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Short communication

SEROPREVALENCE OF *ACTINOBACILLUS PLEUROPNEUMONIAE* INFECTION IN PIGS FROM BULGARIA

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Summary

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Actinobacillus pleuropneumoniae (*App*) is the etiological agent of porcine pleuropneumonia. The purpose of the study was to present a serological report on *App* prevalence among pigs in industrial farms in Bulgaria. Seventy-two pigs from four industrial farms in four districts of Bulgaria – Eastern Bulgaria (Razgrad and Yambol districts) and Western Bulgaria (Lovech and Sofia districts) were included. Animals were divided in two age groups: weaners and fattening pigs. A commercial enzyme-linked immunosorbent assay (ELISA, INgezim APP MIX, Eurofins Ingenasa, Madrid, Spain) for the detection of antibodies against *App* parasuis in porcine serum was used. Microtitre plate was coated with *App* antigen of the serovars 1, 2, 9 and 11. Positive results for anti-*App* antibodies were detected in 32 (44.4%) of all 72 tested sera. The overall seropositivity in weaners and fattening pigs was 22.2% (8/36), and 66.7% (24/36), respectively. The highest *App* seropositivity in pigs was found in Eastern Bulgaria – 61.1% (22/36; P<0.001) in comparison to *App* seropositivity in Western Bulgaria – 27.8% (10/36; P=0.137). This study on anti-*App* prevalence among pigs in Bulgaria gives new insights on *App* epidemiology in our country.

Key words: *Actinobacillus pleuropneumoniae*, Bulgaria, pigs, seroprevalence

Actinobacillus pleuropneumoniae (*App*) is the etiological agent of porcine pleuropneumonia. *App* was originally isolated in 1957 in the United Kingdom and first named *Haemophilus pleuropneumoniae* (Pattison *et al.*, 1957; Shope, 1964). Twenty-six years later it was assigned to the *Pasteurellaceae* family and *Actinoba-*

cillus genus (Pohl *et al.*, 1983). So far, eighteen serovars of *App* are known, differing in their capsular polysaccharide composition, with serovars 16, 17 and 18 identified only recently (Sarkozi *et al.*, 2015; Bosse *et al.*, 2017; 2018a;b). Depending on their requirement for nicotinamide adenine dinucleotide (NAD) to grow, *App* strains can be further classified as biovar 1 (also called "typical") that are NAD-dependent, and biovar 2 (or "atypical") that are NAD-independent (Sassu *et al.,* 2018).

The aim of the study was to present a serological report of *Actinobacillus pleuropneumoniae* infection among pigs in industrial farms in Bulgaria.

Seventy-two pigs (n=72) from four industrial farms in four districts of Bulgaria – Eastern Bulgaria (Razgrad and Yambol districts) and Western Bulgaria (Lovech and Sofia districts) were used in this study. Animals were divided in two age groups: weaners (aged between 1 and 3 months) and fattening pigs (between 4 and 6 months of age). All parameters of the microclimate (ventilation, lighting, temperature) in animal premises were in compliance with Good Management Practices for a modern industrial pig farm. The study was conducted from 1 October to 30 December 2018. Sampling was performed randomly from pigs with clinical signs (fever, cough, asthenia) in the relevant age groups on the farm; unfortunately sex had not been recorded. The investigated farms were located among plains (approximately 42°48'N and 42°69'N latitude, 23°32'E and 26°51'E longitude), and the climate was subtropical/continental (mean annual temperature 13–15 ºC, precipitations about 650 mm/m^2).

Swine blood samples (up to 5 mL per individual) were taken by puncture of the *sinus ophthalmicus*. The farms were far from a diagnostic laboratory, therefore the samples were transported and stored in an electric transport cooler box (220 V/24 V) maintaining a temperature of 4–8 ^{o}C. Blood collection tubes without anticoagulant were kept at room temperature (20 ^{o}C) until visible clot retraction, centrifuged at 1500×*g* for 10 minutes, and the sera were separated and kept at –20 ^{o}C until processing.

The serum samples were tested for *App* antibodies in National Diagnostic and Research Veterinary Medical Institute, Sofia, Bulgaria. A commercial enzyme-linked immunosorbent assay (ELISA, INgezim APP MIX, Eurofins Ingenasa, Madrid, Spain) was used. The INgezim APP MIX was an immunoenzymatic assay for the detection of antibodies against *App* parasuis in porcine serum. Microtitre plate was coated with *App* antigen of the serovars 1, 2, 9 and 11. The test had 99.37% sensitivity and 87.24% specificity at the serovar 1, and 99.29% sensitivity and 87.24% specificity at the serovar 2. The mean optical density (OD) values obtained for the *App* 1, 9, 11 positive controls (mean OD *App* 1, 9, 11), the *App* 2 positive control (mean OD *App* 2), the negative control and each sample (mean OD) were calculated. Then, the arithmetic mean of mean OD *App* 1, 9, 11 and mean OD *App* 2 was calculated (mean OD *App* 1, 9, 11, 2). To obtain the ratio, mean individual sample OD was divided by mean OD *App* 1, 9, 11, 2. Results were interpreted as positive at ratio ≥ 0.50, as negative: at ratio < 0.33 and as doubtful: at $0.33 \leq$ ratio < 0.50.

The study was approved by the Local Ethics Committee at University of Forestry, Sofia, Bulgaria and was conducted according to the ethical principles of animal experimentation, adopted by the Bul-

garian Ministry of Agriculture, Food and Forestry.

