

SEROLOGICAL INVESTIGATIONS REGARDING THE EFFECT OF INFECTION WITH SWINE INFLUENZA VIRUS H1N1 ON THE EVOLUTION OF ENZOOTIC PNEUMONIA IN WILD BOARS FROM WESTERN ROMANIA

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Abstract

The transmission of infectious diseases between wild animals and domestic animals is becoming a global issue of growing interest for the pig producing industry and human public health. In medical literature, there are several reports that wild boars may act as a reservoir for economically important infectious diseases that endemically affect domestic pigs, such as enzootic pneumonia and swine influenza. Although the risk of transmission of these diseases between wild boars and domestic pigs is likely to increase in Western Romania, there is very few data on the seroprevalence of *Mycoplasma hyopneumoniae* and swine influenza virus in wild boar populations. Therefore, the aim of the present study was to determine the seroprevalence of infection caused by *Mycoplasma hyopneumoniae* in wild boar populations from Western Romania in order to contribute to the information necessary for the control of the disease. Also, in order to determine the effect of infection with swine influenza virus H1N1 on the evolution of enzootic pneumonia, the value of seroprevalence for both types of infections was compared. The seroprevalence of infection with *Mycoplasma hyopneumoniae*, reported to the total number of samples tested on nine hunting grounds (which belong to Caraș-Severin County, Timiș County, and Bihor County) was 66.67%. The seroprevalence of swine influenza virus infection, reported to the total number of samples tested on 25 hunting grounds (which belong to Caraș-Severin County, Timiș County, and Bihor County) was 11.80%. Mixed infections with *Mycoplasma hyopneumoniae* and swine influenza virus subtype H1N1 were detected in two out of the three counties included in the study (Timiș County and Bihor County), with a substantial increase in *Mycoplasma hyopneumoniae* seropositivity. The results obtained in this study provide information on the disease exposure and health status of wild boars suggesting that *Mycoplasma hyopneumoniae* and swine influenza virus are widespread in wild boar populations from Western Romania and that these pathogens represent a source of infection for domestic pigs, as well as humans.

Key words: enzootic pneumonia, wild boars, pathogens, hunting grounds, disease exposure

Introduction

Mycoplasma hyopneumoniae is recognized as the causative agent of enzootic pneumonia. The organism is the smallest bacteria and is ubiquitous within swine herds throughout the world. Recent serological studies demonstrated that *Mycoplasma hyopneumoniae* can also infect wild boars (7). According to data from medical literature, transmission of *Mycoplasma hyopneumoniae* between domestic pigs and wild boars is possible in both directions (4).

Therefore, this study had 2 objectives: 1) to determine the seroprevalence of infection caused by *Mycoplasma hyopneumoniae* in wild boar populations with the use of an enzyme-linked immunosorbent assay (ELISA), and 2) to compare the seroprevalence of infection caused by *Mycoplasma hyopneumoniae* with that induced by swine influenza virus subtype H1N1 in wild boar populations from western Romania.

Materials and methods

In order to detect and quantify the antibodies against *Mycoplasma hyopneumoniae* in wild boar populations from western Romania, a total of 45 thoracic fluid samples were harvested and tested. The samples were collected and sent for investigation by the County Sanitary Veterinary Divisions, and samples were harvested in the 2013-2014 hunting three western counties (Caraș-Severin, Timiș, and Bihor).

The processing of samples was performed by ELISA method using the kit CIVTEST SUIS MHYO in the Laboratory of the Department of Infectious Diseases and Preventive Medicine of the Faculty of Veterinary Medicine Timișoara.

Results and discussions

The results obtained after performing ELISA test on the samples collected from wild boars from nine hunting grounds in the three counties (Caraș-Severin County, Timiș County and Bihor County) were systematized and presented in table 1.

Table 1
Positive samples identified from hunting funds of Caraș-Severin, Timiș, and Bihor Counties

County	Hunting grounds	Number positive samples	Total number positive samples/number analyzed samples (% infection)
Caraș-Severin	Bigăr	2	5/8 (62.50)
	Bocșa	1	
	Moldova Nouă	2	
	Slatina Timiș	0	
Timiș	Surduc	2	14/21 (66.67)
	Pișchia	7	
	Luncani	4	
	Traian Vuia	1	
Bihor		11	11/16 (68.75)
Total number positive samples		30	30/45 (66.67)

The results obtained from the serological examinations performed by ELISA method and presented in table 1 show that infection with *Mycoplasma hyopneumoniae* was reported in all three counties monitored (Caraș-Severin, Timiș, and Bihor). Thus, the seroprevalence of infection in the investigated areas was 100%.

