ISBN 978-602-14917-7-5
The First International Conference of Food and Agriculture

Influenza A virus infection in pigs from Mozambique

C Laisse[1,2] M Bianchi[2] P Pereira[2] C De Lorenzo[2] S Pavarini[2]and D Driemeier[2]
[1]Division of Pathology, Veterinary Faculty, Eduardo Mondlane University, Moçambique.
[2]Department of Veterinary Pathology, Federal University of Rio Grande do Sul, Brazil.

E-mail: claudiolaisse@yahoo.com.br

Abstract. Swine influenza (SI) is an acute and highly contagious disease of the respiratory tract of pigs caused by swine influenza A virus (SIA). The disease causes economic losses in swine production and is of great public importance for its zoonotic potential. The aims of the present study were to report SIA infection in pigs from Mozambique and characterize the anatomopathological and immunohistochemical features of associated lung lesions. Lungs from 457 slaughtered pigs were subjected to gross evaluation and 38 (8.3%) lungs with cranioventral consolidation were collected from a slaughterhouse in Matola City, Southern Mozambique. Consolidation areas in each lung lobe were classified in 4 grades according to the lesion extension. Samples with consolidated lung tissue were examined for histopathology and immunohistochemistry for the presence of SIA, Porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumonia*e antigens. The lungs had multifocal to coalescing areas of consolidation observed most frequently in the craniallobes. The lesions involved mainly one or three pulmonary lobes and grade 1 and 2 lesions were the most frequent. The main histopathological findings were necrotizing bronchiolitis (23/38), alveolar neutrophil infiltration (24/38), type II pneumocytes hyperplasia (26/38), peribronchiolar lymphoid tissue hyperplasia (28/38) and interstitial mononuclear cells infiltrate (29/38). SIA antigen was detected by immunohistochemistryin 84.3% (32/38) of lung samples and all lung samples were negative for PCV2 and *Mycoplasma hyopneumoniaantigense*. Pigs that presented a positive result on IHQ were from Matutuine district (5/32), Moamba district (2/32), Namaacha district (21/32), Boane district (3/32) and Matola city (1/32). These results demonstrate that SIA is a cause of pneumonia in pigs in Mozambique.
INDEX TERMS: Swine diseases, Influenza A virus, immunohistochemistry, pulmonary consolidation, Mozambique.

1. Introduction

Swine influenza virus is an important global pathogen in the swine industry. Swine influenza is caused by influenza A virus in the family Orthomyxoviridae. Influenza A viruses are further characterised by subtype by the two major surface glycoproteins, haemagglutinin and neuraminidase. The most important swine influenza A virus (SIA) subtypes that infect pigs are H1N1, H1N2 and H3N2 [1,2]. However, pigs can also be infected by other influenza A subtypes from humans and birds [3].

Natural transmission of SIA between pigs occurs through direct contact with infected animal's secretions or aerosols, and causes an acute clinical disease characterized by abdominal breathing, coughing, fever and depression [4]. The disease affects pigs of any age, and cause high morbidity, however, low mortality rate [5]. The presumptive diagnosis of swine influenza can be based on clinical signs, macroscopic and histological lesions, however, the final diagnosis is based on viral isolation, and detection of antigen and/or genetic material of the virus [5,6].

The Mozambican pig population is around 1,588,325 [7] and pig production is small of scale with many farms with less than 50 pigs. However, there is an increase of the number of medium-sized farms in which breeding animals are kept indoors in close confinement systems what favours the occurrence of respiratory diseases [8]. In contrast to Europe, Asia and the Americas, in African countries, research on SIA infection in swine are scarce or non-existent [9]. In Mozambique, there are no published papers describing the occurrence of this virus in the swine population. The purpose of this study was to report the occurrence of SIA infection in Mozambican pig and perform an anatomopathological and immunohistochemistry characterization of associated lung lesions.

2. Material and methods

2.1. Samples.

Lung samples were collected from slaughtered pigs in a slaughterhouse in Matola City, Maputo Province, Southern Mozambique (25o 55 '26 "S, 32o 27' 57" E), in the period from December 2014 to February 2015 and December 2015 to February 2016. This slaughterhouse was selected because it is the largest pig abattoir in southern Mozambique, and receives animals from several districts in the region.