App positive results among different swine age groups and parts of the country were compared by the Chi-square test. Binary logistic regression analysis was used to evaluate the risk of positive results according to age group. Statistical analysis has been performed by Excel 2007 (Microsoft, USA) and SPSS Statistics 19.0 (IBM Corp., USA). A P-value < 0.05 was considered statistically significant.

Positive results for anti-*App* antibodies were detected in 32 (44.4%) of all 72 tested sera (Table 1). The overall seropositivity in weaners and fattening pigs was 22.2% (8/36), and 66.7% (24/36), respectively. A higher *App* seropositivity in pigs was found in Eastern Bulgaria (61.1%; 22/36; P<0.001) in comparison to *App* seropositivity in Western Bulgaria – 27.8% (10/36; P=0.137). The Chi-square test showed significant differences in *App* seropositivity between the age groups and parts of the country (Table 1). Six percent of all samples were assessed as doubtful.

To estimate the risk for *App* seropositivity, the odds ratio (OR) in different age groups was evaluated by binary logistic regression. The OR of anti-*App* antibodies occurrence in fattening pigs was compared to the group of weaners (Table 2) and it was found out that the risk from *App* infection was 7 times higher in fattening pigs than in the group of weaners.

The results of present study showed that *App* infection was very common in industrial farms in Bulgaria. In some countries as Republic of Korea, Serbia and Czech Republic, low level of occurrence of *App* infection was reported. Lee *et al.* (2015) found 12.2% (54/443) *App* seropositivity rate in Korean pigs. They reported a marked disproportion for *App* infection with serovar 1 and serovar 2: 27.8% (15/54) and 7.4% (4/54), respec-

Table 1. Seroprevalence of *Actinobacillus pleuropneumoniae* infection by age groups in pigs from Bulgaria

Age groups	*App* positive, n (%)	Chi-square	Df	P value
Weaners (n=18)	5 (27.8)	16.83	1	<0.001
Fattening pigs (n=18)	17 (94.4)			
Weaners (n=18)	3 (16.7)	2.22	1	0.137
Fattening pigs (n=18)	7 (38.9)			

App – Actinobacillus pleuropneumoniae; df –degrees of freedom.

Table 2. Logistic regression analysis showing the relationship between *Actinobacillus pleuropneumoniae* positive pigs and age group

Age groups	*App* positive, n (%)	PE	SE	P value	OR	95% CI
Weaners (n=36)	8 (22.2)	NA	NA	NA	1.00	NA
Fattening pigs (n=36)	24 (66.7)	1.95	0.54	<0.001	7.00	2.46–19.96

App – Actinobacillus pleuropneumoniae; PE – parameter estimate; SE – standard error; OR – odds ratio; CI – confidence interval; NA – not applicable.

tively. Zutic *et al.* (2014) declared an overall *App* seropositivity of 12.58% (57/453) in pigs from Serbia. Kucerova *et al.* (2005) notified 18.5% (47/254) positive samples for serovar 2 and 4.7% (12/254) for serovar 11 in the Czech Republic.

Countries from Western Europe communicated a high prevalence of *App* infection among pigs. The incidence of *App* infection in Spain was 89%, in Northwestern Germany – 90%, Belgium – 96%, and Italy – 100% (Krejci & Newberry, 2011). High seropositivity was found in Turkey and China. Metiner & Ak (2007) reported 67.2% (258/384) *App* seroprevalence in 11 farms in Turkey. The highest seropositivity was found out in farms Istanbul–2 (83.3%; 25/30), Tekirdag Corlu–2 (76.2%; 16/21) and Izmir (75.0%; 27/36). In Tibetan pigs, *App* seropositive animals were 55.72% (185/332) (Shi *et al.*, 2012) with 52.02% *App* prevalence in Nyingchi county and 59.75% in Mainling county.

Different serovars of *App* infection were identified in different countries. In Hungary serotype 2 (39.5%; 36/91), serotype 13 (15.4%; 14/91), serotype 8 (8.8%; 8/91) and serotype 16 (8.8%; 8/91) were predominant (Sarkozi *et al.*, 2018). O'Neill *et al.* (2010) reported serovar 8 (78.0%; 295/378), serovar 6 (10.1%; 38/378) and serovar 7 (5.3%; 20/378) as dominant in England and Wales. In a recent study from 2016, Li *et al.* (2016) confirmed that the most common *App* serovars in England and Wales were serovar 8 (71.7%; 81/113), serovar 7 (8.0%; 9/113) and serovar 6 (7.1%; 8/113). Serotype 2 was acknowledged to predominate in many countries in Europe (Dubreuil *et al.*, 2000; Chiers *et al.*, 2002; Stark *et al.*, 2007; Maldonado *et al.*, 2009), that's why we suppose that it could be prevalent in Bulgaria.

This study has some limitations that need to be addressed. Anti-*App* IgG and IgM seroprevalence is crucial in assessing past and recent infection. However, RT-PCR is required to establish an active infection. In addition, the study included a relative small number of pigs. Despite these limitations, this study on anti-*App* prevalence among pigs in Bulgaria gave new insights into *App* epidemiology in our country.

In conclusion, our results showed a high prevalence of *App* infection (44.4%) in swine. Higher prevalence was found in fattening pigs (66.7%). These results emphasised the fact that this infection was a serious health problem in veterinary practice in Bulgaria.

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