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Full Research Article

SEROLOGICAL PROFILE AND PLEURISY LESIONS ASSOCIATED WITH *ACTINOBACILLUS PLEUROPNEUMONIAE* IN PIG FARMS IN NORTH MACEDONIA

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Abstract

Actinobacillus pleuropneumoniae (*App*) is one of the most important swine respiratory pathogens that causes porcine pleuropneumonia and massive financial losses in pig industry. The objective of this study was to investigate App infection in five pig herds from North Macedonia experiencing clinical respiratory infections by serological testing and a slaughterhouse pleurisy evaluation system (SPES). In total, 250 blood samples were taken from pigs aged 6, 10, 14, 18, and 22 weeks. Ten animals per age category from each farm were sampled and analyzed for presence of antibodies against *App*. At the slaughterhouse, 50 lungs per herd from slaughtered age pigs were scored by the SPES for the presence of lesions associated with chronic pleurisy. The overall seroprevalence to *App* was 65.6%, ranging from 34% to 98% at the farm level. The highest seroprevalence was found in 6-week-old pigs in all farms, while significant differences were observed between farms in the 10-, 14-, 18-, and 22-week-old pig

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groups. Pleurisy associated with *App* was found in 26.4% of all examined lungs, with the mean SPES score being 0.75 (0.14 - 1.10). The percentage of SPES scores of 0, 1, 2, 3, and 4 in all lungs were 73.4%, 1.6%, 8.8%, 8.4%, and 7.6% respectively. A significant difference in mean SPES score was obtained between two farms. High seroprevalences of *App* detected on the tested farms were probably due to constant natural infection. The highest seroprevalences, measured in the youngest pigs, could be due to maternally-derived antibodies. Higher seroprevalence against *App* and lower SPES scores in some farms suggests immunity resulting from infection by corresponding field serotypes.

Keywords: *Actinobacillus pleuropneumoniae,* lung, seroprevalence, slaughterhouse, SPES

INTRODUCTION

Actinobacillus pleuropneumoniae is a primary etiological agent of porcine pleuropneumonia (Galdeano et al., 2019; Gottschalk and Broes, 2019; Kim et al., 2016; Merialdi et al., 2012). It is one of the most important swine respiratory bacteria found worldwide, associated with enormous losses due to increased mortality and treatment and decreased growth and conversion rate (Gottschalk and Broes, 2019; Fraile et al., 2010). The most virulent strains often induce severe dyspnea, blood-tinged discharge from nostrils and mouth, and severe fibrinohaemorrhagic pleuropneumonia in pigs of all age categories (Sipos et al., 2021; Gottschalk and Broes, 2019). Additionally, *App* can act as a secondary bacterial pathogen, contributing to the etiology of porcine respiratory disease complex (PRDC), and it is primarily responsible for pleural lesions in finishing pigs (Yoeger and VanAlstine, 2019; Fraile et al., 2010). Dorsocaudal pleurisy over the diaphragmatic lung lobes is most suggestive of previous *App* pleuropneumonia (Rodrigues da Costa et al., 2020; Merialdi et al., 2012; Fraile et al., 2010).

Lung inspection of fattening pigs at the slaughterhouse is an excellent approach to quantify the presence and severity of pulmonary lesions. Several pleurisy-scoring systems are reported in the literature, among which the slaughterhouse pleurisy evaluation system (SPES) is widely used for evaluating pleurisy caused by *App* (Dottori et al., 2007). Lungs from a minimum of 30 fattening pigs should be examined at slaughter to obtain a reliable picture of respiratory infections at farm level (Fraile et al., 2010). Although, lung inspection at slaughter is mostly associated with detection of chronic pleural lesions, it can give valuable data on the presence of subclinical respiratory infections during the finishing phase (Yoeger and VanAlstine, 2019).

