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POTENTIAL BENEFITS OF SERUM PROFILING FOR RESPIRATORY DISEASE CONTROL

IRENA GOLINAR OVEN[1]

SUMMARY: On one Slovenian large pig farm 36 litters were selected from a herd to make serum profiles to selected (important) respiratory pathogens for preparation of specific control measures. 36 serum samples from breeding sows and 342 serum samples from pigs (38 serum samples; 9 samplings) were tested for antibodies to Porcine Circovirus Type 2 (PCV2), Swine Influenza Virus (SIV), Mycoplasma hyopneumoniae, Actinobacillus pleuropneumoniae and Haemophilus parasuis. The same pigs were bled at 2, 4, 6, 8, 11, 14, 17, 22 and 28 weeks of age. Commercial ELISA kits of different producers were used. In breeding sows seroprevalence to SIV, A. pleuropneumoniae was 100 %, to PVC2 94 %, to M. hyopneumoniae 83,3 % and to H. parasuis 36 %. Colostral antibodies in pigs against SIV and PCV2 persisted for about 4 weeks. The lowest seroprevalence was detected in 6 weeks old pigs against both viruses. According to serum profiles vaccination of sows against SIV and PCV2 can be proposed. Colostral antibodies against A. pleuropneumoniae persisted for almost 8 weeks (94,8 % prevalence). The lowest prevalence was detected in 14 weeks old pigs (53,8 %). According to serum profiles vaccination against A. pleuropneumoniae around 11-14 weeks of age can be proposed. Till 8 weeks of age pigs were seronegative against H. parasuis. The seroprevalence increased at 11 weeks of age. Vaccination against H. parasuis around 8-9 weeks of age can be proposed. The seroprevalence against M. hyopneumoniae at 6 weeks of age decreased to 0 % and at 11 weeks of age started to increase. Second vaccination between 11.-14. weeks of age can be proposed.

Key words: *pig, respiratory diseases, serum profile, control measure.*

INTRODUCTION

Respiratory infections occur with a high prevalence in all swine-producing areas (Sörensen et al., 2006). The economic impact of respiratory diseases is considerable,

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mainly due to reduced growth and feed efficiency (Stärk, 2000).

Clinically significant disease is seldom the result of an infection with one pathogen. Several pathogens are very often involved in respiratory diseases (Sörensen et al., 2006). Combinations of bacteria and viruses work synergistic in producing more severe respiratory diseases than those induced by each individual agent (Choi et al., 2003).

In developing intervention strategies the interaction between different pathogens on a farm should be considered (Thacker, 2001). Although the structure of modern pig production is rapidly changing, preferably toward all-in/all-out and multisite systems, farrow-to-finish operations still exist. Continuous flow of animals through the system leads to steady transmission of respiratory pathogens from sows to piglets, and from older to younger pigs (Sörensen et al., 2006).

The main respiratory pathogens of concern are Porcine Circovirus Type 2 (PCV2), Swine Influenza Virus (SIV), Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae* and *Haemophilus parasuis*.

Serological data in studying respiratory-disease dynamic in herds may be useful. Clinical occurrence of disease is of little value because most pigs in chronically infected herds are subclinically infected (Andreasen et al., 2000). Serum profiles are those serial studies performed in order to know the immunological status of a farm. Serum profiles are based on the detection of circulating antibodies. The same serum can be tested against specific antibodies for different respiratory pathogens (Golinar Oven and Valenčak, 2010). In practice, cross-sectional serological testing of different age groups from different housing units is the easiest way to gather such information (Andreasen et al., 2000). We chose to use a longitudinal study design which is more precise.

The objective of this study was to make the serum profiles to selected (important) respiratory pathogens for preparation of specific control measures on one Slovenian large pig farm.

MATERIAL AND METHOD OF THE STUDY

A farm was one site unit from farrow-to-finish production with 7000 breeding sows at the time of collection of samples. Pigs were vaccinated at the age of 10-14 days against *M. hyopneumoniae*. Blood samples were collected between December 2007 and July 2008.

36 litters were selected from a herd. Piglets were randomly selected (using a computer-generated list of random numbers) from each of these litters. A cohort consisted of pigs born within the same week. The sows were chosen according to the week they farrowed.

