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SEROPREVALENCE OF ACTINOBACILLUS PLEUROPNEUMONIAE IN SWINE ORIGINATED FROM COMMERCIAL FARMS IN SERBIA

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Abstract: Porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae* (*A.pleuropneumoniae)* is one of the most important respiratory disease of pigs and causes worldwide severe losses in pig farming. For *A.pleuropneumoniae* control and monitoring, the detection of ApxIV antibodies in the serum is the most frequently used serological method. The aim of this study was to investigate presence of antibodies against *A. pleuropneumoniae* in blood sera of gilts and sows using the ELISA test. Samples were taken from gilts and sows originating from four commercial swine farms in Serbia. For detection of ApxIV antibodies, commercial ELISA kit was used. A total of 453 blood sera samples of gilts (207) and sows (246) were examined. Antibodies against *A. pleuropneumoniae* were detected in 57 (12.58%) sera. Antibodies were present in 22 (10.62 %) sera of gilts and in 35(14.22%) sera of sows. Percentage of positive sera differed among the farms, ranging in gilts from 3.33-17.77 % and in sows from 8.95-22.64%. Serological methods is one of the most important procedures in the diagnosis of porcine pleuropneumonia particularly suitable for the control of animal health status in a large breeding.

Key words: *Actinobacillus pleuropneumoniae*, gilt, sow, antibodies, ELISA.

Introduction

The most significant problems in contemporary pig production are in connection with diseases of the respiratory system **(***Baker,2005; Hansen et al., 2010*). It is a characteristic of the current manner of production to set up agglomerations with concentrations of large numbers of animals within a small space. As a multifactorial disease, environmental conditions, population size, management strategies and pig-specific factors such as age and genetics also play significant roles in the outcome of respiratory disease (*Opriessing et al., 2011*).

Such conditions are especially favourable for respiratory pathogens and continuous presence of a high degree of virulence *in vivo*. As a result, there are increasingly frequent outbreaks of respiratory infections which are more difficult to control, with the maintenance conditions in large agglomerations exerting an extremely unfavorable effect (*Žutić et al., 2009*). The prevalence of respiratory disease is affected by the following: the presence of respiratory pathogenic organisms, the virulence of the pathogens present, the level of the pathogens in the house environment, the immunity of the pig and the time of exposure to the organisms, the presence of secondary opportunistic bacteria, the interactions between management, environment, the diseases and the pig (*The Pig Site Pig Health,2013*).

Porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae (A.pleuropneumoniae)* is one of the most important respiratory disease of pigs and is widely spread among pig-keeping countries (*Vidić et al., 2004; Bochev, 2007; Vaduva et al., 2011*). *A. pleuropneumoniae* is a small, gram-negative, encapsulated rod belonging to the family Pasteurellaceae.

There are two biotypes of *A.pleuropneumoniae,* differentiated on the basis of their requirement for factor V, (NAD) in biotype I (NAD-dependent) and biotype II (NAD-independent). There are 13 serotypes of biotype I and 2 serotypes of biotype II , based on surface polysaccharide antigens (*Maldonado et al., 2009; Bessone, 2010; Gotschalk, 2012*). The four major RTX toxins ApxI, ApxII, ApxIII and ApxIV are secreted by the different serotypes in various combinations (*Shin et al.,2010*). All serotypes can secrete ApxIV which can be produced only during infection, but not in vitro conditions. Presence of the Apx toxins is strongly correlated with virulence.

Growing pigs are most likely to be affected when they are 12-16 weeks old, but the disease can occur in all ages of swine (*Gotschalk and Taylor, 2006*). This disease is causing significant loss to farmers particularly when finisher pigs are involved. Pigs, asymptomatically infected with *A.pleuropneumoniae* in their upper respiratory tract, can transmit the infection. The main route of spread is by direct contact from pig to pig or by aerosol within short distances, although some authors reported that airborne transmission between closely located pig units is possible but rare (*Woeste and Grosse, 2007*). The clinical course of the disease can vary widely, ranging from the acute forms with severe clinical signs and a high mortality to the more chronic forms with few or even without any clinical symptoms (*Shi et al., 2012*). In the absence of treatment, the disease can progress very rapidly and death can occur within a few hours. Main clinical signs of the acute disease are anorexia, depression, fever, dyspnea and/or polypnea. Chronic infections are characterized by coughing and pleuritis in lungs (*Gotschalk et al., 2010*). The recent epidemiological studies indicate a very high rate of exposure reaching up to 100% of seropositivity of investigated farms (*Krejci and Newberry, 2011).* Confirmation by laboratory testing is essential for diagnosis, especially in monitoring schemes.