2.2. Macroscopic examination

Slaughtered pig's lungs were submitted to visual evaluation and palpation to detect changes in colour and consistency of the seven pulmonary lobes: right cranial (RC); left cranial (LC) right middle (RM), left middle (LM), accessory (A), right caudal (RCA) and left caudal (LCA). The percentage of extension of the lung consolidation area was estimated based on the imaginary division of lobes into four equal parts and classified in four scores, according to the Madec and Kobisch method [1]. 0 = no lesions (0%), 1 = mild pneumonia (ranging from 1 to 25%), 2 = moderate pneumonia (ranging from 26 to 50%), 3 = severe pneumonia (ranging from 51 to 75%), and 4 = very severe pneumonia (ranging from 76% to 100%). The percentage of consolidation in each pulmonary lobe, origin and live weight of the slaughtered pigs were recorded.

2.3. Histopathology and immunohistochemical examination

Samples of lungs with consolidation were fixed in buffered formalin solution at 10% for 48 hours, routinely processed for histology, included in paraffin blocks, cut at 3µm and stained with haematoxylin and eosin (HE). To detect SIA antigens, all lung samples with pneumonia were submitted to immunohistochemistry (IHC). Antigen retrieval was obtained with protease XIV by 25 minutes at 37 ° C. Inhibition of non-specific binding was performed with 5% skim milk, for 15 minutes at room temperature. The slides were incubated overnight in primary monoclonal antibody anti-IA at 1: 500 dilution (Millipore, Billerica, MA, USA). The MACH 4 Universal HRP-Polymer was used as secondary antibody (Biocare Medical, Concord, CA). The reaction was revealed with chromogen 3'-diaminobenzidine (DAB, Dako). Sections were counterstained with Harris haematoxylin. Negative controls were established by omission of primary antibody. As positive control IAV positive lungs previously tested were used [10]. Lung samples were also submitted to IHC for Swine Circovirus type 2 (PCV2) using an polyclonal antibody anti-PCV2 (Iowa State

University, USA) [11] and for Mycoplasma hyopneumoniae using an autoimmune antiserum anti-Mycoplasma hyopneumoniae, kindly provided by Nelson Morés (CNPSA / Embrapa) [12].

2. Results

3.1. Macroscopic examination

A total of 457 pigs were slaughtered (average: 22 pigs/ day) from 9 districts of Southern Mozambique. The slaughtered pigs were of local breeds (Landim) and exotic breeds, mainly Large White and Landrace.

The live weight of the slaughtered pigs that presented lung consolidation (n = 38) ranged from 5 to 120 kg (mean of 64.3 kg) and 13.2% (5/38) were from Matutuine, 5.3% (2/38) Moamba, 68.4% (26/38) Namaacha, 2.6% (1/38) Chibuto, 7.9% (3/38) Boane and 2.6% (1/38) Matola. All pigs with pneumonia from the Namaacha district (n = 26) were from a commercial farm and were slaughtered between December 2014 and February 2015.

Thirty-eight (8.3%) slaughtered pigs presented consolidation in at least one pulmonary lobe, and these were multifocal to coalescing, well delimited, reddish to greyish, with increased consistency compared to the normal lung parenchyma (Fig. 1). Distribution and severity of the lung lesions are shown in table 1.

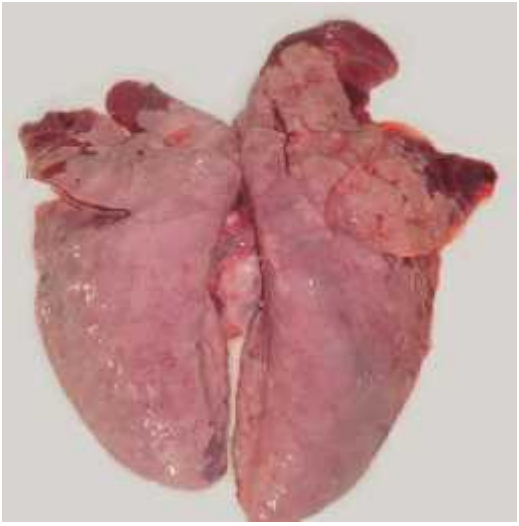

Figure 1. Gross lesion typical for SIA infection presents as purple to dark red consolidated area in right cranial (score 2), left cranial (score 1), right medial (score 2) and left medial (score 1) lobes.

