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Serological evidence of *Erysipelothrix rhusiopathiae* antibodies in wild boars *(Sus scrofa)* in Poland

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Abstract

Introduction: This study aimed to evaluate the seroprevalence of *Erysipelothrix rhusiopathiae* among wild boars inhabiting Poland. **Material and Methods:** Serum samples were collected from 281 wild boars of varying ages (juveniles, adolescents and adults) and subjected to testing using a commercial indirect ELISA assay. **Results:** The prevalence of *E. rhusiopathiae* antibodies was notably high, reaching 75.1% overall and varying slightly across different age categories (90.0% in juveniles, 70.7% in adolescents and 79.6% in adults). Despite these variations, no statistically significant differences were observed among age groups (P-value > 0.05). **Conclusion:** The elevated prevalence of *E. rhusiopathiae* identified in this study underscores the significance of wild boars as natural reservoirs of this bacterium. Further investigations are warranted to identify the risk factors associated with disease transmission to other species, including humans.

Keywords: wild boars, age category, seroprevalence, Poland.

Introduction

Erysipelothrix rhusiopathiae, a rod-shaped Gram-positive bacillus, is the primary causative agent of swine erysipelas (SE). Members of the Suidae family serve as reservoirs for *E. rhusiopathiae* (7). The disease is prevalent in pigs and outbreaks carry significant economic consequences (2). While wild boars are susceptible to infection, there remain gaps in our understanding of their role in spreading *E. rhusiopathiae*. These animals often dwell in close proximity to humans, particularly in cultivated areas where livestock is commonly raised (7). Recent findings from an Italian study reinforce the notion of wild boars as reservoirs for *E. rhusiopathiae*, suggesting they can harbour the bacterium without displaying symptoms (13). Additionally, this study highlights a connection between SE infections in pigs and wild boars and anthropogenic factors, namely that higher human population and pig farm densities are linked to increased SE seroprevalence (13). It has been documented that wild boars can become infected with serovars commonly associated with the disease in pigs and humans, as well as with some others isolated from diseased pigs (26, 31). Therefore, it is reasonable to assume that wild boars can serve as a reservoir for disease transmission (7). The spread of *E. rhusiopathiae* among Suidae can occur through direct means, such as ingestion of faeces, urine or other secretions from infected individuals; or indirectly through consumption of contaminated water or feed (7). The risk of disease transmission between wild boars and pigs is highest in the case of backyard or free-range pigs, as these animals may have direct or indirect contact with wild boars (7); this is a concern because in the EU, a significant number of pigs are raised in backyard systems (4) and there is a growing consumer demand for free-range pork (19). In such settings, infected wild boars can be an important source of infection for pigs and a cause of the attendant economic losses.

In addition to infecting Suidae, *E. rhusiopathiae* has the capability to infect a broad spectrum of mammals, birds and fish (1). Furthermore, documented cases of human infection with *E. rhusiopathiae* have occurred, primarily among animal handlers (3, 28). The existing literature suggests that individuals exposed to animals or animal products in their occupation are at a significantly higher risk of infection (2). Infected individuals typically recall prior animal contact regardless of their profession, emphasising the pivotal role of human–animal interactions in disease transmission (24). *Erysipelothrix rhusiopathiae* infections in humans are relatively uncommon (2); nevertheless, recent data indicate a rising trend in confirmed human cases, with the majority occurring in developed and high-income countries and likely being attributable to improved diagnostics, the lack of which in developing and underdeveloped nations implies underestimation of the incidence of human infections globally (24). While human erysipelas is a rare and typically self-limiting condition, certain predisposing factors such as immune system depression may lead to more severe outcomes like endocarditis resulting from bacteraemia (28).