Serological testing is commonly applied for tracking diseases in pigs since it is faster and cost-effective compared to other diagnostic procedures (Fitzgerald et al., 2020). In serology, enzyme-linked immunosorbent assay (ELISA) is one of the most suitable and very sensitive tests (Calsamiglia et al., 1997). However, ELISA results must be interpreted cautiously, since antibodies detected could be due to vaccination or field infection, or be maternally-derived antibodies. For these reasons, serology tests are usually applied in populations with known vaccination status. Regarding *App* infection in pigs, herd seroprevalence is positively correlated with the severity of chronic pleurisy (Rodrigues da Costa et al., 2020; Meyns et al., 2011). Thus, lung inspection at slaughter

and serology screening of different pig categories can provide helpful data regarding respiratory disease status in pig herds.

The aim of this study was to evaluate, by ELISA testing and SPES evaluation at slaughter, clinical App infections in five commercial pig farms from North Macedonia with histories of clinical pneumonia.

MATERIALS AND METHODS

Farms

This survey was carried out from September 2018 to April 2019 in five farrow-to-finish commercial pig farms in North Macedonia. The farms selected (A, B, C, D, and E) showed clinical signs of pneumonia:

- Farm A was a herd with 80 breeding sows with history of enzootic pneumonia seen in pigs at 16 weeks of age. The farm was positive for porcine reproductive and respiratory syndrome virus (PRRSV), and neither medication nor vaccination were applied.
- Farm B was a herd with 150 sows and severe clinical pneumonia was seen in pigs at 10 weeks of age. PRRSV and *App* were laboratory-confirmed and no vaccination was conducted. Weaned pigs were medicated via feed with a combination of sulfamethoxazole-trimethoprim (400 ppm) and tiamulin (150 ppm) for 10 days in pre-starter feed and 7 more days in starter feed.
- Farm C held 170 sows. Nursery pigs (20 to 30% of animals) at around 8 weeks of age showed poor growth rate, severe dyspnea, cyanosis, and dehydration. The farm was positive for PRRSV, and vaccination was not performed. Medication protocols were not used.
- Farm D held 170 sows in a breeding herd with severe pneumonia seen in weaned pigs at 9 weeks of age. Respiratory clinical signs continued in grower pigs until 14 weeks of age. This farm was seropositive for PRRSV in all pig categories, and no medication was used.
- Farm E was a herd with125 breeding sows. Pigs aged 16 to18 weeks (10 to 20% of animals) showed dry and nonproductive cough in the fattening stables. The farm was positive for PRRSV, and vaccination was not implemented. Medication and vaccination were not used.

A one-site pig production system was implemented by all farms included in this study, while all-in-all-out (AIAO) practices were managed only at the pen level in nursery and fattening units.

Samples and serology

During a single farm visit, blood samples were collected from 250 pigs. From each farm, 50 pigs from different age groups (6, 10, 14, 18, and 22 weeks) were randomly selected and sampled. From each age category, 10 animals were restrained and bled

via puncture of the external jugular vein by using 19-gauge needles and vacuum blood tubes for serum retrieval. Serum samples were analyzed for the presence of specific *App* antibodies. For serological testing, we used commercial ELISA kit IDEXX (Westbrook, ME, USA) APP-ApxIV Ab Test (97.8% sensitivity, 100% specificity) with differentiation between infected and vaccinated animals based on the manufacturer's recommendations. The antibody level in each serum sample was determined by calculating the sample to positive (S/P) ratio. Samples were considered positive when cut-off values were ≥ 0.5.

Assessment of lung lesions

At the abattoir, 250 lungs (50 lungs from each farm) from market weight pigs were scored and examined for pleural lesions. The slaughterhouse pleurisy evaluation system (Dottori et al., 2007) was used to evaluate lungs and the presence of pleurisy. According to SPES, lungs were scored with five different grades (0-4) depending on the severity and location of the lesions (Table 1).

