Immunopathological Changes Induces by Porcine Circovirus Type 2 on Pigs

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Abstract. The aim of this paper was to evidence and evaluating the changes induced by porcine circovirus type 2 to different floors of the immune system. Immunological analysis showed a substantial changes in leukocytes subsets mainly to 6-weeks-old PCV2 positive clinically healthy pigs, PMWS and PDNS affected pigs. Total leukocytes counts were in relatively normal limits to PMWS and PDNS affected pigs. The circulating immune complexes levels were extremely high in case of PDNS affected pigs, other categories has no statistical differences of this parameter. Whole immunoglobulin counts follows the infection according to age, lower levels were recorded only in PMWS affected pigs. From leukocytes populations an increase in the percentage of monocyte particularly in PMWS and PDNS affected pigs were observed. PDNS and PMWS affected pigs shows a deficit of lymphocyte/neutrophil ratio in favor of the last, this event is in direct relationship to increased levels of serum lysozyme. These data support the notion that PCV2-positive pigs might have an impaired immune response.

Key words: immunopathology, porcine, PCV2

INTRODUCTION

Porcine circovirus type 2 (PCV2) is a virus from Circoviridae family that can cause morbid entities with high economic effects, with a main target for lymphoid tissues, influencing in a negative sense the functionality of the immune system and thereby inducing a very severe immunosuppression. The effects of PCV2 on the immune system of the pig have not been widely studied. Analyses on field cases of acute PMWS (Segalés et al., 2001) and PCV2-infected pigs from a conventional farm with histories of PMWS (Darwich et al., 2002) showed that some immunological parameters were being modulated.

The aim of this study was to evidence the changes induced by porcine circovirus type 2 to different floors of the immune system, actually watching how the changes induced by infection or disease (PCVAD – porcine circovirus associated diseases) affecting the nonspecific immune cell system (white blood cell count, leucocytes subsets) as well as unspecific humoral defense (lysozyme, whole immunoglobulin and circulating immune complexes) both at PMWS/PDNS affected and subclinical infected pigs, respectively.

MATERIAL AND METHODS

In this study were included all categories of swine from flow technology. Thirty clinically healthy PCV2 positive sows, 4,6,7,8,10,12,14,16,22-week-old pigs, PMWS and PDNS affected pigs were selected from a farm with history of porcine circovirus associated...
Peripheral blood samples were collected from the anterior vena cava cranialis on vacutainer with and without EDTA.

For detection of PCV2 specific antibodies were used ELISA (1.1.PCV2.K2, Ingenasa, Madrid) and in our lab made indirect-fluorescent antibody procedure. The optical densities obtained to detection of antibodies from sera by ELISA were processed statistically. The titres of sera PCV2 antibodies were registered and processed.

Measurement of the level of circulating immune complexes (CIC) allows evaluation of the molecular clearance capacity at a particular moment. Part of the collected blood was allowed to clot for 30-min at 37°C and then centrifuged at 1308·g for 10-min. Sera were removed and kept at –20°C until tested. A 4.2% polyethylene glycol (PEG) solution in borate buffer was used as the precipitating agent, while buffer-treated samples served as controls for borate-induced precipitation.

Serum lysozyme concentrations were determined used method of Spinu et al. (2). Briefly 10 ml of 2% agarose dissolved in phosphate buffer (Sigma) was mixed with 20 ml suspension of 24 hours culture of Micrococcus lysodeicticus (Sigma) at 56°C. This mixture was poured out in Petri’s dish. After solidifying at room temperature 12 wells were made. Twenty microliters of undiluted sera were poured out in each well. The samples were incubated for 24 hours at 37°C and lytic diameters were measured. The final lysozyme concentrations were calculated and expressed as mg/l.

Total immunoglobulin, known as opsonins, play an important role in the ‘first line of defense’ against aggressors, that is innate immunity. At a pH 7.4, the electric charge and colloidal stability of gammaglobulins are lower than those of serum albumins.

Part of the heparinized blood was diluted 1:20 with Türk solution, kept at room temperature for 3-min and then leukocytes were counted in a Bürker–Türk chamber, counting the elements in four corner squares. The mean value was multiplied by 20 for the dilution degree. The values were expressed in number of cells/mm³.

The leucocyte differential count was performed, using light microscopy, by identification of 200 cells on a blood smear for each sample. All blood smear samples were stained using a Diaquick Panoptic staining kit (Reagent Ltd., Budapest, Hungary).

RESULTS AND DISCUSSIONS

The IgM and IgG levels analysed to all studied categories suggests that young pigs are exposed to infection with PCV2 throughout their life but the pressure of infection is maximum at the age when PMWS evolves, namely within 7-10 weeks, during which the pigs are immunologically naive.
Immunological analysis showed a substantial change in leukocytes subsets in peripheral blood mainly to 6-weeks-old PCV2 positive clinically healthy pigs, PMWS and PDNS affected pigs. Total leukocytes counts were in relatively normal limits to PMWS and PDNS affected pigs, the lower values being recorded in 7-10-week-old clinically healthy PCV2 positive pigs.

Figure 1. Comparision of □-IgM and ●-IgG serological profiles to different swine categories on flow technology on a farm with PCVAD history. The quadruped represent the critical period for PCV2 infection.

Figure 2. Comparision of whole immunoglobulin serological profiles to different swine categories on a farm with PCVAD history.
The circulating immune complexes levels were extremely high in case of PDNS affected pigs (Fig. 3), other categories has no statistical differences of this parameter. Whole immunglobulin counts follows the infection according to age (Fig. 2), lower levels were recorded only in PMWS affected pigs. From leukocytes populations an increase in the percentage of monocyte particularly in PMWS and PDNS affected pigs were observed. PDNS and PMWS affected pigs shows a deficit of lymphocyte/neutrophil ratio in favor of the last, this event is in direct relationship to increased levels of serum lysozyme (Fig. 4). Thus, the antibacterial defense of these pigs, at least towards gram positive germs, was ensured by the increased activity of these effectors. Their death was caused mainly by gram negative bacterial co-infection, hardly destroyed by lysozyme.
In the case of clinically healthy pigs the values of serum lysozyme can be found at lower levels, probably on one hand due to a lack of a bacterial aggression (as a result of a balance between clearance and the ability of defense immune response), and on the other hand the lymphocyte/neutrophil ratio which is found in the normal range (Fig 5,6).

PCV2 specific IgM and IgG dynamics in the investigated conventional swine supported the increased vulnerability of 6 to 8 weeks old animals to PCV2 infection, this period being the critical one (lowest levels of IgM and IgG), concordant with the appearance
of post-weaning multisystemic wasting syndrome (PMWS). PDNS appeared at older ages (10 to 12 weeks) than PMWS, due to unknown enhancing factors. Vascular lesions encountered in the skin and were the indirect result of PCV2 infection, mediated by high levels of CIC that entirely compromised the tissue structure. These data support the notion that PCV2-positive pigs might have an impaired immune response.

REFERENCES

