

## Mixed Mycotic Rhinitis and Pneumonia in Wild Boars

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### ABSTRACT

**Background:** Wild boar population is present worldwide. Contact between wild boars and domestic pigs may occur occasionally, and several diseases, as well as the occurrence of opportunistic infections are observed in both species. Mycotic rhinitis and pneumonia were reported before in pig herds, mainly associated with immunosuppression caused by viral infection. This study reports the occurrence of mycotic rhinitis in two wild boars due to *Aspergillus fumigatus*, *A. flavus* and *Candida albicans*, together with *Pneumocystis* sp. in the lungs, originating from a herd infected with PCV2.

**Cases:** In a commercial wild boar herd, poor body condition, sneezing and diarrhea were observed. Three animals were euthanized and, in two of them, yellow and green plaque-like masses of fungal growth in the mucosal and in cartilage surface and accentuated atrophy of nasal turbinates were observed. Additionally, multifocal subcutaneous abscesses in the maxillary area and bilateral reddening of the ocular mucosa with muco-purulent discharge were noted. Microscopically, in fragments from the nasal cavity of the two affected wild pigs, massive ulceration of the mucosal surface and presence of hyphae with septations and dichotomous branching and pseudohyphae were observed. Multifocal moderated interstitial pneumonia and alveolar edema were the main histological lesions founded in the lungs of 3 animals. In the lymph nodes multifocal moderated lymphoid depletion and lymphohistiocytic infiltrated was the main microscopical lesion. *Aspergillus fumigatus*, *A. flavus* and *Candida albicans* were isolated in nasal cavity. *Pseudomonas aeruginosa* was isolated from the subcutaneous abscesses and *Staphylococcus hyicus* and *Streptococcus equisimilis* from ocular swab. *Pneumocystis* was detected in lungs from the three wild boars by nested PCR, Grocott's staining and immunohistochemistry (IHC). Porcine circovirus 2 (PCV2) was detected in lungs by PCR. Virus detection by IHC was only confirmed in one wild boar.

**Discussion:** Diagnostic of mycotic rhinitis and pneumonia was based on macroscopical and microscopical findings, as well as mycological analysis, IHC and Grocott's methenamine staining. *Pneumocystis carinii*, *Aspergillus* spp. and *Candida* spp. are considered as opportunistic fungal pathogens commonly associated with immunosuppression in animals and humans and have been found in lungs and in muco-cutaneous tissue of PMWS affected pigs. Clinically, immunodeficiency is usually associated with illness caused by organisms of low pathogenicity or well-know secondary pathogens, among other factors. Besides immunodeficiency, prolonged antimicrobial therapy is another predisposing factor to the development of mycotic infections, well described in animals. In the present report, antimicrobial therapy was performed when respiratory signs were noted in therapeutic doses, suggesting that massive antibiotic use was not the trigger of mycotic rhinitis. PCV2 IHC result positive only in one wild pig, although all the samples were positive by PCR. This finding could indicate a subclinical infection or a recovery phase of the disease in the IHC negative cases, as previously suggested for domestic and wild pigs using *in situ* hybridization. PCV2 load in wild boar was lower when compared with domestic pigs. A viral load higher than 10<sup>8</sup> PCV2 genomes per 500 ng DNA was required to give a visible IHC staining in swine. Although quantitative PCR it was not used in order to detect PCV2 in the present report, the viral load could be another possible explanation for the IHC negative cases observed. The role of PCV2 as a cause of immunosuppression, facilitating the infection with secondary agents as *Aspergillus*, *Candida* and *Pneumocystis* cannot be ruled out.

**Keywords:** *Pneumocystis* sp., *Aspergillus* spp., *Candida albicans*, wild boars, porcine circovirus type 2 infection, mixed mycotic infection.

## INTRODUCTION

Wild boar population is present worldwide. Contact between wild boars (*Sus scrofa*) and domestic pigs (*Sus scrofa domestica*) may occur occasionally, and several diseases, as well as the occurrence of opportunistic infections are observed in both species. There is a major concern to monitor the epidemiological situation of wild boars diseases especially when control measures in domestic pigs are implemented [7].