Table 1. Pleurisy scoring by the slaughter pleurisy evaluation system (SPES)

Score	Lesion description
0	Absence of lesions
1	Ventrocranial lesion: pleural adherence between lobes or at ventral border of lobes
2	Unilateral focal lesion of the diaphragmatic lobe
3	Bilateral type 2 lesion or extended monolateral lesion (at least 1/3 of a diaphragmatic lobe).
4	Severely extended bilateral lesion of both diaphragmatic lobes.

Statistical analysis

For statistical analyses, we used STATISTICA software (version 8.0; StatSoft, Inc). To find differences in the frequency of seropositive pigs between the same age categories among herds, Chi square-test and the Fisher's exact test were used. For the data obtained from lung examination at the slaughterhouse, descriptive statistics were applied. A non-parametric Kruskal-Wallis test was performed to find statistical differences among SPES scores between each farm. Significant differences were considered at $P<0.05$.

RESULTS

Serology

All farms held pigs that were seropositive against *App*, with antibodies detected in 164 (65.6%) of the 250 tested sera. The highest seroprevalence among all pig age groups was noted in 6-week-old pigs on all farms. Seropositivity results of animals per age group are shown in Table 2. The overall seroprevalence in farms A, B, C, D, and E was 94% (47/50), 58% (29/50), 44% (22/50), 98% (49/50), and 34% (17/50), respectively.

The frequency of pigs seropositive against *App* between age categories in each farm is shown in Figure 1. Significant differences in the prevalences of seropositive pigs among farms were observed in 10-, 14-, 18-, and 22-week-old groups (Figure 2). On farm E, no seropositive pigs were detected at 14, 18, or 22 weeks of age. On farm B, low prevalences of seropositive pigs were observed at 14 and 18 weeks of age, but the seroprevalence of 22-weeks-old pigs was higher. Farms A and D had higher prevalences of seropositive pigs in the 14- to 22-week-old groups in contrast to the same age groups on other farms.

Table 2. Number and percentage of pigs that were seropositive against *Actinobacillus pleuropneumoniae* as measured by ELISA and according to pig age group in all farms

Age	App	Total of pigs tested
6 weeks	50 (100%)	50
10 weeks	39 (78%)	50
14 weeks	23 (46%)	50
18 weeks	23 (46%)	50
22 weeks	29 (58%)	50

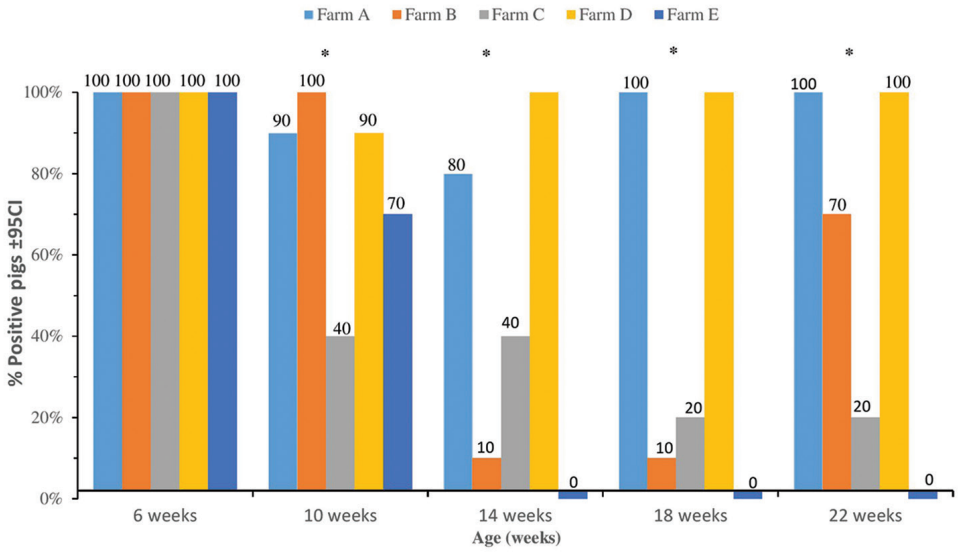

Figure 1. Seroprevalence to *Actinobacillus pleuropneumoniae* by age groups on five pig farms. The P values were obtained using Chi square-test. Differences in 10 weeks pigs (P = 0.007) and 14-, 18- and 22-weeks pigs (P<0.001) are indicated with *.

