservatories. Overall, six samples were reagent for two or more serovars. The reactions to multiple serovars were more frequent in RS samples. The coinfection by different serovars may be associated to expositions to different sources of infection and/or reservoirs or even to infections by multiple serovars from the same animal or environmental source but other studies must be performed to elucidate these questions

The reactive samples to *T. gondii* and *Leptospira* spp were not significantly associated with gender or age. Seroprevalence of these pathogens in wild population indicate the environmental health, including sympatric livestock and wildlife. Ecological factors should be considered to understand the role of wild boars in the cycle of these diseases.

Conclusions

Our results showed that there are no *Brucella* spp circulation in the studied population but the seroprevalence of *Toxoplasma gondii*, although low, showed the circulation of the pathogen pointing the boar as a potential source of infection to animal and human.

The seroprevalence of *Leptospira* spp and high titers to serovares tested, especially from the RS samples, indicate that wild boar were persistent exposit to the agents. These results raise questions relevant to both, livestock, wildlife and public health.

Hunters and consumers of wild boar meat should be aware of the risk in carcass management and consumption of boar meat and meat products, raw or poorly cooked.

Table 1. Apparent serorevalence of *Leptospira* spp and *Toxoplasma gondii* in RS and SC boars.

Agent	State	Apparent prevalence	95% CI	
<i>Toxoplasma gondii</i>	RS/SC	2,7%	0,1%	5,3%
<i>Leptospira spp</i>	RS/SC	6,7%	3,2%	10,3%
<i>Toxoplasma gondii</i>	RS	3,3%	0,0%	6,9%
<i>Toxoplasma gondii</i>	SC	1,75%	0,0%	5,3%
<i>Leptospira spp</i>	RS	12,5%	4,3%	20,7%
<i>Leptospira spp</i>	SC	3,1%	0,1%	6,1%

Table 2. *Leptospira* species and serovars antibodies detected in wild boar from SC and RS.

State	<i>Leptospira interrogans</i> Serovars				
	Autumnalis	Grippotyphosa	Hardjo	Icterohaemorrhagiae	Pomona
RS				1:400	1:3200
	1:200			1:400	1:400
	1:1600			1:1600	1:12800
		1:400			
				1:200	
		1:400		1:800	1:6400
SC				1:200	
				1:200	
			1:200	1:400	1:3200
				1:100	
				1:100	
		1:800		1:200	
			1:100		
				1:200	

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