In Caraș-Severin County, positive specimens were identified in three out of the four hunting grounds taken into the study; the seroprevalence of mycoplasma infection reported to the total number of hunting grounds monitored in the county was 75%. In the case of infection with swine influenza virus subtype H1N1, negative samples were identified in all four hunting grounds investigated. Thus, the value of seroprevalence of viral infection was 0%. The analyzed results regarding infection with *Mycoplasma hyopneumoniae* had shown that in the four hunting grounds of Caraș-Severin County, five positive samples were identified out of the eight samples taken into the study. Thus, the seroprevalence of infection with *Mycoplasma hyopneumoniae* was 62.50%.

In Timiș County, positive samples were identified in all four hunting grounds investigated; the seroprevalence of infection reported to the number of hunting grounds monitored in the county was 100%, which is higher compared to that reported for infection with swine influenza virus subtype H1N1, which was 30%. In the four hunting grounds of

Timiș County, there were 14 positive samples identified out of the 21 samples taken into the study; the seroprevalence of infection with *Mycoplasma hyopneumoniae* reported to the number of samples monitored within the county was 66.67%, which is higher than the seroprevalence of infection with swine influenza virus subtype H1N1, at 22.40%, reported to the total number of samples monitored within the hunting grounds in Timiș County.

Bihor County was represented by only one hunting ground from which 16 samples were harvested and tested. After analyzing the samples using ELISA method, 11 positive samples were identified; the seroprevalence of infection reported to the total number of samples monitored within Bihor County was 68.75%. In the case of infection with swine influenza virus subtype H1N1, the seroprevalence was 5.80%, reported to the total number of samples tested within the hunting ground.

Overall, the seroprevalence of infection induced by *Mycoplasma hyopneumoniae*, reported to the total number of samples tested on the hunting grounds, was 62.50% in Caraș-Severin County, 66.67% in Timiș County, and 68.75% in Bihor County. Average values of seroprevalence of infection with *Mycoplasma hyopneumoniae*, reported to the total number of samples tested on the nine hunting grounds in Caraș-Severin County, Timiș County, and Bihor County, was 66.67% (30/45). The seroprevalence of infection caused by swine influenza virus subtype H1N1 on the hunting grounds investigated was 0% in Caraș-Severin County, 22.40% in Timiș County, and 5.80% in Bihor County. Average values of seroprevalence of infection with swine influenza virus subtype H1N1, reported to the total number of samples tested on the 25 hunting grounds in Caraș-Severin County, Timiș County, and Bihor County, was 11.80% (Figure 1).

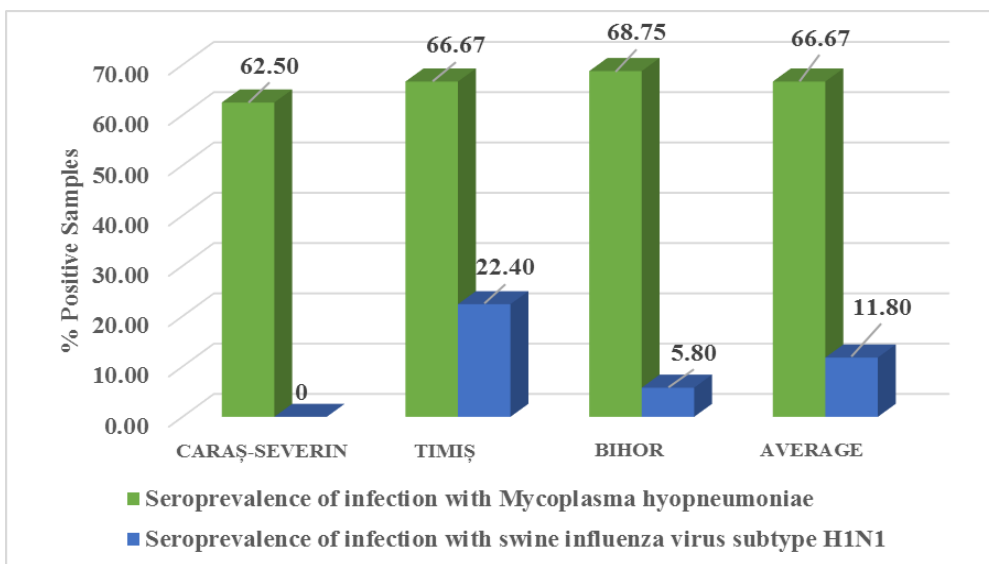


Figure 1. Comparative results of seroprevalence of *Mycoplasma hyopneumoniae* and swine influenza virus infection H1N1 within hunting grounds from monitored counties

The seroprevalence of infection with *Mycoplasma hyopneumoniae* in wild boar populations was reported in several countries in Europe and throughout the world, varying from one country to another. In this context, the value of seroprevalence of mycoplasma infection of 66.67% that was obtained in the present study was higher than that achieved in Slovenia

(15.80%), France (58%), and in United States, where the seroprevalence of infection was 32% (8).