The health monitoring of herds is extremely important, firstly because of the need for the adequate strategy to be chosen for controlling the porcine pleuropneumonia and, at the same time, in order to prevent economic losses that this disease may cause. Identification of seropositive animals is an important measure to control and eliminate the disease from the swine farms. Also, serological tests showed seroconversion of each animal according to certain technological categories within the herd. Using ELISA, it is possible to detect ApxIV antibodies against all serotypes of the *A. pleuropneumoniae*, without cross-reaction with other bacterial species (*Dreyfus et al., 2004; Nussbaumer et al., 2008; Eamens et al., 2012*). The aim of the study was to investigate the presence of ApxIV antibodies against *A. pleuropneumoniae* in gilts and sows in four pig farms in Serbia.

Material and methods

For *A.pleuropneumoniae* monitoring purposes, the detection of ApxIV antibodies in the serum is currently the most frequently used serological method. ELISA test can be used to evaluate the *A. pleuropneumoniae* status of commercial herds especially for the diagnosis of latently infected, without clinical signs. For the investigations, samples were taken of the blood of 207 gilts and 246 sows originating from 4 commercial swine farms in Serbia. The capacity of each farm is about 700-1.000 sows. Investigations were carried out using the method of ELISA with the following diagnostic kits: Chekit APP-Apx IV: *A. pleuropneumoniae* antibody test Kit
(IDEXX APP-ApxIV Ab Test).

Results and Discussions

A total of 453 blood sera samples of gilts (207) and sows (246) were examined. Results of determination of antibodies against of *A. pleuropneumoniae* are given in Table 1.

The results of the investigations have shown that infection with *A.pleuropneumoniae* is present on all four examined farms. Antibodies against *A. pleuropneumoniae* were detected in 57 (12.58%) sera. Antibodies were present in 22 (10.62%) sera of gilts and in 35 (14.22%) sera of sows. Percentage of positive sera differed among the farms, ranging in gilts from 3.33-17.77% and in sows 8.95-22.64%**.** The results of this study show a significant decrease of seropositive animals. In previous study, antibodies against *A. pleuropneumoniae* were detected in 69.86% of sows and 76.72% of gilts (*Žutić J, et al.,2008)*.

Table 1. Results on presence of antibodies against *Actinobacillus pleuropneumoniae* in gilts and sows blood sera

Farms	Category	No. investigated	No. positive	% positive
1	gilts	49	7	14.28
	sows	55	9	16.36
2	gilts	53	5	9.43
	sows	61	8	13.11
3	gilts	60	2	3.33
	sows	67	6	8.95
4	gilts	45	8	17.77
	sows	63	12	22.64
Total gilts		207	22	10.62
Total sows		246	35	14.22
Total animals		453	57	12.58

It is probably result of intensive serological monitoring of gilts prior to fertilization and improving the living conditions of animals. A higher percentage of positive animals on the farm 4 are the result of old buildings and poor conditions.

Porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae* is still a problem today in herds of many countries where the swine industry is important (*Gotschalk et al.,2010*).The recent epidemiological studies indicate a very high rate of exposure, reaching up to 100% of seropositivity of investigated farms. Of those, 90% in Northwest Germany, 96% in Belgium, 89% in Spain and 100% in Italy and Belarus (*Krejci and Newberry,2011*). In Belgium, seroprevalence for serovar 2 was 58% (range 0 to 100 %), 53% for serovar 3 (range 10 to 95%), and 35% for serovar 9 (range 5 to 100%) (*Maes et al., 2002*). In the first report of the presence of *A. pleuropneumoniae* in Turkey, 258 out of 384 blood samples (67 %) were positive (*Metiner,2007*). Shi (*Shi et all, 2012*) reports of 55.72% seropositive of the Tibetan pigs. The prevalence ranged from 42.68-71.11%. This agrees with Assavacheep (*Assavacheep et al., 2003*), who had reported that 60% pigs were seropositive to at least one serotype and 45% of the pigs were seropositive to more than one serotype**.**