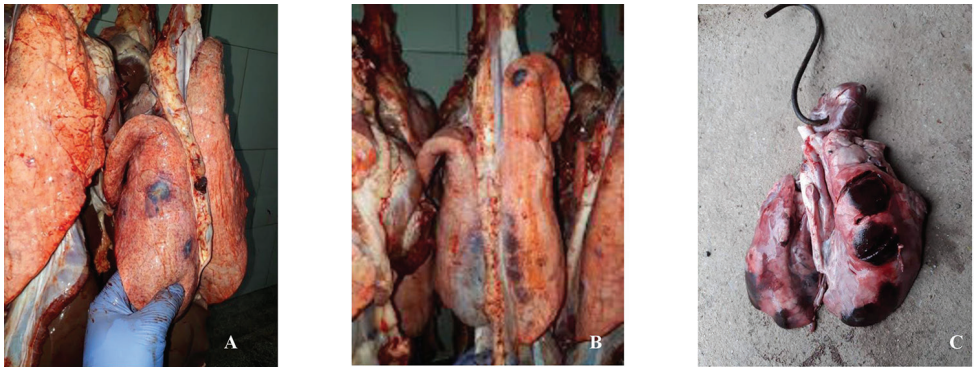

Figure 2. Pig lungs: (A) Unilateral focal lesion of the diaphragmatic lobe (slaughterhouse pleurisy evaluation system (SPES) score 2); (B) Bilateral type 2 lesion or extended monolateral lesion (SPES score 3); (C) Severely extended bilateral lesion of both diaphragmatic lobes (SPES score 4).

Lung assessment

From all lungs examined at the slaughterhouse, 26.4% (66/250) presented macroscopic changes associated with chronic pleurisy. The mean SPES score for all farms was 0.75 (0.14 - 1.10), while the percentage of pigs with pleural scores of 0, 1, 2, 3, and 4 were 73.4%, 1.6%, 8.8%, 8.4%, and 7.6% respectively. Examples of lungs examined in this research with SPES scores 2, 3, and 4 are presented in Figure 2. The SPES scores for different farms are summarized in Table 3. The highest percentage of healthy lungs was observed in farm D (96%), while the highest prevalence of lungs with pleurisy lesions was found in farm C (40%; 20/50) (Table 3). The highest percentage of lungs with the worst SPES score of 4 was noted in farm E (18%), whilst farm D had significantly lower SPES scores than the scores found in farm C ($P<0.001$) (Figure 3). SPES scores did not differ between other farms in the study.

Table 3. Percentage of tested lungs with slaughter pleurisy evaluation system scores of 0, 1, 2, 3, and 4 for all farms

Farm	Score 0 (%)	Score 1 (%)	Score 2 (%)	Score 3 (%)	Score 4 (%)
A (n = 50)	36 (72)	2 (4)	4 (8)	5 (10)	3 (6)
B (n = 50)	38 (76)	0 (0)	4 (8)	6 (12)	2 (4)
C (n = 50)	30 (60)	1 (2)	9 (18)	6 (12)	4 (8)
D (n = 50)	48 (96)	0 (0)	0 (0)	1 (2)	1 (2)
E (n = 50)	33 (66)	1 (2)	4 (8)	3 (6)	9 (18)

