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Posted Date: 30 June 2023

doi: 10.20944/preprints202306.2246.v1

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Seroprevalence Study of Brucellosis in Wild Boars Hunted for Private Consumption in Northeast Portugal

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Simple Summary: The northeast region of Portugal is considered an endemic area of brucellosis for small ruminants, representing a (public) health risk for humans, domestic and wild animals. The potential role of wild boar (*Sus scrofa*) in the epidemiological dynamic of brucellosis is largely unknown. A total of 332 wild boar was investigated using serology (i-ELISA). Eighty-eight wild boars showed that had been exposed to *Brucella* spp., pointing to a seroprevalence of 26.5% % (95% CI: 21.8% – 31.3%). These results suggest wild boar as a potential reservoir and spreader of this disease to other animals (e.g., livestock, hunting dogs) and to hunters, deserving greater awareness from the livestock/hunting sector and Veterinary Competent Authorities.

Abstract: Brucellosis is an important infectious disease caused by bacteria of the genus *Brucella*. In the northeast region of Portugal, brucellosis is endemic in small ruminants, and there are also human cases. However, the epidemiological role of the wild boar in the dynamics of this disease in this region is unknown. For this reason, a total of 332 blood samples were collected from wild boar hunted in thirty-six hunting areas during the 2022/2023 hunting season. All were taken by the hunters for private consumption, with no evisceration and examination in the field. Serum samples were tested by i-ELISA. It was observed that 88 wild boars were exposed to *Brucella* spp., pointing to a seroprevalence of 26.5% (95% CI: 21.8% – 31.3%). This high prevalence underlines the importance that wild boar may have in the dynamics of this disease in the region and its potential transmission to other animals and to human, for example, during the handling of carcasses. Increased awareness and knowledge of brucellosis in wild boar is essential for the implementation of effective practices and habits and, consequently, for the control and prevention of this important zoonosis.

Keywords: *Sus scrofa; Brucella* spp.; zoonosis; one health

1. Introduction

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Brucella species one of the main highly pathogenic zoonotic agents that infect humans [1], as well as domestic animals [2,3], including dogs [4], mainly hunting dogs [5] and wild animals [6], cause a serious public health threat and an economic impact [7]. In 2019, 310 confirmed brucellosis cases in humans were reported in the European Union. From those, 10% were from Portugal [8].

Excreted material as vaginal excretions and aborted material from infected animals are the major sources of contamination in feeding areas, pastures and water, constituting the main sources of infection among animals [9]. The disease is associated with reproductive losses in animals worldwide [7], but in general, most infected animals do not demonstrate clinical illness on visual examination [10]. Human are foremost infected through consumption of unpasteurized dairy products and uncooked meat [11] or through direct contact with infected animals [12].

Brucellosis still has a high incidence in some regions of Portugal, especially in northeast Portugal, where brucellosis in sheep and goats is still endemic [9,13]. Brucellosis is known to be an important disease in wildlife and all *Brucella* species can also infect wild species [14,15]. Furthermore, it is known that having a reservoir of the disease in wildlife can complicate eradication efforts [11]. Within wildlife, large game species, like wild boar (*Sus scrofa*) which may be in closer contact with human, may constitute an important threat in the transmission of zoonotic diseases, that must be addressed. Wild boar is known as an important reservoir of *Brucella* [3,12,16–26]. In Portugal, the potential role of wild boar as a reservoir of *Brucella* infection is largely unknown. In northeast region of Portugal, wild boar is currently the most important wild species hunted. Furthermore, there is evidence of wild boar/domestic animal (e.g., pigs raised outdoor, hunting dogs)/human sympatric interactions that may contribute to the interspecies transmission of this agent. Under this epidemiological scenario, what could be the role of wild boar? This study aimed to identify the seroprevalence and spatial distribution of *Brucella* spp. in wild boars hunted in the northeast of Portugal where brucellosis is still endemic in livestock, increasing knowledge about the disease in wild boars in Portugal.

2. Materials and Methods

2.1. Ethical Approval

Our study did not require ethical approval, because all samples were collected from wild boars legally hunted. No live animals were used for this study.

2.2. Area of study

The present study was performed in the district of Bragança (**Error! Reference source not found.**), in the northeast of Portugal where brucellosis is an endemic disease [27].