Diagnosing the disease in humans can pose challenges because of the nonspecific symptoms and diverse clinical manifestations (2), making effective prophylaxis crucial. Education about the potential threat, particularly educating individuals at risk, stands as one preventive measure. Among the members of the at-risk group, hunters are particularly vulnerable to contracting *E. rhusiopathiae* from wild boars (7). In some countries, the majority of culled wild boars are used for private consumption (7); in Portugal, according to a questionnaire conducted by Vieira-Pinto *et al*. (30), the majority of hunters (86%) confirmed their intention to use wild boar meat for private consumption, and 93% of those intending to consume it also planned to sell a portion of the meat, mostly doing so without post-mortem inspection. In the EU, post-mortem inspection is not mandatory for game intended for private consumption and not intended for the market (12, 30). This underscores a lack of awareness among hunters regarding the potential health risks associated with game consumption.

Given the potential threat of SE that wild boars pose to domestic pigs and humans, there is a pressing need to expand our understanding of *E. rhusiopathiae* infections in wild boars, particularly in terms of their epidemiology. To date, there have been no data amassed on *E. rhusiopathiae* prevalence in wild boars. Therefore, the current study aimed to estimate the prevalence of *E. rhusiopathiae* antibodies in the sera of wild boars in Poland.

Material and Methods

Wild boar of various ages from 46 forestry management areas under the jurisdiction of 16 Regional Directorates of State Forests in Poland were included in the study (Table 1). Blood samples were collected during the hunting seasons of 2017/2018 and 2019/2020 from wild boar immediately after they were shot, primarily as blood clots extracted from the main vein trunks or the animal's chest cavity. Upon arrival at the laboratory, serum was separated by centrifugation of coagulated blood (at 4°C and 1,000 × *g* for 10 min) and stored frozen at −20°C until further analysis was carried out. The boar were categorised into three age groups on the body weight criterion: juveniles (less than 1 year old) were up to 35 kg, adolescents (between 1 and 2 years old) were 36 to 70 kg, and adults (over 2 years old) were more than 70 kg in weight.

Table 1. The number of animals culled by area under a particular Regional Directorate of State Forests' control

Regional Directorate of State Forests	Province	Number of tested animals
Katowice	Opolskie, Śląskie	27
Olsztyn	Warmińsko-Mazurskie	31
Szczecin	Lubuskie, Zachodniopomorskie	28
Szczecinek	Pomorskie, Zachodniopomorskie	22
Białystok	Podlaskie, Warmińsko-Mazurskie	8
Gdańsk	Pomorskie, Warmińsko-Mazurskie	12
Krosno	Podkarpackie	21
Lublin	Lubelskie, Podkarpackie	6
Łódź	Łodzkie, Mazowieckie	19
Piła	Wielkopolskie	25
Poznań	Wielkopolskie	15
Radom	Mazowieckie, Świętokrzyskie	5
Toruń	Kujawsko-Pomorskie	21
Kraków	Małopolskie	10
Warszawa	Mazowieckie	15
Zielona Góra	Lubuskie	16
Total		281

Table 2. Prevalence of antibodies against *Erysipelothrix rhusiopathiae* detected in wild boar across Poland (n = 281)

Age category	Number of tested animals	Number of seropositive animals	Seroprevalence (%)	95% CI
Juveniles	20	18	90.0	68.0–98.8
Adolescents	157	111	70.7	62.9–77.7
Adults	103	82	79.6	70.5–86.9
Total	281	211	75.1	69.6–80.0

A commercial indirect ELISA (Civtest Suis SE/MR; Hipra, Amer, Spain) was employed to detect antibodies in the wild boar sera following the manufacturer's instructions. The optical density (OD) was measured at 405 nm, and the results were interpreted by calculating the IRCP (Relative Index × 100) value (IRPC = [(sample OD − mean znegative control OD/mean positive control OD − mean negative control OD)] × 100). Sera were considered positive when the IRCP value exceeded 40.0.

The proportion of seropositivity and the corresponding 95% confidence intervals (95% CI) were calculated using a binomial confidence intervals calculator (https://statpages.info/confint.html). Differences in the presence of anti-*E. rhusiopathiae* antibodies among different age groups were determined using a chi-squared test, with the significance level set at P-value < 0.05. Data analysis was conducted using Microsoft Excel with the Real Statistics Resource Pack for Excel Release 9.0 (Microsoft, Redmond, WA, USA).