Piglets were individually identified at 2 weeks of age with numbered ear tags. The same pigs were bled at 2, 4, 6, 8, 11, 14, 17, 22 and 28 weeks of age. The last blood sampling (at 28 weeks of age) was done in a slaughterhouse. 38 pigs finished the trial. One blood sample was taken from each sow at weaning.

Blood samples were drawn from the anterior *vena cava* by venipuncture. Serum was harvested by centrifugation for 10 min at 3000 rpm and stored at - 20°C until testing for the presence of antibodies.

36 serum samples from breeding sows and 342 serum samples from pigs (38 serum samples; 9 samplings) were tested for antibodies to PCV2, SIV, *M. hyopneumo-*

niae, *A. pleuropneumoniae* and *H. parasuis*.

Commercial ELISA kits of different producers were used:

- INGEZIM CIRCO IgG (Ingenasa) – indirect ELISA,
- INGEZIM INFLUENZA PORCINA (Ingenasa) – indirect ELISA,
- INGEZIM MHYO COMPAC (Ingenasa) - blocking ELISA,
- CHEKIT-APP-ApxIV ELISA Test Kit (IDEXX),
- *Haemophilus parasuis* Antibody Test Kit (ELISA) Swinecheck® HPS (Biovet).

Ingezim Circo IgG: Samples with optical density (OD) higher than positive cut off value were considered positive to PCV antibodies. Samples with OD lower than negative cut off value were considered negative.

Ingezim Infuenza Porcina: Samples with S/P values greater or equal 0,2 were positive for antibodies to influenza A viruses. Samples with S/P values less than 0,2 were considered negative for antibodies to influenza A viruses.

Ingezim Mhyo Compac: The results of the test were expressed as OD value. A blocking percentage of sample was calculated. Samples were considered positive when the OD value was equal or lower than 40% of negative control. Samples were considered negative when the OD value was equal or higher than 45% of negative control.

Chekit -APP-ApxIV ELISA: The diagnostic relevance of the result was obtained by comparing the OD of the samples, with OD of the positive control. Samples were considered positive when the value (%) was equal or higher than 40%. Samples were considered negative when the value (%) was lower than 30%. If the samples were suspect (≥30% to <40%) they were tested in a second run.

Haemophilus parasuis ELISA: For each sample and control we subtracted the OD obtained in the well containing antigen from the well without antigen. A ratio was calculated. Sample ratio less than 0,6 was considered negative and sample ratio greater or equal to 0,9 were considered positive. Sample ratio less than 0,9 but greater or equal to 0,6 was considered suspicious and sample was tested in a second run.

RESULTS

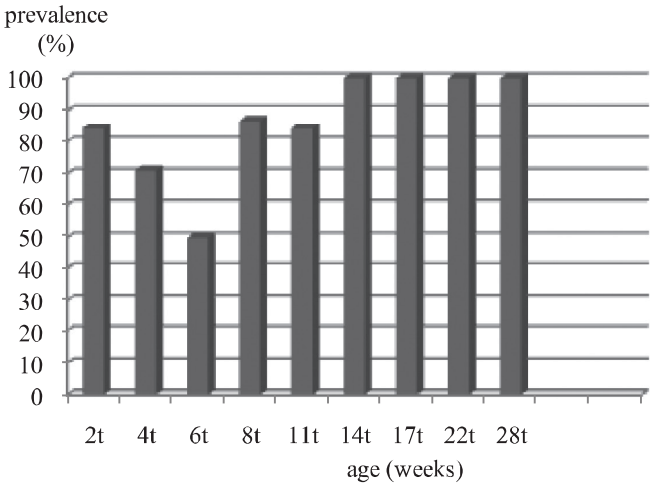
Breeding sows

Seroprevalence to SIV, *A. pleuropneumoniae* was 100 %, to PVC2 94 %, to *M. hyopneumoniae* 83,3 % and to *H. parasuis* 36 %.