Wild boars and domestic pigs are susceptible to infection with porcine circovirus type 2 (PCV2), agent of postweaning multisystemic wasting syndrome (PMWS) and of a number of other diseases or syndromes designed as PCVAD. It has been proposed that this is an acquired immunodeficiency syndrome of pigs [9] and wild boars [10]. The lack of antimicrobial therapy response against the disease, the concurrence of other disease syndromes and well-known secondary pathogens, such as *Pneumocystis* sp., *Chlamydia* spp., *Aspergillus* spp., and *Candida* spp. may indicate the immunosuppression observed in PMWS affected pigs [7]. A diagnostic of PMWS in a pig is made following three criteria: presence of clinical signs and macroscopical findings suggestive of the disease (wasting, enlargement of lymph nodes, diarrhea, pneumonia), microscopical lesions in lymphoid tissues (lymphoid depletion) and the presence of the virus in those lesions, detected by IHC or *in situ* hybridization [8].

PCV2 is apparently ubiquitous in domestic pigs and has also been demonstrated in wild pigs either by immunohistochemical and molecular techniques [3].

This study reports the occurrence of mycotic rhinitis in two wild boars originating from a herd infected with PCV2 due to *Aspergillus fumigatus*, *A. flavus* and *Candida albicans*, together with *Pneumocystis* sp. in the lungs.

## CASE REPORT

Necropsy was performed in 3 wild boars, male, ranging from 180 to 252 days old, from a herd in the State of Rio Grande do Sul composed of about 1250 animals. Sections from several tissues were collected and fixed in neutrally buffered 10% formalin, prepared by standard histological methods and stained with haematoxylin and eosin.

In lung sections for detection of *Pneumocystis* organisms staining with Grocott's methenamine silver technique and immunohistochemistry (IHC) were performed. The presence of *Pneumocystis* DNA was determined by nested PCR in lung samples at the *mtLSU rRNA* gene. For gene amplification, the primer sets pAZ102H-pAZ102E and pAZ102X-pAZ102Y were used [11]. IHC of *Pneumocystis* was performed using monoclonal antibody<sup>1</sup> on formalin fixed paraffin-embedded sections of lung [2].

PCV2 was detected by PCR technique [6] in lung tissues and by IHC in mesenteric lymph node using polyclonal rabbit antibody to PCV2<sup>2</sup> at 1:1000 dilution, incubated for 1 h and stained by the streptavidin-biotin immunoperoxidase technique using diaminobenzidine<sup>3</sup> as chromogen.

Plaques fragments from the nasal cavity surface were streaked onto Sabouraud's dextrose agar with 0.5 g/L chloramphenicol and incubated for a period of up to 4 weeks at a temperature of 25°C to 30°C. Micromorphology was made employing lactophenol cotton blue staining. Whenever an initial identification was not possible due to the absence of characteristic structures, the isolate would be picked onto Potato agar to stimulate the development of reproductive structures. Yeasts were characterized through physiological routine assays and differential tests, such as chlamydoconidia production and germ tube tests.

Fragments from the nasal cavity surface, ocular swab and exsudate from the abscess in the facial area were submitted to bacteriological identification.

Unthriftiness, decreased feed intake, wasting and diarrhea were observed in wild boars from a commercial herd in Rio Grande do Sul state, Brazil. Sneezing non responsive to antimicrobial treatment, lasting 2 weeks was observed in two animals.

The main macroscopical findings were observed in the nasal cavity (sagittal section) of two animals showing yellow and green plaque-like masses of fungal growth in the mucosal and in cartilage surface (Figure 1), together with accentuated atrophy of nasal turbinates. Additionally, multifocal subcutaneous abscesses in the maxilar surface area ranging from 0,5 to 2,0 cm of diameter were noted in one wild boar with mycotic rhinitis. Bilateral reddening of the ocular mucosa with muco-purulent discharge and presence of *Trichuris suis* in the large intestine was noted in another case

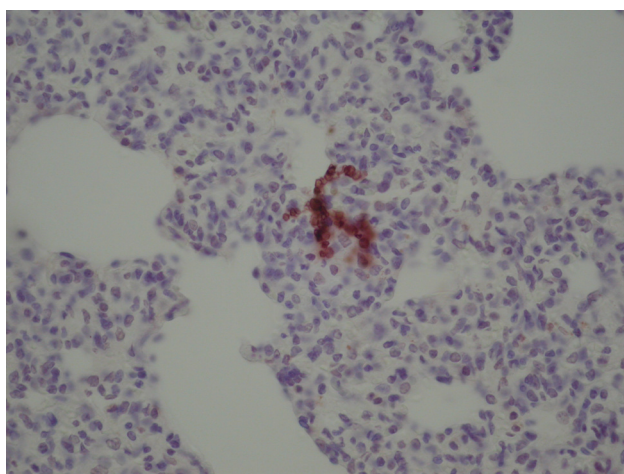
where mycotic rhinitis was observed. Pulmonary edema was observed in 3 wild boars.