Results

A total of 281 blood samples were gathered from wild boar across various age categories spanning Poland's territory. The prevalence of *E. rhusiopathiae* antibodies in serum samples from these wild boars was notably high at 75.1% (95% CI: 69.6–80.0%). There were slight variations in antibody prevalence across different age groups. However, no statistically significant differences were observed among age categories (P-value > 0.05). The youngest group of wild boar exhibited the highest rate of seropositivity at 90.0% (95% CI: 68.0–98.8%). Meanwhile, both adolescent and adult groups displayed a comparable pattern of seropositivity, with rates reaching 70.7% (95% CI: 62.9–77.7%) and 79.6% (95% CI: 70.5–86.9%), respectively.

Discussion

The European wild boar population is on the rise (7). It is speculated that over the last 15 years in Poland, the number of wild boars has doubled and their exact numbers and density are underestimated in available data (21). In Poland, the number of wild boars culled varies annually; in 2003, 122,000 were culled, whereas in 2013 this number increased to 242,000, reflecting a 98% growth (6). During the 2021/2022 hunting season, 143,800 wild boar were hunted (27). Additionally, the Polish Minister of Agriculture and Rural Development has mandated the targeted removal of wild boar as part of efforts to combat African swine fever (ASF). Hunters are often permitted to use the game obtained in this manner for their private consumption. This increases the likelihood of direct or indirect contact between wild boars and humans or pigs (15). In such circumstances, the lack of knowledge regarding the potential health threats posed by wild boar or game meat is alarming and could be considered a public health concern. Therefore, the present study offers data on *E. rhusiopathiae* antibodies within the Polish wild boar population. Information on the seroprevalence of this pathogen among wild boars worldwide is scarce, with only a few studies addressing the presence of anti-*E. rhusiopathiae* antibodies in this species. Additional reports originate from Portugal, Spain, Greece, Sweden, Italy and Japan (13, 15, 17, 18, 25, 29). Importantly, in most of the referenced research, the observed seroprevalence was notably lower compared to the findings presented herein. Studies conducted in Japan, akin to ours, revealed a high seroprevalence among the country's wild boar population. In 2016, 2019 and 2022 studies, 66.7%, 95.6% and 100% of sampled animals tested positive for anti-*E. rhusiopathiae* antibodies, respectively (15, 25, 26). This elevated seropositivity rate is likely attributed to a high proportion of carriers (26). Moreover, strains isolated from infected farmed wild boars in Japan displayed antimicrobial resistance to certain antibiotics (31). In Italy, the observed seroprevalence was also notably high, reaching 69.4% (13). In contrast, the overall seroprevalence in Portugal, Greece, and Sweden stood at 16.2%, 2.4%, and 17.5%, respectively (17, 18). The highest concentration of studies on this subject occurred in Spain, where seroprevalence ranged from 5% in 2002 to 15% in 2011 (5, 8, 9, 29). Additionally, the presence of *E. rhusiopathiae* antibodies was confirmed annually during a seven-year serological survey by Cano-Manuel *et al.* (8) conducted in one of the Spanish national parks, indicating the endemicity of this infection. Those authors suggested a significant and positive correlation between wild boar population density and *E. rhusiopathiae* seroprevalence. In Poland, wild boar density varied considerably across regions (21). However, in most regions, it appeared to be higher than those in Spain, Sweden, Greece, Portugal or Italy and was believed to be vastly underestimated (21). Intensive management practices such as fencing or feeding are thought to

influence the prevalence of various pathogens in wild boars (17). Since 2014, Poland has been grappling with ASF and fencing is a commonly employed strategy in the country in the fight against it (21). National legislation prohibits wild boar feeding throughout the country and restricts the amount of feed used for baiting to 10 kg/km^2/month (14).