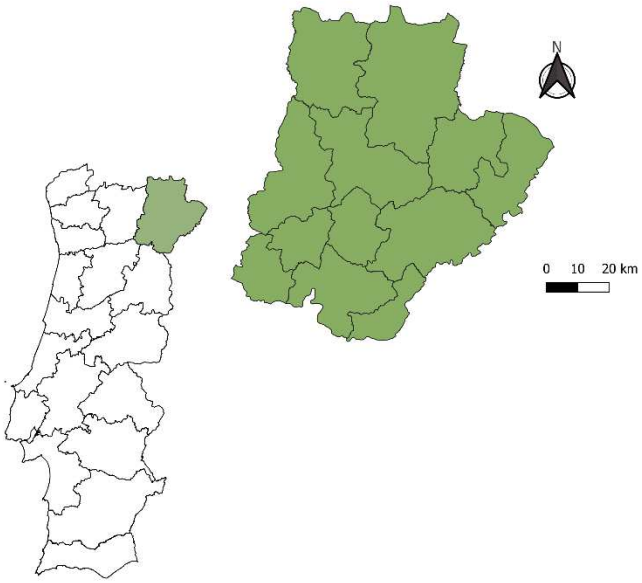

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Figure 1. The map of Portugal showing the area of study (Bragança district).

The territory is very mountainous with abundant wild species, especially wild boar. Oak acorns represents the basic diet of wild boars [28] and the study area is favorable for wild boar maintenance. The region is characterized mainly by oaks, chestnut trees, shrub vegetation like heather *Erica* spp., gum rockrose and fragmented by cultivated fields.

There are several herds of ruminants and domestic pigs raised outdoor favoring possible contact between wildlife directly or through the common natural resources (food and water).

2.3. Sampling and laboratory analysis

A cross-sectional study was carried out during the 2022/2023 hunting season (October – February) to determine the seroprevalence of *Brucella* spp. in wild boars (*Sus scrofa*). Thirty-six hunting associations from the area of study were contacted to collaborate in the study. All hunting associations accepted to participate in the study.

A non-probabilistic sampling method (convenience sampling) was used in this study. A total of 332 blood samples from wild boars were collected in eight municipalities (Bragança, Freixo de Espada à Cinta, Macedo de Cavaleiros, Miranda do Douro, Mogadouro, Torre de Moncorvo, Vimioso and Vinhais) of the Bragança district. Blood samples were obtained using a 10 ml syringe, tubes containing clot activator (BD Vacutainer®) and a 80 mm long needle (1x280mm, BOVIVET, Kruuse®, Denmark), by ocular puncture, described by Arenas-Montes et al. (2013) [29]. Samples were refrigerated to be taken to the laboratory.

No animals were eviscerated and examined after the hunt. All were taken by the hunters for private consumption to different parts of the country.

After coagulation the blood samples were centrifugated and serum stored at -20ºC until analyses. The samples were analysed for antibodies against *Brucella* spp. using a multi-species i-ELISA test kit (ID Screen® Brucellosis Serum Indirect Multi-species, ID vet Innovate Diagnostics, Grabels, France), following the instructions of the manufacturer. The optical density (OD) was read at 450 nm and results were evaluated by calculating the S/P $[OD_{sample} - OD_{NC}] / [OD_{PC} - OD_{NC}] \times 100$. Samples with S/P%≤110% were considered negative, 110 - 120% doubtful, and ≥120% positive.

2.4. Statistical data analysis

Seroprevalence of *Brucella* spp. was estimated from the ratio of positive samples to the total number of samples analysed. The 95% confidence interval (CI) for seroprevalence proportions was calculated.

3. Results

Seven out of eight municipalities were positive for *Brucella* spp. antibodies. Eighty-eight wild boars were exposed to these bacteria, pointing to a seroprevalence of 26.5% (95% CI: 21.8% – 31.3%). The number of positive and doubtful samples results and prevalence of *Brucella* spp. in wild boars per municipality is shown in **Error! Reference source not found.**.

Table 1. Seroprevalence of Brucella spp. in wild boars from 8 municipalities of Bragança district.

Municipality	Number of tested	Number of doubtful	Number of positives	Percentage of positives	95% CI (%)
Bragança	99	0	22	22.2	14.0 – 30.4
Freixo de Espada à Cinta	10	0	5	50.0	19.0 – 81.0
Macedo de Cavaleiros	38	1	13	34.2	19.1 – 49.3
Miranda do Douro	51	0	7	13.7	4.3 – 23.2
Mogadouro	29	0	7	24.1	8.6 – 39.7
Torre de Moncorvo	5	0	0	0.0	0.0
Vimioso	37	1	10	27.0	12.7 – 41.3
Vinhais	63	0	24	38.1	26.1 – 50.1

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TOTAL	**332**	**2**	**88**	**26.5**	**21.8 – 31.3**

Prevalence of antibodies to *Brucella* spp. was 50.0% (95% CI: 19.0% – 81.0%) in wild boars from Freixo de Espada à Cinta municipality, followed by Vinhais (38.1%, 95% CI: 26.1% - 50.1%), Macedo de Cavaleiros (34.2%, 95% CI: 19.1% - 49.3%), Vimioso (27.0%, 95% CI: 12.7% - 41.3%), Mogadouro (24.1%, 95% CI: 8.6% - 39.7%), Bragança (22.2%, 95% CI: 14.0% - 30.4%) and Miranda do Douro (13.7%, 95% CI: 4.3% - 23.2%). We had two results doubtful in Macedo de Cavaleiros and Vimioso municipalities and no positive samples were registered in the municipality of Torre de Moncorvo (**Error! Reference source not found.**).