Piglets

PCV2

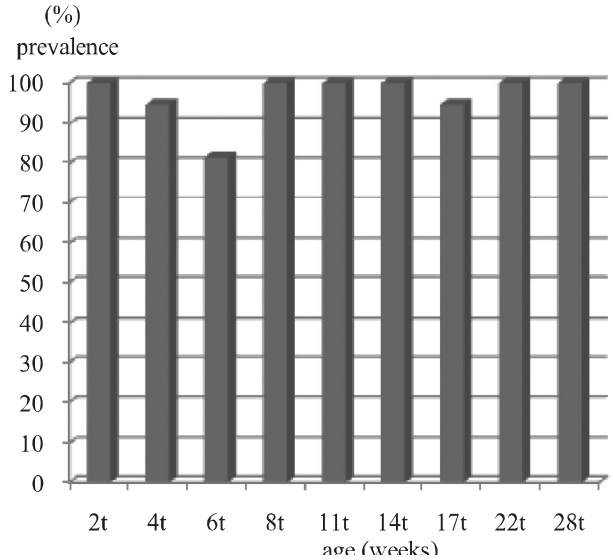
At 2 weeks of age the seroprevalence against PCV2 from 84,2 % decreased to 50 % (at 6 weeks of age). At 8 weeks of age increased to 86,8 % and at 14 weeks of age to 100 %.



Graph. 1. Serum profile of PCV2.

SIV

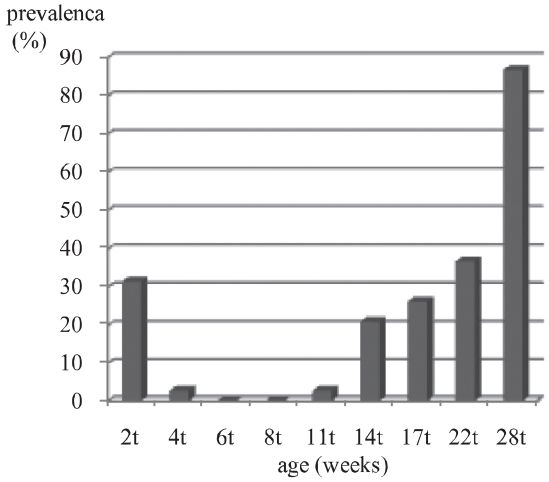
The seroprevalence against SIV was 100 % at 2 weeks of age, 94,7 % at 4 weeks of age; the lowest seroprevalence was at 6 weeks of age (81,5 %). The seroprevalence then increased to 100 %, only at 17 weeks of age was a little lower (94,7 %).



Graph 2. Serum profile of SIV.

M. hyopneumoniae

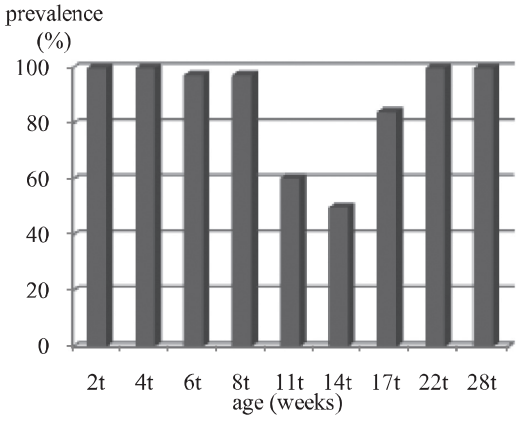
The seroprevalence against *M. hyopneumoniae* was 31,5 % at 2 weeks of age and decreased to 0 % at 6 and 8 weeks of age. At 11 weeks increased to 2,6 % and 28 weeks of age increased to 86,8 %.



Graph 3. Serum profile of *M. hyopneumoniae.*

A. pleuropneumoniae

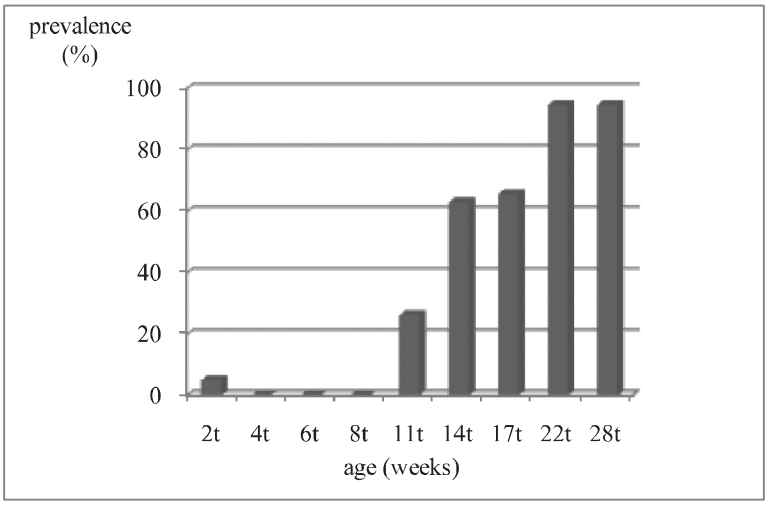
All pigs had specific antibodies against *A. pleuropneumoniae* at 2 and 4 weeks of age. The seroprevalence was lower at 6 and 8 weeks of age (97,4 %), and decreased to 50 % at 14 weeks of age. At 17 weeks of age increased to 84,2 % and at 22 and 28 weeks of age to 100 %.



