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C-reactive Protein and Haptoglobin as A potential Biomarkers of Health and Welfare in a Herd of Wild Boars (*Sus scrofa* L.) from a Game Reserve

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Abstract: The aim of this study was compare serum level of C-reactive protein (CRP) and haptoglobin (Hp) between conventional farms piglets and wild boar piglets from a game reserve. The serum CRP was assayed by a solid phase sandwich Immunoassay, Hp was determinated by colorimetric assay. The mean values of CRP and Hp in wild boars were 280.71 ± 52.62 µg/ml and 3.05 ± 0.88 mg/ml, respectively. They were significantly higher than mean values found in clinicaly healthy piglets 94.83 ± 58.21 µg/ml and 1.11 ± 0.91 mg/ml, respectively ($p<0.001$). There is no significant difference between them and piglets with confirmed pathological process. The results indicate that adequate attention should be paid to the veterinary care of wild boars in game reserve.

Key words: CRP · Game Reserve · Hp · Piglets · Wild Boar

INTRODUCTION

C-reactive protein (CRP) and haptoglobin (Hp) belongs to acute phase proteins (APPs). APPs are blood proteins that can be used to assess the innate immune system's systemic response to infection, inflammation or trauma [1-3]. By definition, these proteins change their serum concentrations by >25% in response to proinflammatory cytokines stimulated during the disease process [4]. Under the influence of interleukin (IL), i.e., IL-1, IL-2 and tumor necrosis factor - alpha (TNF-α), liver cells synthesize and secrete APPs [5]. The maximum serum concentration of positive APPs is 24 to 48 h after the initiation. Feed-back regulations will limit the response leading to its resolution within 4-7 days after the initial stimulus. Chronic inflammation can be perceived as a consecutive series of separate inflammatory stimuli. In such conditions, increased serum concentrations of APPs are generally observed [5, 6]. In swine, C-reactive protein, haptoglobin, serum amyloid A and pig major acute phase protein (Pig-MAP) are the main positive APP [7-8] and they are possible candidates to monitor the health status of pigs herds [9-10].

The aim of this study was compare serum level CRP and Hp between conventional farm piglets and wild boar piglets from game reserve.

MATERIAL AND METHODS

Serum samples were collected in a conventional farm from Large white piglets after weaning. Piglets were classified in groups: animals with good body condition and without signs of disease group A (n=16), animals with poor body condition group B (n=16). Boar-piglets group C (n=14). Boar were caught in the game reserve and placed into smaller fenced pens. Blood samples were taken from the *sinus opthalmicus*. The blood samples were centrifuged and sera stored at -20 °C until use.

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The serum CRP was assayed by a solid phase sandwich Immunoassay, using a commercially avaiable kit for pigs (Tridelta PHASE porcine CRP kit, Tridelta Development Ltd, Ireland). For CRP The immobilized antibody, bind specifically to any CRP in the well. After washing to remove any unbound material tetramethylbenzidine (TMB) substrate solution were added. The intensity of the colour produced is proportional to the concentration of CRP present in the original specimen. The value of haptoglobin in the samples was determined by a colorimetric method using the commercially available kit (Tridelta PHASE Haptoglobin Assay kit, Tridelta Development Ltd, Ireland). Analysis was based on Hp-haemoglobin binding and preservation of the peroxidase activity of the bound haemoglobin at low pH [11]. The optical densities were read on automatic microplate reader (Opsys MR, Dynex Technologies, USA) at an optical density of 630 nm.

All analyses were carried out using the GraphPad Prism 6.0 program. The differences between the groups were found using Tukey's Multiple Comparison Test and evaluated at the same time. P value of <0.001 was considered significant.

RESULTS AND DISCUSSION

Serum concentrations of acute phase proteins were determinate on 46 piglets. Results are presented in Table 1. Mean value of CRP and Hp in group B was significant higher than in group A ($p<0.001$). Wild boar piglets in group C had no visual signs of disease and mean value of CRP and Hp in this group was significantly higher then mean in group A ($p<0.001$), but there is no significant diference between group B. This shows Figures 1. and 2.