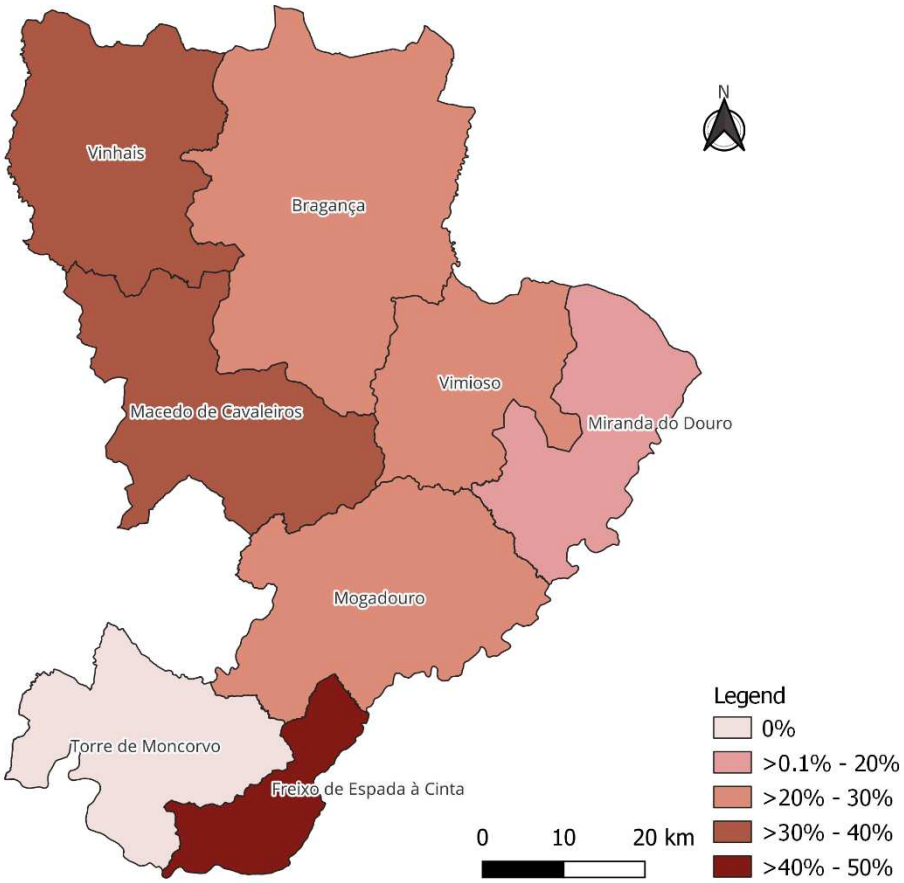

Figure 2. Seroprevalence of Brucella spp. in wild boars in eight municipalities of the district of Bragança.

4. Discussion

Brucellosis in wildlife has been neglected [24]. Our study aimed to determine the seroprevalence of brucellosis in wild boars in the district of Bragança, Portugal, during the 2022/2023 hunting season, using a multi-species i-ELISA test kit. From a total of 332 samples of wild boars, 88 (26.5%, 95% CI: 21.8% – 31.3%) were positive to *Brucella* spp. antibodies.

In contrast to what happens in Portugal, there are some studies on the seroprevalence of brucellosis in wild boars in Spain, neighboring country of Portugal. In 2006 Ruiz-Fons et al.,[23] studied the seroprevalence of six reproductive disease pathogens in wild boar females and revealed a seroprevalence of 29.7% for *Brucella* spp. In other study, wild boar showed a high prevalence of brucellosis (33.0%) in all Spanish territory [21]. A lower seroprevalence was found in northeast of Spain, this study was conducted by Closa-Sebastià et al. [22] in 2010 and detected in 28 of the 256 (10.9%) wild boars *Brucella* antibodies.

 doi:10.20944/preprints202306.2246.v1

High seroprevalences were detected in Belgium and Switzerland, with 54.9% and 28.8%, respectively [18,20].

Our study reveals a higher seroprevalence than in Italy and the Netherlands. In Italy, antibodies to *Brucella* spp. were found in wild boars in Sardinia region (2015) and in Campania region (2020) with seroprevalences of 6.1% and 13.6%, respectively [19,26]. In Netherlands, the prevalence ranged from 4.1% to 11.6%, in different provinces [25]. Poland and Ukraine also had lower seroprevalences, registering 24.5% and 11.3%, respectively [16,17].