Graph. 4. Serum profile of A. pleuropneumoniae.

H. parasuis

Only 5,2 % of piglets had specific antibodies against *H. parasuis* at 2 weeks of age. Pigs were seronegative against *H. parasuis* at 4, 6 and 8 weeks of age. The seroprevalence increased to 26,3 % at 11 weeks of age and to 97,4 % at 22 weeks of age.



Graph. 5. Serum profile of *H. parasuis*.

DISCUSSION

Serum profiling is a method which can contribute to creation of an effective vaccination program on farm against selected agents.

Colostral antibodies against SIV and PCV2 persisted for about 4 weeks. The lowest seroprevalence was detected in 6 weeks old pigs against both viruses. The animals were probably infected around 6 weeks of age with both viruses. According to serum profiles vaccination of sows against SIV and PCV2 can be proposed to prolong colostral immunity and better protect animals against postweaning multisystemic wasting syndrome (PMWS) and swine influenza.

Colostral antibodies against *A. pleuropneumoniae* persisted for almost 8 weeks (94,8 % prevalence). The lowest prevalence was detected in 14 weeks old pigs (53,8 %), and the highest at 22 and 28 weeks old pigs (100 %). According to serum profile the best time for vaccination against *A. pleuropneumoniae* is around 11-14 weeks of age.

Samples were examined for antibodies against *A. pleuropneumoniae* using ApxIV-ELISA. ApxIV is expressed by all serotypes of *A. pleuropneumoniae* only after infection of pigs, but not under in vitro conditions (Dreyfus et al., 2004).The ApxIV-ELISA does not differentiate serotypes of *A. pleuropneumoniae*. The previous survey in years 1997-2001 showed that in Slovenia are present pathogen serotypes. The advantage of ELISA for *A. pleuropneumoniae* over other serological tests is that it does not cross-react with other bacterial species and the test differentiate between pigs infected with *A. pleuropneumoniae* and vaccinated pigs against *A. pleuropneumoniae* (Dreyfus et al., 2004).

Colostral antibodies against *H. parasuis* were found only in 5,1 % at 2 weeks

old piglets. Circulating antibodies as result of natural infection were detected from 11 weeks (25,6 % prevalence) to 28 weeks old pigs (94,7 % prevalence). According to serum profile the best time for vaccination in this farm against *H. parasuis* is around 8-9 weeks of age.

Specific antibodies against *M. hyopneumoniae* found at 2 weeks of age are maternal antibodies, but prevalence (31,5%) in piglets is unexpectedly low according to prevalence in sows. Following vaccination with one dose product frequently no serum antibodies are detected. Serum antibody levels decline and pigs frequently become seronegative 4-6 weeks following vaccination (Thacker and Thanawongnuwech, 2002). This is also evident from our serum profiling. Up to 25% of the pigs may express antibodies to *M. hyopneumoniae* at the age of 10-12 weeks. At the time of slaughter approximately 90% of the animals become seropositive to the microb (Wallgren et al., 1998). In our case the seroprevalence started to increase at 11 week of age and at time of slaughter 86,8% animals had specific antibodies. Increasing of antibodies to *M. hyopneumoniae* should be due to decreasing immunity against *M. hyopneumoniae* and result of infection with *M. hyopneumoniae*. According to serum profiling second vaccination around 11-14 weeks of age can be proposed.

CONCLUSION

In our study was established, that cross-sectional testing is sufficient for preparation of control measures for the farm, but only with supposition that no new disease is entering the farm. Cross-sectional method must be repeated quarterly or at least twice a year, to follow the efficiency of performed measures.

