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LETTER TO THE EDITOR

Prevalence of porcine circovirus 2 and virus-specific antibodies in wild boars (*Sus scrofa*) in Slovakia

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Porcine circovirus 2 (PCV-2) is a causative agent of several diseases of pigs that are collectively termed porcine circovirus diseases. The virus has a single-stranded DNA genome of a size approximately 1.8 kb, virions of 17–20 nm in diameter and belongs to the genus *Circovirus* from the family *Circoviridae* (*1*). In most cases PCV-2 infection has a subclinical course; however the outcome may result as postweaning multisystemic wasting syndrome (PMWS). PMWS affects domestic pigs between the age of 5 weeks and 14 weeks, causing wasting and growth retardation that are often associated with anemia of the skin, respiratory distress, diarrhoea and/or icterus. Interstitial pneumonia, enlarged lymph nodes, hepatitis and nephritis are frequent pathological findings in the necropsy. Depletion of lymphocytes in lymphoid tissues suggests immunosuppressive properties of the pathogen (*2*). In addition to PMWS, PCV-2 has been attributed to various other clinical conditions such as reproductive disorders, porcine respiratory disease complex, porcine dermatitis and nephropathy syndrome, proliferating necrotizing pneumonia and congenital tremors (*3–5*). PCV-2 has also been detected in wild boars in several other European countries, such as Belgium, Spain, Croatia, the Czech Republic, Hungary and Germany, either by antibody or virus detection (*6–8*). Sporadic cases of PMWS were noted in feral pigs (*9*) and that places them in the position of reservoir hosts of PCV-2. The prevalence of specific antibodies and PCV-2 in the population of domestic pigs in Slovakia is 54% and 35.5%, respectively (*10*); however, no information about the prevalence of PCV-2 in wild boars has been published. The aim of the present work is to establish the prevalence of PCV-2 and PCV-2 antibodies in different age categories of wild boars in Slovakia.

The sampled open-hunting area is 6,110 ha in size and is located in the southern part of central Slovakia. Lungs and inguinal lymph nodes were collected from 51 wild boars of different ages and blood was collected from 27 of these animals. The age of each animal was estimated based on its tooth eruption pattern, and animals were categorized into adults (>2 years), subadults (1–2 years) and yearlings (<1 year). Tissue samples and sera were stored at -80°C and -20°C, respectively.

The protocol for DNA extraction from lung or lymph node tissue was adopted from Pearson and Stirling (*11*). For PCR, 25 µl of recombinant Taq DNA polymerase (Invitrogen) reaction mixture composed of 0.5 µl of sample DNA, 200 mmol/l of each dNTP and 2.5 U of polymerase was used. The primers (ORF2.PCV2.S4 and ORF2.PCV2.AS4) at the concentration of 300 µmol/l and the cycling conditions were adopted from Ouardani (*12*). The 494 bp target sequence was

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Abbreviations: ISH = in situ hybridisation; PCV-2 = porcine circovirus 2; PMWS = postweaning multisystemic wasting syndrome

Table 1. Prevalence of PCV-2 and PCV-2 antibodies in different age categories of wild boar

Age categories (years)	No. of samples	PCV-2 DNA			PCV-2 antibodies		
		No. of positive lungs (%)	No. of positive lymph nodes (%)	Prevalence (%)	No. of samples	No. of positive samples	Prevalence (%)
Yearlings (<1)	9	1 (11.1)	0	11.1	8	1	12.5
Subadults (1-2)	21	4 (19.0)	4 (19.0)	38.1	13	5	38.5
Adults (>2)	21	8 (38.1)	8 (38.1)	76.2[a, b]	6	4	66.7
Total	51	13 (25.5)	12 (23.5)	49	27	10	37

[a]p = 0.016 between yearlings; [b]p = 0.0278 between subadults.

electrophoresed in 1.5% agarose gel and stained with 1 mg/l of ethidium bromide.

Despite the endemic nature of PCV-2 in the population of domestic pigs, there is no published information on its occurrence in wild boars in Slovakia. Viral DNA was detected in 49% of the tissue samples, with the positive correlation increasing with the age of animals, where the highest positivity was noted in adults. Correlation between presence of PCV-2 in different age groups was assessed with χ^2 and Fisher´s exact test, where P ≤0.05 was considered as significant difference. There was significant difference in the PCV-2 occurrence among the age categories yearlings and adults and between subadults and adults (Table 1). The prevalence of PCV-2 in feral pigs in Slovakia differs from the prevalence in neighboring countries. Cságola (*13*) reported a 20.5% prevalence in Hungary, Cadar (*14*) reported 13.5% in Romania and PCV-2 has been detected in 75.6% of tested wild boars in Poland (*15*). Prevalence similar to our results was reported in a study carried out in Germany by Reiner (*16*), where the viral genome was detected by quantitative PCR with an average concentration of $10^{2.8}$ PCV-2 copies/μg extracted DNA in 45% of lymph nodes from hunted wild boars. In a similar study conducted in Spain, only 5% of tested lymph nodes were positive for PCV-2 by in situ hybridization (ISH) (*7*). The distinct prevalence of the PCV-2 genome compared to our study may result from several factors. On one hand, it is the epizootological situation in the geographical region from which samples were obtained; on other hand, different laboratory methods were used for detection of PCV-2 in our study and in the Spanish one. The variance between PCR and ISH in detection of PCV-2 was described by Calsamiglia (*17*). In cases where the viral load is very low, ISH is not able to detect PCV-2. Segalés concluded that ISH gives negative results when the concentration of PCV-2 is 10^4 genome copies/ng or less of total DNA (*16*). Taking these observations into account, ISH is not the method of first choice for detecting PCV-2 in wild boars.

PCV-2 antibodies were detected by immunoperoxidase monolayer assay on persistently infected PK15 cells with PCV-2 Stoon-1010 strain seeded on 96 well plates (*18*). The overall seroprevalence of PCV-2 antibodies was 37% (Table 1). The proportion of seropositivity was similar to the occurrence of PCV-2. The highest positivity was noted in adults (66.7%), followed by subadults (38.5%) and then yearlings (12.5%; Table 1). There was no significant difference in the occurrence of PCV-2 antibodies among the age categories, which can be attributed to the low number of analyzed serum samples. Similar results of PCV-2 antibodies were published by Sanchez (*6*) in Belgium (30%). A higher seroprevalence was reported by Sedlak (*8*) from the Czech Republic (47%) and by Fabisiak (*19*) from Poland (47.9%). The relatively low percentage of PCV-2 antibodies in our study is not a surprising result. Vicente (*7*) compared seroprevalence in different wild boar subject to different management methods. In intensively-managed wild boars the seroprevalence rate ranged from 70-80%, followed by fenced management at 45–55% and about a 30% seroprevalence rate in wild boars living in open hunting areas. Another large-scale study carried out in Spain supports our results, in which the highest seroprevalence rate was noted in adults (32%) and in subadults (approximately 25%). The overall seroprevalence rate was higher in fenced estates compared with open hunting areas, 58.1±3% and 28.1±6%, respectively (*20*).

The results of our study show a high prevalence of PCV-2 in the population of wild boar in the investigated area of Slovakia, with a significantly increasing rate corresponding with the age of the animals. It can be concluded that wild boars may act as a reservoir host for PCV-2, a pathogen which may cause high economic losses in domestic pig farms.

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