A comparative analysis of results obtained in our study to data published by other authors in other countries from Europe had shown that the value of seroprevalence of mycoplasma infection in these countries were lower. Thus, a study conducted by Sibila et al., in 2010, demonstrated that the seroprevalence of infection induced by *Mycoplasma hyopneumoniae* in wild boar populations was 21% (7).

In a serological ELISA testing performed by Chiari et al., in 2013, antibodies against *Mycoplasma hyopneumoniae* were detected in 655 out of the 2.177 analyzed wild boars, so the value of seroprevalence of mycoplasma infection was 30% (2).

In another study conducted by Vengust et al., blood samples were taken from wild pigs and subjected to an examination by ELISA for the detection and quantification of antibodies against several pathogens, including *Mycoplasma hyopneumoniae*. The obtained results concluded that the seroprevalence of infection induced by *Mycoplasma hyopneumoniae* was 21% (9).

In Russia, a study done by Kukushkin et al., in 7 regions of the country, showed the presence of antibodies to *Mycoplasma hyopneumoniae* in 52% of samples (4).

Serological studies conducted worldwide have revealed that *Mycoplasma hyopneumoniae* may persist in infected pigs more than 28 months' post infection. In this context, the results of the current study demonstrated that mycoplasma infection occurred in wild boars monitored many months prior to collection of blood samples (10).

Currently, serological studies conducted worldwide have emphasized that there is an increasing tendency of infection with *Mycoplasma hyopneumoniae* in wild boar populations. Given this context, in the present study the relatively high value of seroprevalence of infection with *Mycoplasma hyopneumoniae* (66.67%), recorded in wild boar populations in the investigated area, shows that the infection is prevalent in the sylvatic environments. However, the danger of transmission of disease from wild animals to domestic animals is not sufficiently known. Therefore, a thorough investigation is needed to explore the genetic linkage that exists between infection caused by *Mycoplasma hyopneumoniae* detected in wild boar populations and mycoplasma infection reported in domestic swine herds. It is important to note that this investigation is of fundamental importance to further establish whether wild boars can serve as a reservoir of the disease for domestic pigs in that location (10).

From the literature reviewed, it is well-known that age class is involved in transmission of mycoplasma infection in wild boars. Thus, the value of seroprevalence of mycoplasma infection is higher in young and adult wild boars (3).

It is well-understood that nowadays, globalization, climate change, increased animal movements, and trade have led to an increasing number of emerging diseases. Given this context, in order to understand the role of environmental change in disease emergence and transmission, the expertise of specialists working in several areas such as ecology, zoology, microbiology, human medicine, and veterinary medicine is required. Meanwhile, veterinarians and other public health professionals have to become an integral part of the research teams involved in control of mycoplasma infection in wild boar populations. More specifically, the major role of these professionals is to understand and prevent the transmission of disease from wild boars to domestic animals and humans (6).

The probability of transmission of infection induced by *Mycoplasma hyopneumoniae* from wild boars to domestic pigs is affected by prevalence and mode of transmission of the agent. Determining the likelihood of transmission of mycoplasma infection from wild boars to domestic pigs plays a special role in the implementation of biosecurity measures in order to

decrease or even to eliminate the risk of exposure of domestic pigs to mycoplasma infection transmitted by wild boars (5).

Given this context, close and continue collaboration among biologists, ecologists, veterinarians, and epidemiologists is crucial for successful prevention interventions concerning mycoplasma infection and other respiratory diseases that are prevalent in wild boar populations. Additionally, dispersion ability of wild boars should be considered in developing surveillance programs. Knowledge of host dispersal rates is considered vital in understanding the spread of diseases in wild animals. The combination of higher dispersal ability of wild boars and the fact that wild pigs are known to be an important reservoir for many zoonotic pathogens can facilitate the transmission of diseases that affect wild boars to domestic pigs and humans (1).

All things considered, taking into account the higher rate of prevalence of *Mycoplasma hyopneumoniae* infections in wild boar populations, a combination of serological tests are required to establish an accurate diagnosis of mycoplasmosis in order to implement successful prevention interventions in the future (10)

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Conclusions

- Infection with *Mycoplasma hyopneumoniae* in wild boar populations was reported in all three counties monitored (Caraș-Severin, Timiș, and Bihor); the seroprevalence of infection in the investigated area was 100%.
- The seroprevalence of infection induced by *Mycoplasma hyopneumoniae*, reported to the total number of samples tested on the hunting grounds investigated, was 62.50% in Caraș-Severin County, 66.67% in Timiș County, and 68.75% in Bihor County.
- The seroprevalence of infection with *Mycoplasma hyopneumoniae*, reported to the total number of samples tested on the nine hunting grounds in Caraș-Severin County, Timiș County, and Bihor County, was 66.67%.
- The seroprevalence of infection with swine influenza virus subtype H1N1, reported to the total number of samples tested on the 25 hunting grounds in Caraș-Severin County, Timiș County, and Bihor County, was 11.80%.

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