THE 3rd INTERNATIONAL SYMPOSIUM ON HUNTING

"Modern aspects of sustainable management of game populations"

Proceedings of 3rd International Symposium on Hunting with Abstract book

Organizers

Balkan Wildlife Scientific Society

Zemun-Belgrade, 26-28. September, 2014.

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AMOVA analysis showed no significant differentiation between presumed „west" and „east" subpopulations, indicating that no genetic structure is present in grey wolves from Bosnia&Herzegovina. This result, however, should be confirmed in further analyses, since low number of individuals is analysed in this research.

Constructed median-joining network showed that number of mutational steps among haplotypes vary between 1 and 5 (Fig. 2)

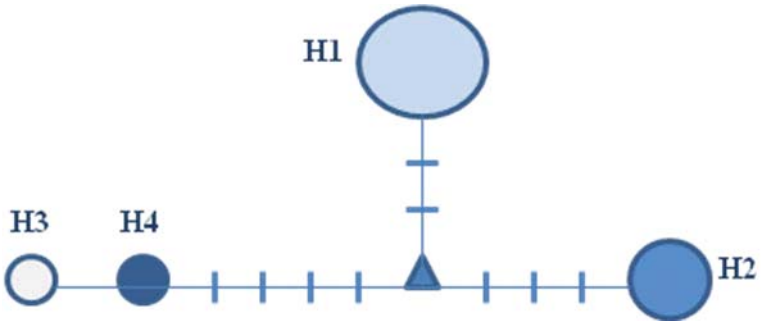

Figure 2. Median-joining network of 4 determined mtDNA control region haplotypes. Circle sizes correspond to the frequencies of the haplotypes.

Conclusion

Conducted research indicates high genetic variability within analysed grey wolf population, as expected for the Dinaric-Balkan grey wolf populations, as compared with other European wolf populations. Detection of present genetic diversity and demographic history is important for determination of population structure and sustainable management of the population.

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ANTIBODIES TO SELECTED VIRAL DISEASE AGENTS IN HUNTED WILD BOARS IN VOVODINA REGION

Prodanov-Radulović J.,[1] Došen R.[1], Petrović T., [1]
Polaček V. [1], Lupulović D.,[1]Stojanov I.,[1] Grubač S.[1]

Summary: The aim of the current study was to investigate the presence of antibodies to selected viral disease agents (PRRSV, PPV and CSFV) in hunted wild boars in Vojvodina region. Blood samples from 300 hunted wild boars from hunting ground were collected during the hunting season from October 20011 until March 2012. Presence of anti-PRRSV and anti-CSFV antibodies was determined using a commercially available ELISA test kits while the presence of anti-PPV antibodies was determined using the method of hemagglutination inhibition (HI test). Out of total 300 examined blood samples, in 49.33% antibodies against PPV, 1.33% antibodies against PRRSV and 10.33% antibodies against CSFV was detected. These results, support the hypothesis that wild boar are reservoirs of certain viral infectious agents, but some infections in wild boars originate from domestic swine. Having in mind this fact, the special attention should be given to active surveillance of wild boars population in the areas where close contact with domestic swine is possible.

Key words: wild boars, swine viral diseases, Vojvodina

Introduction

Wild boar (*Sus scrofa scrofa*) numbers have dramatically increased over the past 60 years and the species also shows a more widespread distribution (Sedlak et al., 2008; Wu et al.,2011). The increase in population density of wild boar raises concerns regarding the welfare and an increasing prevalence of infectious diseases and parasites (Ruiz-Fons et al., 2008). The parallel increase of outdoor piggeries has led to a higher risk of contacts, and thus of disease transmission, between wild boars and domestic pigs. Because pigs and wild boars belong to the same species, they share the same pathogens (Wu et al., 2011).

The overabundance of wildlife, recognized as a relevant risk factor for disease transmission between wildlife and domestic animals, compromises the health surveillance programs carried out both population (Frölich et al., 2002; Prodanov-Radulović et al.,2013; Ruiz-Fons et al., 2008). Among the agriculturally important pathogens known to be prevalent in wild boars are Classical swine fever virus (CSFV), Pseudorabies virus (PRV), African swine fever virus (ASFV), Porcine circovirus type 2 (PCV2), Porcine reproductive and respiratory syndrome virus (PRRSV) and Porcine parvovirus (PPV) (Albina et al., 2000; Meng et al., 2009; Montagnaro et al., 2006; Kaden et al., 2006; Roic et al., 2012; Ruiz–Fons et al., 2006). Knowledge of diseases circulating in wildlife populations can be important not only for conservation and livestock production but also for public health (Boadella et al., 2012; Meng et al., 2009; Ruiz-Fons et al., 2008).