The successful control of porcine pleuropneumonia depends on the efficiency of preventing intra- and inter-farm transmission of the infection. One of the ways to successfully control and eliminate the porcine pleuropneumonia is a timely and fast diagnostic procedure with the implementation of immunodiagnostic tests (*Žutić et al., 2008).*

Serological diagnostics of infections caused by *A.pleuropneumoniae* is essential measure for the identification of latently infected herds and the determination of multiplicity of serotypes within the herd. The serological control

of gilts is of particular importance to detect infected animal prior to fertilization, because the mothers can transmit the infection to their offspring at birth.

Conclusions

It is very important to identify the presence of individual agents in the etiopathogenesis of respiratory disease of pigs in a large agglomerations. Based on our results, ELISA is a reliable method for serological diagnosis of porcine pleuropneumonia. This method is specific and may be used for the routine surveillance of health status in pig herds.

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Seroprevalencija *Actinobacillus pleuropneumoniae* kod svinja poreklom sa komercijalnih farmi u Srbiji

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Rezime

Pleuropneumonija svinja jedna je od najznačajnijih respiratornih bolesti svinja koja širom sveta dovodi do gubitaka, posebno u intenzivnoj svinjarskoj proizvodnji. Uzročnik bolesti je *A.pleuropneumoniae*.Prema korištenju β-nikotinamid adenin dinukleotida (β-NAD) za rast, uzročnik je podeljen na biovar 1 (β-NAD ovisan) i biovar 2 (β-NAD neovisan). Svaki biovar je dalje podeljen na serotipove. Do sada je otkriveno 15 različitih serotipova, među kojima su ustanovljene znatne razlike u virulenciji ali isto tako i varijacije u virulenciji između sojeva istog serotipa.Glavne faktore virulencije predstavljaju toksini.Sojevi A.pleuropneumoniae stvaraju četiri tipa egzotoksina označenih ApxI, ApxII, ApxIII i ApxIV. Za razliku od ostalih, ApxIV toksin proizvode svi serotipovi i visoko je specifičan za *A.pleuropneumoniae*.Bolest se klinički manifestuje kašljem, teškim disanjem i visokom temperaturom, a patoanatomski hemoragično-nekrotičnim promenama na plućima.U brojnim je istraživanjima dokazano da ovaj patogen često učestvuje u interakcijama bilo sa bakterijskim ili virusnim patogenima kao što su Mycoplasma hyopneumoniae, PRRSV i PCV2.Glavni put širenja je direktni kontakt među svinjama ili aerosolom na kratkoj distanci kao i mogućnost prenosa uzročnika sa majki na prasad.U cilju praćenja pojave i kontrole pleuropneumonije, najčešće se koriste serološke metode koje otkrivaju prisustvo

antitela za ApxIV toksin *A.pleuropneumoniae*.Cilj je ovog rada bio da se ispitaju krvni serumi nazimica i krmača poreklom sa 4 svinjarske farme u Srbiji,na prisustvo specifičnih antitela za *A.pleuropneumoniae*. U ispitivanju je, za otkrivanje antitela korišten komercijalni ELISA kit (IDEXX APP-ApxIV Ab Test). Ukupno je serološki pregledano 453 uzorka krvnih seruma, i to 207 uzoraka od nazimica i 246 uzoraka od krmača. Antitela za *A.pleuropneumoniae* ustanovljena su u 57 (12,58%) od ukupno 453 pregledanih seruma životinja. Kod nazimica, antitela su ustanovljena u 22 (10,62%) a kod krmača u 35(14,22%) seruma.Procenat pozitivnih seruma životinja bio je različit među farmama i kretao se kod nazimica od 3,33-17,77 % a kod krmača 8,95-22,64%. U dijagnostici pleuropneumonije svinja, serološke metode predstavljaju jedan od najznačajnijih postupaka, posebno pogodnih za kontrolu zdravstvenog stanja životinja u velikim aglomeracijama, pri čemu je moguće otkriti i latentno inficirana grla.

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