Microscopically, in fragments from the nasal cavity of the two affected wild pigs, massive ulceration of the mucosal surface and presence of hyphae with septations and dichotomous branching and pseudohyphae were observed. Multifocal moderated interstitial pneumonia and alveolar edema were the main histological lesions founded in the lungs of 3 animals. In the lymph nodes multifocal moderated lymphoid depletion and lymphohistiocytic infiltrated was the main microscopical lesion.

The lungs tested showed positivity for *Pneumocystis* in PCR, Grocott staining and IHC (Figure 2). Mycological examination from plaques fragments from the nasal cavity surface detected *Aspergillus fumigatus*, *A. flavus* and *Candida albicans*.



**Figure 1.** Wild boar nasal cavity sagittal section. Yellow and green plaque-like masses of fungal growth in the mucosal surface.



**Figure 2.** Wild boar lung sample labeled by anti-*Pneumocystis* sp. Monoclonal antibody. Streptavidin-biotin-immunoperoxidase technique counterstained with haematoxylin (40x).

All samples tested by PCR were positive for PCV2. However, PCV2 IHC was positive only in mesenteric lymph node in one wild boar where mycotic rhinitis was not observed.

In the bacteriological isolation *Staphylococcus hyicus* and *Streptococcus equisimilis* were isolated from ocular swab and *Pseudomonas aeruginosa* from the facial abscess and plaques in the nasal cavity.

## DISCUSSION

In the state of Rio Grande do Sul, the free wild boar population is growing fast in the last years, mainly in the Southeastern and Northeastern regions, representing a risk of disease transmission to commercial swine herds [4]. The studied commercial herd of wild boar is located in the Northeastern region of the state, a know area of free herds of wild boars. Previously reports suggested that although the free wild boar population represents a risk for disease transmission to swine farms, in the case of PCV2 infection it seems that wild pigs became infected trough the contact with commercial pigs [10]. Considering this, commercial herds of wild pigs could also act as a possible source of transmission of diseases to free living wild boars herds.

Secondary immunodeficiency is a well known consequence of some viral infections in animals and humans. Field and experimental evidence have suggested that severely PMWS affected pigs may develop immunosuppression [9]. *Pneumocystis carinii*, *Aspergillus* spp. and *Candida* spp. are considered as opportunistic fungal pathogens commonly associated with immunosuppression in animals and humans that have been found in lungs and in muco-cutaneous tissue of PMWS affected pigs [2,12].

Clinically, immunodeficiency is usually associated with illness caused by organisms of low pathogenicity or well-know secondary pathogens, lack of response to antimicrobial therapy, presence of concurrent diseases or syndromes among other factors [9]. In our cases, fungal pathogens observed are described as well-know pathogens associated with immunodeficiency. Besides immunodeficiency, prolonged antimicrobial therapy is another predisposing factor to the development of mycotic infections, well described in animals [5]. In the present report, antimicrobial therapy was performed when respiratory signs were noted in



therapeutic doses, suggesting that massive antibiotic use was not the trigger of mycotic rhinitis.

PCV2 IHC result positive only in one wild pig, although all the samples were positive by PCR. This finding could indicate a subclinical infection or a recovery phase of the disease in the IHC negative cases, as suggested for domestic and wild pigs [10] using *in situ* hybridization. PCV2 load in wild boar was lower when compared with domestic pigs in a study performed by Vicente *et al.* [10]. Besides, in previously reports a viral load higher than 108 PCV2 genomes per 500 ng DNA was required to give a visible staining in IHC in swine [1]. Although we do not use quantitative PCR to detect PCV2 in the present report, the viral load could be another possible explanation for the IHC negative cases observed in this work.

This work has described the occurrence of four opportunistic fungus *Pneumocystis* sp., *Aspergillus fumigatus*, *A. flavus* and *Candida albicans* in two wild boars from a commercial herd infected with PCV2.

Although the participation of PCV2 as a cause of immunosuppression in the described cases could not be comprovred it should be considered since the virus was circulating in the herd.

#### SOURCES AND MANUFACTURES

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**Acknowledgments.** To Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) Brazil for scholarship (PNPD) of P. Zlotowski.

**Declaration of interest.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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