In our country a certain number of wild boars are reared in controlled and enclosed hunting grounds, while a number of free-ranging populations are mainly unknown. One of the characteristics of outdoor swine production in some regions is raising free-roaming domestic pigs, where they share forest habitat with wild boars (Prodanov-Radulović et al., 2010; Prodanov-Radulović et al., 2013). Contacts between wild boars and domestic pigs kept in outdoor farms may occur occasionally (Albina et al., 2000; Prodanov et al., 2009; Roic et al., 2012; Sedlak et al., 2008). Since both animals have the same susceptibility to various infections including CSFV, PRRSV and PPV, there is a major concern to monitor the epidemiological situation of wild boars especially when control measures in domestic pigs

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are implemented (Lupulović et al., 2007; Montagnaro et al., 2010; Roic et al., 2012; Vengust et al., 2006). Animal health surveillance is routinely applied to domestic animals, but limited data exist on the prevalence and distribution of infectious agents of wild boars in Vojvodina (Lupulović et al., 2007; Prodanov-Radulović et al., 2010; Prodanov-Radulović et al., 2013)..

The objective of our study was to conduct a serologic survey of the hunted wild boars in Vojvodina region, focusing on selected viral diseases (CSFV, PRRSV, PPV) that are epidemiologically and economically important to the health of both wild boar and domestic swine populations.

Material and Methods

Sera samples

Blood samples from 300 hunted wild boars from hunting ground of Vojvodina were collected during the hunting season from October 2011 until March 2012. The samples were collected on shot wild boars by the veterinarians or by hunters. Hunters were previously trained to collect the blood from the heart and asked for doing the sample as soon as possible after death. Blood samples were sent to the Scientific Veterinary Institute "Novi Sad" either immediately or after storage at 48 °C for few days. In the laboratories, the samples were centrifuged and only sera of minimal quality (limited hemolysis and absence of protein denaturation) were selected for serology. All sera were collected under the National Classical Swine Fever Virus Surveillance Program and, therefore, no age or sex data were available for these animals.

Serology testing

Enzyme-linked immunosorbent assay (ELISA)

Commercially available ELISA test kit-INGEZIM PRRS Universal (Ingenasa, Madrid, Spain) was used for detecting anti-PRRSV antibodies in serum samples. The ELISA was performed according to the manufacturer's instructions, and in the ISO/IEC 17025:2005 standard accredited laboratory. This kit is based on an indirect enzymatic immunoassay (Indirect ELISA). The reaction was read at a wavelength of 405 nm, and specific antibody levels were expressed as optical density values (OD). The results were expressed as positive or negative based on producer's recommended cut off value.

For CSFV serum antibody detection the commercial indirect immunoenzyme test (ELISA) kit (Classical Swine Fever Virus Antibody Test Kit, HerdChek CSFVAb, IDEXX Laboratories, USA) was used according to manufacturer′s instruction.

Haemagglutination inhibition test (HI test) for PPV

The blood serum samples to be tested for PPV antibodies were heat-inactivated at 56 °C for 30 min. Haemagglutination inhibition (HI) test was carried out on 96 – U-bottom plates. Two-fold dilution of the treated serum were made in 25 µl volumes of PBS (pH 7.2), and 25 µl of viral suspension containing 4 haemagglutinating units of PPV (strain TEEN, American Bioresearch, USA) was added to each dilution. After incubating for 1 h at 37 °C, 50 µl of 0.6 % suspension of guinea pig erythrocytes was added and the plates were incubated at room temperature for additional 1-1.5 h. Apropriate serum, virus and erythrocytes controls were added to the test. The HI titer of anti-PPV antibodies was expressed as the reciprocal value of the highest sera dilution at which haemagglutination was inhibited. A wild boar was considered to be infected with HI titers higher than 1:256. Wild boars with titers equal to or lower than 1: 256 were considered to be non-infected (Huysman et al. 1992; Lupulović et al., 2007; Sorensen et al. 1988).

Results

In cooperation with the hunting societies and local veterinary service gathering of sera samples of hunted wild boars was organized from six districts in Vojvodina (Severnobački, Zapadnobački, Južnobački, Sremski, Severnobanatski and Srednjebanatski district). It should be noted that there are many domestic swine herds in the examined area; these are mainly large or medium-sized swine farms. Also, one of the characteristics of Srem district is outdoor swine production i.e. raising free-roaming domestic pigs. It is especially important that the owners of the free-roaming domestic pigs in the same time have backyard pigs (Prodanov et al., 2009). Having in mind that the domestic pigs are raised as free-

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roaming animals and the fact that they share the forest habitat with the wild boars, contact between wildlife and domestic livestock can easily occur (Prodanov–Radulović et al., 2013).