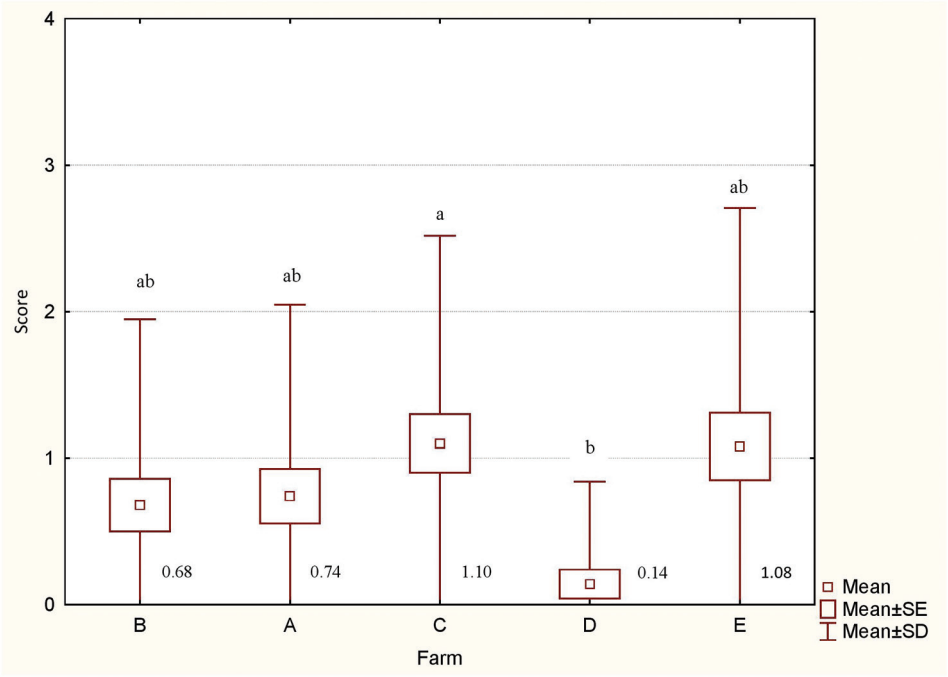

Figure 3. Mean slaughter pleurisy evaluation system (SPES) scores within farms. The P value for the scores between each farm was obtained using Kruskal-Walli's test. Farms with different small letters are significantly different (P<0.001).

DISCUSSION

In this study, five pig farms from North Macedonia exhibiting respiratory infection were assessed for *App* infection by using ELISA and pleurisy scoring by SPES. According to the best of the authors' knowledge, this is the first survey evaluating *App* infections in commercial pig farms from North Macedonia.

For serology analysis, we used a commercial APP-ApxIV antibody test, which detects only antibodies produced by *App* infection. Although the sample size for serological testing in this research is not sufficient for determining prevalence of disease, nevertheless, the dynamics of *App* infection in herds was evaluated. The commercial antibody test used is highly sensitive, although it has no ability to detect antibodies produced by specific serotypes (Giménez-Lirola et al., 2004). The high seroprevalence of 65.6% that we found in our study is in line with other authors. Similar results were obtained in Belgian pig herds in the research of Meyns et al. (2011) where 63% of samples were seropositive against *App*. Rodrigues da Costa et al. (2020) also found quite a high percentage of *App* seropositive pigs (76%) in their survey conducted in 56 Irish pig farms. On the other hand, our result for overall seroprevalence disagrees with findings reported by other researchers. Baraldi et al. (2019) reported low *App*

seroprevalence of 18.7%, while Pepovich *et al.* (2022) obtained 44.4% *App* seropositive samples taken from industrial pig farms in Bulgaria.