This study revealed that in the northeast of Portugal the role of wild boar could be of relevance, possibly serving as reservoir of brucellosis and spillover infections to sympatric domestic animals and humans.

Brucellosis in wild boar can be widespread in the northeast of Portugal, thus representing an important threat for domestic pigs, in particular, Bísaro pig, an autochthonous breed in the northeast of Portugal. This breed is mostly reared in a semi-extensive system where breeders have pigsties but the animals are still free to spend most of their time roaming the adjacent parks. Therefore, there is a higher risk of interactions between pigs outdoors and wild boars in the study area. In Switzerland, swine brucellosis was detected on two outdoor pig farms after contact with wild boar [30].

Wild boar hunting has been considered a source of *Brucella* spp. infection in hunting dogs [31]. Dogs are infected when in contact with body fluids and tissues from infected wild boars. There are a few studies on the occurrence of brucellosis in hunting dogs. Brucellosis should be considered in the differential diagnosis of abortion, testicular/epididymal enlargement, lameness and discospondylitis [32]. The prevalence found in this study should raise awareness among owners of hunting dogs about possible exposure to the disease.

The prevalence found highlights that brucellosis in wild boars may represent a significant threat to public health, as it was previously referred for other geographical regions. In France, seven cases of *Brucella suis* in humans have been reported and all patients had direct contact with wild boars while hunting or preparing wild boar meat for consumption, which proves the occupational threat to humans, principally hunters [12].

Despite, private consumption on game meat not examined/inspected can be considered a public health risk [33], there is no mandatory initial examination of the carcasses of wild boars hunted in northeast of Portugal. All 332 carcasses were not eviscerated and examined after the hunt. All were taken by the hunters for private consumption (with no inspection). These results suggest that hunters or other people during the carcasses' handling may be exposed at home if no protective measures are adopted. The low brucellosis awareness and knowledge level and incorrect practices in handling, cooking and preserving animal-based food, poses a great threat to public food safety[12].

Also, due to the fact that hunters take wild boars for private consumption, the disposal of by-products may not be done correctly. A study carried out in Portugal during the three hunting seasons (from the year 2020 to 2023) by Abrantes et al. [34], concluded that 11% of hunters or managers of hunting areas do not correctly dispose of by-products, endangering public health. Proper disposal of by-products is critical to preventing the spread of brucellosis and other diseases [35].

Knowledge of the epidemiology of brucellosis is of paramount importance for the protection of public health, particularly among high-risk groups such as hunters. Knowledge/training allows people to take protective measures and to actively participate in disease control programs by actively contributing to the development of brucellosis control strategies [36].

Our study had several limitations due to the lack of initial examination of wild boar carcasses in this region. Therefore, samples were collected quickly, because people wanted to transport wild boar carcasses to their homes as quickly as possible. The lack of data on risk factors generated insufficient data to provide the basis for a representative statistical description and analyses.

The role of wild boar can be of great importance, but is often largely neglected. Furthermore, wildlife brucellosis inspection is not mandatory and data are scarce. This study aimed to get insights and increase knowledge into the occurrence of brucellosis in wild boars hunted in a brucellosis endemic region in livestock in Portugal.

This information should trigger increased attention from the competent national veterinary authorities who should encourage surveillance and control actions for this important zoonotic disease. Furthermore, more information/training should be given to hunters so that they can implement effective protection measures. Protection should be used while handling wild boar animals and awareness of by-products elimination should be raised among hunters.

In future, it would be highly desirable to collect more accurate epidemiological information on the prevalence of wild boar brucellosis and its etiology.

5. Conclusions

Our study contributed for the first report to the knowledge of *Brucella* seroprevalence in wild boars hunted in a brucellosis endemic region in livestock in Portugal.

Given the fact that in wild boar hunts in Portugal the initial examination of the hunted pieces is not always performed [34], the risk of transmission of brucellosis to humans, hunting dogs and livestock can be considered high. Attention should be given to biosecurity measures for wild boar hunters and livestock farms to prevent brucellosis infection. Also, health education about the disease for high-risk groups, like hunters, could be of capital importance. Furthermore, more information/training should be given to hunters so that they can implement effective protection measures.

This information should trigger increased attention from the Competent National Veterinary Authorities who should encourage surveillance and control actions for thus important zoonotic disease.

More studies on the identification of brucellosis are essential to provide epidemiological data for control of brucellosis in the northeast of Portugal.

Author Contributions: Z.M.R. and A.C. did the wild boars sampling. Z.M.R. and S.S-S. performed the laboratory analyses. Z.M.R., T.L.M. and M.V.P. wrote the first draft of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

Funding: This work was supported by the projects UIDP/CVT/00772/2020 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT).

Institutional Review Board Statement: All samples were collected from wild boars legally hunted. No ethical approval was deemed necessary.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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