Out of 300 sera tested, in total 31 (10.33%) were found positive in the CSFV- ELISA. The positive samples were from 4 different districts in Vojvodina. Clinically, no abnormal mortality has been reported in the analyzed districts. By additional epizootiological evaluation, it was discovered that some of the examined sera samples from certain hunting grounds that tested positive were a consequence of previous vaccination against CSFV in the past, with modified live (China strain) vaccine (Prodanov et al., 2009). Therefore, we cannot exclude the possibility that vaccinated wild boars released from closed farm systems in the field may have been sampled and detected as positive in our survey. This could explain the presence of antibodies against CSFV in some of examined sera samples. In previous research conducted in 2013, applying reverse transcription-polymerase chain reaction (RT-PCR) analysis the presence of CSFV genome was not established in tissues samples deriving from shot wild boars in Vojvodina (Prodanov-Radulović et al., 2013).

Classical swine fever virus is a small single-strand positive-sense RNA virus, which belongs to the genus *Pestivirus* in the family *Flaviviridae* (Le Potier et al., 2006; Meng et al., 2009). The disease in the wild boar population was diagnosed and/or serologically confirmed in several Central and Eastern European countries (Artois et al., 2002; Montagnaro et al., 2010; Prodanov et al., 2009; Roic et al., 2012; Rossi et al., 2005; Vengust et al., 2006). Outbreaks are generally self-limiting in most wild boar populations (Rossi et al., 2005) but, in other cases, CSFV circulates for years (Ruiz-Fons et al., 2008). In some European countries, CSFV has been reintroduced periodically into domestic pigs via contact with infected wild boars (Le Potier et al. 2006). Moreover, epidemiological links between CSF virus infections in wild boars and domestic pigs have been repeatedly reported, mainly in Germany (Ruiz-Fons et al., 2008; Kaden et al., 2006). At present, CSF monitoring program in Serbia is primarily focused on the serological investigations of blood samples from hunted wild boar. Sampling is performed randomly based on the density of the wild boar population in different regions (Prodanov –Radulović et al., 2013).

In only 4 out of 300 sera tested were found seropositive in the PRRSV-ELISA. Seropositive boars were essentially detected in Juznobacki district where the domestic pig density and PRRSV prevalence are high. Positive results were not obtained elsewhere, which indicates that PRRSV has little or no importance in examined wild boar populations. This finding is similar to reports from Croatia (Roic et al., 2012), but it differs from other European studies (Albina et al., 2000; Montagnaro et al., 2010; Ruiz-Fons et al., 2006). In France, it is considered that PRRSV infection was introduced by movements of domestic pigs. The virus has probably been transmitted to wild boars due to the high infectious pressure existing in domestic pig farms (Albina et al., 2000). Taken together, these results suggest that when PRRSV enters the wild boar population, its subsequent spread is rather limited, probably because the virus is not easily transmitted within a population of low or medium density (Montagnaro et al., 2010; Roic et al., 2012). The contamination of the wild population is not surprising since PRRS virus is known to spread by air. Currently, the transmission of PRRSV from domestic swine to wild boar is more probable than vice versa. There is lack of convincing evidence to suggest that wild boars serve as a reservoir for PRRSV (Meng et al., 2009; Ruiz-Fons et al., 2008). Owing to this fact, further studies aimed at elucidating whether the origin of the detected seropositivity is a true contact with the virus or the result of false positives will be needed (Boadella et al., 2012).

Antibodies against PPV were widely distributed among the wild boar in the present study: 148 (49.33%) of the 300 examined samples tested PPV positive by the HI test. The highest prevalence of seropositive animals was associated with the hunting areas in Bačka and Srem districts. We believe that this is connected with the tradition of keeping domestic pigs in woods (extensive grazing, especially in Srem district), thus increasing possible contact and transmission of diseases between wild boars and domestic swine (Prodanov et al., 2009; Prodanov-Radulović et al., 2010; Roić et al., 2005). Infections with PPV represent one of the major causes of reproductive failure in domestic pigs in Vojvodina and systematic monitoring and vaccination programs are implemented (Lupulović et al., 2007). The high prevalence of PPV antibody suggests this virus is endemic in our wild boar populations. However, virus transmission between wild boars and domestic pigs could occur in both directions when contact between these two species occur (Roic et al., 2012).