There is no precise data regarding *E. rhusiopathiae* prevalence in the Polish pig population. In the last decades, the widespread use of vaccines against erysipelas in breeding herds and on grow-finish farms where there is perceived high erysipelas pressure has eliminated the manifestation of the clinical form of the disease as a significant problem for pig producers (11). The occurrence of erysipelas cases was commonly observed whenever established erysipelas vaccination programmes were discontinued in an effort to reduce production costs or when human error occurred during vaccination (20). In Poland, a recent report by Dec *et al*. (10) demonstrated that the *E. rhusiopathiae* strains which developed erysipelas (clinical or post-mortem signs of disease) were mainly isolated from unvaccinated pigs on backyard farms. The occurrence of erysipelas in unvaccinated backyard pigs may be associated with using straw as bedding, unpaved paddocks and insufficient manure removal, all of which can be sources of *E. rhusiopathiae* (10). Data on the frequency of *E. rhusiopathiae* infection in humans in Poland is also scarce. An analysis of human erysipeloid in one of the Polish provinces during 2005–2008 demonstrated strict disease association with occupational exposure, while in the same publication reporting that analysis, erysipeloid was noted as being one of the most common zoonoses in Poland (16) and one which could prove fatal. The disease does not, however, attract much attention and clinical reports have only been published sporadically up to the present time (16). Between 1966 and 2008, reporting and registration of human erysipeloid to the designated institution was mandatory. During the last 10 years of the monitoring programme, 1,121 cases were reported across the country; however, it is speculated that the true number of cases was underestimated in the number of notified cases (16). The high seroprevalence of anti-*E. rhusiopathiae* antibodies observed in the present study suggests that wild boars may represent a significant human infection risk, particularly for backyard pigs and individuals in high-risk occupations.

In the present study, age showed no statistically significant effect on seroprevalence, which was consistent with the findings of Canotilho *et al.* (7). While no statistical significance was found with regard to age, the researchers did report a contrasting age observation to ours: that anti-*E. rhusiopathiae* antibodies were predominantly found in adult wild boars, with only 2 out of 18 positive wild boars belonging to the subadult age category. The number of juvenile animals in our study was much lower than the numbers in the adolescent and adult categories, which may have influenced the observed results. Additionally, the high seroprevalence in the juvenile group could be due to the presence of maternal-derived antibodies (MDA) and may not necessarily indicate active immunity acquired through exposure to *E. rhusiopathiae* bacteria. In pigs, MDA can remain detectable up to eight weeks of age (23). This is important to consider, as we do not have precise data regarding the age of the juvenile wild boars included in this study.

Besides age, it has been shown that sex also did not have a statistically significant impact on seroprevalence (8, 9). However, in the study by Cano-Manuel *et al*. (8), it was found that seroprevalence in positive areas was significantly higher in females despite the lack of a significant correlation between total *E. rhusiopathiae* seroprevalence and sex. This higher prevalence among females may be attributed to more frequent contact between individuals or an earlier breeding age compared to males (8). Since we did not have data regarding the sex of the sampled wild boars, the evaluation of this finding was not feasible in the present study.

Conclusion

The present study uncovered elevated levels of *E. rhusiopathiae* seropositivity among Polish wild boars, suggesting that they serve as natural carriers of this bacterium. Considering the zoonotic potential of *E. rhusiopathiae*, these findings underscore the necessity for a standardised risk management strategy to mitigate transmission from wild boars to humans, particularly those involved in activities such as hunting, carcass dressing and preparing and selling game meat. Given this heightened seroprevalence and the ecological role of wild boars, these animals should be recognised as potential sources of infection for pigs and other species susceptible to *E. rhusiopathiae*.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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Animal Rights Statement: Ethical approval was not required for this study as blood samples were obtained from dead wild boars legally hunted as part of the hunting economy program and within hunting limits (Dz. U. z 1995 r. Nr 147 poz. 713, Ustawa z dnia 13 października 1995, "Prawo Łowieckie" z póź zm. (Journal of Laws 1995, No. 147, item 713; Bill of 13 October 1995, "The Hunting Law" as amended)).

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