In our study, we found high percentages of seropositive animals on all farms at 6 weeks (100%) of age followed by 10 weeks (78%) old nursery pigs. This high percentage conforms to the result of Baraldi et al. (2019), who observed high seropositivity in the nursery phase in contrast to finishing pigs (58% vs. 7.1%). This finding is most probably due to maternally-derived antibodies that originated from immune sows, and which circulate in the piglets for around the first 5 to 12 weeks of life (Vigre et al., 2003). The high seropositivity found in farms A and D over time is most likely due to constant infections occurring during the growing and finishing stages. On the contrary, in farm B, the high percentage of positive animals detected in the 22-week-old group indicates late infection during the fattening period. Natural infection stimulates the immune system to produce antibodies, starting at 10 to 14 days post-infection and reaching the highest level at 4 to 6 weeks after infection (Desrosiers, 2004). Serologically negative pigs identified in farm E and the low percentage of positive animals in farm C at 14, 18, and 22 weeks of age could be associated with certain strains that do not induce antibody production (Tegetmeyer et al., 2008). In addition, good management practices including appropriate environmental temperatures, appropriate seasonal ventilation, and appropriate stocking density minimizes *App* outbreaks in infected farms (Yoeger and VanAlstine., 2019). According to Tegetmeyer et al. 2008, some *App* strains in their genome have insertion sequences that abolish production of ApxIV toxin and do not induce an immune response. Moreover, interpretation of the ApxIV serological test should be interpreted with caution, since subclinically infected herds with carrier pigs may not induce high levels of antibody against *App* toxins (Chiers et al., 2002).

In our study, the low overall percentage of lungs affected by *App-like* lesions was very similar to findings reported by Fraile et al. (2010), where pleurisy lesions (SPES $\geq$ 1) were found in 26.8% of all examined lungs. On the contrary, lower prevalences of pleurisy (SPES score $\geq$ 1) from 6.7% to 15% were reported by Rodrigues da Costa et al. (2020), Baraldi et al. (2019), Fablet et al. (2012) and Galdeano et al. (2019). However, the prevalence of pleurisy in our study (26.4%) was much lower in contrast to the findings of other authors. Meyns et al. (2011) in their research reported 62.3% lungs with pleurisy lesions, while Di Provvido et al. (2019) detected 50.46% of lungs with pleurisy. Additionally, Merialdi et al. (2012) and Liao et al. (2017) in their studies documented a higher percentage of lungs with chronic pleurisy of 47.5% and 37.6% respectively. This variability in the prevalence of pleural lesions among studies could be related to many factors, such as virulence of the strain and coinfection with other agents like *Mycoplasma hyopneumoniae*, PRRSV, pseudorabies virus, and swine influenza virus (Rodrigues da Costa et al., 2020; Yoeger and VanAlstine, 2019; Merialdi et al., 2012). Influenza virus was the main pathogen related to pleurisy in pigs in the survey of Rodrigues da Costa et al. (2020). Moreover, Merialdi et al. (2012) found a higher proportion of lungs with cranioventral pleurisy in farms that were also seropositive for pseudorabies virus.

The highest mean SPES scores in our study were obtained for farm C and farm E. These two farms had the lowest seroprevalence in 22-week-old pigs (slaughter weight), which disagrees with the findings reported by other authors who found strong positive associations between seropositive animals and pleurisy lesions in slaughter-aged pigs (Baraldi et al., 2019; Liao et al., 2017; Merialdi et al., 2012; Meyns et al., 2011). On the other hand, our results for these two farms are consistent with the survey conducted in Goiás State, Brazil, in which the highest prevalence of pleurisy lesions (50%) and very low seroprevalence to ApxIV toxin in slaughter-aged pigs (3.3%) were noted on one pig farm (Galdeano et al., 2019). Low seroprevalence to ApxIV antibodies in slaughter-aged pigs and a high level of pleurisy could be associated with infection induced by some *App* strains that do not produce antibodies against this toxin (Tegetmeyer et al. 2008). In addition, pleural lesions in pigs also can be frequently related to dissemination by the hematogenous route to the serosal surfaces of bacteria, such as *Haemophilus parasuis, Streptococcus suis*, or *Mycoplasma hyorhinis*, or could be related to an extension of underlying pneumonia or abscess (Yoeger and VanAlstine., 2019). However, the severity and type of pleurisy lesions in fattening pigs are the result of complex and dynamic interactions between multiple infectious agents and mainly are associated with co-infection of *App* and *Pasteurella multocida* (Meyns et al., 2011). Factors including crowding and rapid variation in environmental conditions, such as temperature, humidity, and poor ventilation, also promote development of *App* infection (Fraile et al., 2010).