REFERENCES

SØRENSEN, V., JORSAL, S.E., MOUSING, J.: Diseases of the respiratory system. In: Diseases of swine (B.E. Straw, J.J. Zimmerman, S. D'Allaire, D.J.Taylor, eds). Blackwell Publishing, Ames, pp.149-177, 2006.

STÄRK, K.D.C.: Epidemiological investigation of the influence of environmental risk factors on respiratory diseases in swine: a literature review. Vet. J., 159:37-56, 2000.

CHOI, Y.K., GOYAL, S.M., JOO, H.S.: Retrospective analysis of etiologic agents associated with respiratory disease in pigs. Can. Vet. J., 44:735-737, 2003.

THACKER, E.L.: Porcine respiratory disease complex-what is it and why does it remain a problem? Pig J., 48:66-70, 2001.

ANDREASEN, M., NIELSEN, J.P., BÆKBO, P., WILLEBERG, P., BØTNER, A.: A longitudinal study of serological patterns of respiratory infections in nine infected danish swine herds. Prev. Vet. Med., 45:221-235, 2000.

GOLINAR OVEN, I., VALENČAK, Z.: The use of serum profiles for control Haemophilus parasuis and Actinobacillus pleuropneumoniae. Proceedings of the 21st International Pig Veterinary Society Congress, Vancouver, Canada, 18-21 July, 2010, pp.195.

DREYFUS, A., SCHALLER, A., NIVOLLET, S. et al.: Use of recombinant ApxIV in serodiagnosis of *Actinobacillus pleuropneumoniae* infections, development and prevalidation of the ApxIV ELISA. Vet. Microbiol., 99:227-238, 2004.

THACKER, E., THANAWONGNUWECH, R.: Porcine respiratory disease complex

(PRDC). Thai J. Vet. Med., 32: 125-134, 2002.
WALLGREN, P., BÖLSKE, G., GUSTAFSSON, S., MATTSSON, S., FOSSUM, C.: Humoral immune responses to *Mycoplasma hyopneumoniae* in sows and offspring following outbreak of mycoplasmosis. Vet. Microbiol., 60:193-205, 1998.

POTENCIJALNA KORIST ANALIZE SERUMA U KONTROLI RESPIRATORNIH BOLESTI

IRENA GOLINAR OVEN

Izvod

Na jednom slovenačkom velikoj farmi svinja, odabrano je 36 legala za analizu serumua na respiratorne patogene, radi pripreme mera kontrole. Uzeto 36 uzoraka seruma priplodnih krmača i od 342 uzorka seruma prasadi (38 uzoraka seruma, po 9 uzorakovanja). Uzorci su testirani na antitela svinje na Circovirus Tip 2 (PCV2), virus svinjskog gripa (SIV), Micoplasma hiopneumoniae, Actinobacillus pleuropneumoniae i Haemophilus parasuis. Krv je uzimana od istih prasadi, 2, 4, 6, 8, 11, 14, 17, 22. i 28. nedelje starosti. Komercijalni ELISA kompleti različitih proizvođača su korišćeni. Kod priplodnih krmača seroprevalencija na SIV i A. pleuropneumoniae je bila 100%, na 94% PVC2, M. hiopneumoniae na 83,3% i na H. parasuis 36%. Kolostralnih antitela u svinja protiv SIV-a i PCV2 traje već oko 4 nedelje. Najniži seroprevalencija je otkrivena kod 6 nedelja stare prasad protiv oba virusa. Prema serumskom profilu krmača, vakcinacija protiv SIV-a i PCV2 može se predložiti. Kolostralnih antitela protiv A pleuropneumoniae traje već skoro 8 nedelja (94,8% prevalenca). Najniža prevalencija je otkrivena kod prasadi starih 14 nedelja (53,8%). Prema serumu profilu, vakcinisanja protiv A. pleuropneumoniae oko 11-14 nedelja starosti može se predložiti. Do 8 nedelja starosti, prasad imaju seronegativni profil protiv H. parasuis. Seroprevalencija se povećana sa 11 nedelja starosti. Vakcinacija protiv H. parasuis oko 8-9 nedelja starosti može se predložiti. Seroprevalencija protiv M. hiopneumoniae sa 6 nedelja starosti se smanjila na 0%, a sa 11 nedelja starosti je počela da raste. Druga vakcinacija između 11.-14. nedelja starosti može se predložiti.

Ključne reči: svinja, respiratorne bolesti, serum profil, kontrolne mere.

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