Due to lack of scientific reference values for APP in wild boars, data acquired by us was compared with results of analogical studies, where subjects of study were domestic pigs of the same age. Phylogenetically domestic pig *Sus scrofa domestica* and wild boar *Sus scrofa scrofa L* are the same animal species. Most often the domestic pig is classified as a sub-species of wild boar, but also sometimes as an integral part of the species from which it evolved. In domestication centres in Europe it comes generally about wild boars *Sus scrofa scrofa L*. Also some attributes of game reserve, like closed area, higher concentration of animals, stimulate conditions of

Table 1: Serum concentration of CRP and Hp in each group that wax examinated.

APP	CRP µg/ml			HP mg/ml		
Group	Mean	Median	SD	Mean	Median	SD
A	94.83	67.60	58.21	1.11	0.75	0.91
B	317.22	318.0	92.25	2.67	3.01	1.08
C	280.71	295.3	52.62	3.05	2.95	0.88

A – clinically healthy piglets (n = 16), B – piglets with signs of disease (n = 16) and C – wild boar piglets (n = 14), mean – average value, median – middle value, SD – standard deviation

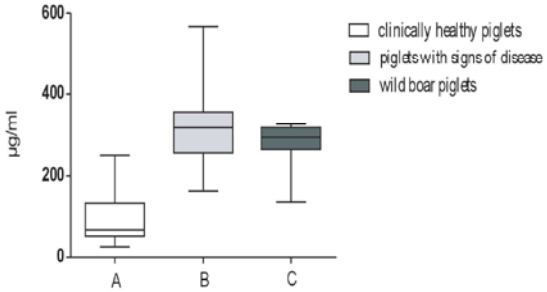

Fig. 1: Comparison of CRP, The plot shows median (line within box), 25th and 75th percentiles (box), 5th and 95th percentiles (whiskers). Group C differ significantly to group A ($p<0,001$). No significant different is between group C and B.

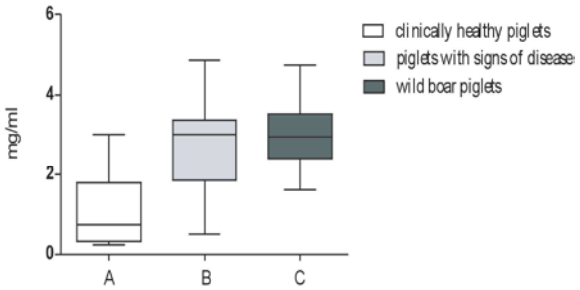

Fig. 2: Comparison of Hp, The plot shows median (line within box), 25th and 75th percentiles (box), 5th and 95th percentiles (whiskers). Group C differ significantly to group A ($p<0,001$). No significant different is between group C and B

conventional pig breeding. Results of this study mention to significantly higher values of CRP in wild boar piglets 280.71 μg/ml against group of clinically healthy piglets. By contrast, value in group of wild boar-piglets matches with value in group of piglets with processing pathological process of various ethiology 317.22 μg/ml This fact is possible accredit to non-apparently processing diseases of parasitic origin (coprologic confirmation of infestation with *Metastrongylus spp*. *Trichuris suis*, *Oesophagostomum sp*. and *Capillaria sp*.) and also to environmental factors, mainly to stress induced by regular disturbance and manipulation. High values of CRP can also mention of other inflammatory processes or hidden bacterial infection. In case of Hp, data acquired by us have the highest average value in complex of compared groups. As serum concentration of Hp in wild boar-piglets has a value of 3.05 mg/ml it is highly probable though not an apparent process of disease in most of them. Serum concentration of Hp is significantly increased with age in conventional breeding of pigs, but not in pigs from SPF breeding (Specific Pathogen Free SPF-x). What indicate the higher values could be subclinical processing diseases in older categories [5]. This shows that Hp could be a potential marker for detection of subclinical processin diseases, however, to get more complete and valuable information it might be advisable to perform APPs profiles including another APP, such as SAA or AGP.

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