CONCLUSION

Our results showed high seroprevalences of *App* infection on the tested farms. Higher seroprevalences in the youngest pigs at 6 weeks of age indicates natural infection of sows, which passively have transferred antibodies to their offspring. Herds with a high percentage of pigs seropositive against *App* but with low SPES scores suggest immunity has developed from infection of corresponding farm serotypes. Nevertheless, the limitation of our research is that it includes only a small number of farms and samples and so cannot demonstrate the real situation regarding *App* infections. Additional studies with higher numbers of samples should be conducted to reveal more comprehensive data on these pulmonary infections in commercial pig farms.

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Ethical Statement

The farm owners gave written consent for the study, and the blood sampling procedures performed complied with the Law of North Macedonia on the protection and welfare of animals, which is in accordance with the European Directive (DIRECTIVE 2010/63/EU) on the protection of animals used for scientific purposes. The samples were part of the regular serological survey within the Annual Animal Health Order 2019 (No.02-20/1, 03.01.2019) issued by Food and Veterinary Agency, North Macedonia.

Authors' contributions

BA conceived the study, conducted the pleurisy evaluation at slaughterhouse and wrote the manuscript. AJ collected samples and contributed to manuscript writing. CMO revised the manuscript. EAP participated in data analysis and took part in the writing of the manuscript. JB was involved in data analyses and critically reviewed the final version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

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SEROLOŠKI PROFIL I PRISUSTVO PLEURITISA UDRUŽENOG SA *ACTINOBACILLUS PLEUROPNEUMONIAE* NA FARMAMA SVINJA U SEVERNOJ MAKEDONIJI

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Kratak sadržaj

Actinobacillus pleuropneumoniae (App) je jedan od glavnih respiratornih patogena svinja koji izaziva pleuropneumoniju. Nanosi velike ekonmoske gubitke u industrijskoj proizvodnji svinja širom sveta. Cilj ovog rada bio je da se serološkim testiranjem i sistemom za procenu pleuritisa (SPES) na liniji klanja ispita prisusustvo *App* infekcija na pet makedonski farmi svinja na kojima su bile prisutne respiratorne infecije. Na farmama su uzetiti uzorci krvi svinja 6, 10, 14, 18, i 22 nedelje starosti. Deset životinja svake starosne grupe su uzorkovani itesturani za prisitvo *App* antitela. Na liniji klanja ukupno 50 uzoraka pluća je pregledano na prisustvo lezija udruženi sa hroničnim pleuritisom. Korišćen je sistem za procenu pleuritisa(SPES). Svi prelgedani uzorci pluća su poticali od svinja sa farmi na kojima su bile prisutne respiratorne infekcije. Ukupna seroprevalencija na *App* bila je 65,6% u rasponu od 34% do 98% na nivou farme. Najveća seroprevalenca je utvrđena kod svinja starih 6 nedelja na svim farmama, dok je značajnost otkrivena između farmi kod svinja od 10, 14, 18 i 22 nedelje. Pleuritis udružen sa App pronađen je u 26,4% svih pregledanih pluća sa srednjim SPES skorom od 0,75 (0,14 - 1,10). Procenat SPES rezultata od 0, 1, 2, 3 i 4 u svim plućima bio je 73,4%, 1,6%, 8,8%, 8,4% i 7,6%. Visoka seroprevalencija koja je ustanovljena verovatno je posledica stalne prirodne infeklcije. Najveća seroprevalenca kod najmlađih kategorioja svinja bila je povezana sa antitelima dobijenim od majke. Veća seroprevalencija na *App* na nekim farmama je rezultat infekcije odgovarajućim serotipovima na terenu.

Ključne reči: *Actinobacillus pleuropneumoniae,* linija klanja, pluća, seroprevalencija, SPES