Table 1: Percentage of cases of different score lesion in each pulmonary lobe

	% of cases of diferente score lesion in each pulmonar lobe						
Score	**RC**	**LC**	**RM**	**LM**	**A**	**RCA**	**LCA**
0	54.1	56.8	24.3	40.5	51.4	73	73
1	13.5	18.9	21.6	8.1	18.9	24.3	24.3
2	18,9	10.8	18.9	27	8.1	2.7	2.7
3	2.7	2.7	18.9	8.1	10.8	0.0	0.0
4	10.8	10.8	16.2	16.2	10.8	0.0	0.0
Total	100	100	100	100	100	100	100

3.2. Histopathology

The most frequent microscopic lesions observed in lungs with pneumonic lesions were necrotizing bronchiolitis (23/38) (Fig. 2A) neutrophils infiltrate in the alveoli (24/38), type II pneumocytes hyperplasia (26/38), peribronchiolar lymphoid tissue hyperplasia (28/38), and mononuclear interstitial pneumonia (29/38). The interstitial infiltrate consisted mainly of lymphocytes and plasma cells, which were often perivascular and peribronchiolar. Some lungs presented constrictive bronchiolitis (Fig. 2B), alveolar edema, pleuritis, hyperemia, atelectasis and emphysema.

3.3. Immunohistochemistry

SIA antigens were detected in 84% (32/38) of lungs. Positive pigs were from Matutuine (5/32), Moamba (2/32), Namaacha (21/32), Boane (3/32) and Matola (1/32). Immunostaining was visualized in the nucleus of bronchioles (Fig.2C) and / or bronchi (25/32, 78%) epithelial cells (Fig.2D), or in macrophages located in the alveolar septa or in alveoli lumen (27/32, 84.4%). In 20/32 (62.5%) samples, the immunostaining was concomitant in epithelial cells of bronchioles and / or bronchi and in alveolar macrophages (Fig.2D). Eleven samples also showed immunostaining in the epithelium of bronchial glands. All lungs that presented pneumonia (n = 38) were negative for PCV2 and *M. hyopneumoniae*.

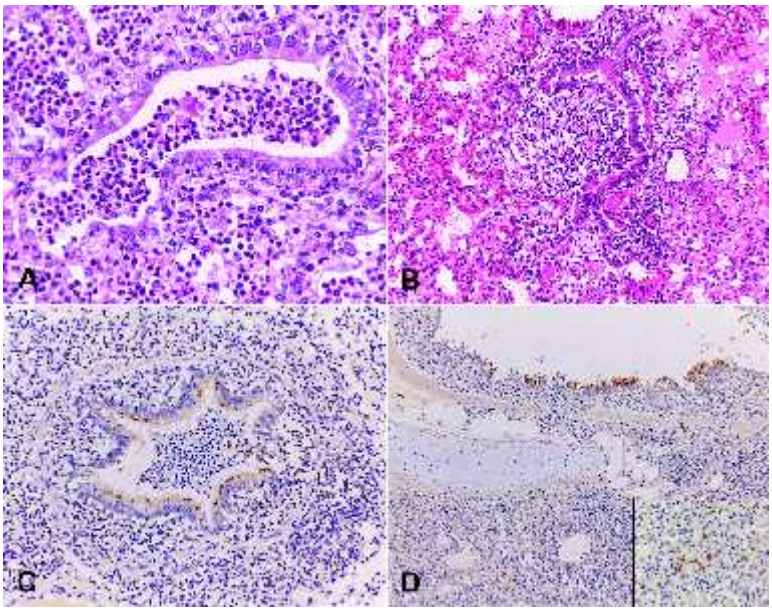

Figure 2. Histology and anti–SIA immunohistochemistry (IHC) of lungs from slaughtered pigs.**A**. Necrotizing bronchiolitis. HE, obj. 40x. **B**.Constrictive bronchiolitis. HE, obj.20x. **C**. SIA antigens in bronchial epithelial cells. IHQ, obj.20x. **D**.SIA antigens inbronchial epithelial cells and macrophages. IHQ, obj.10x (detail = 40x).

4. Discussion

This paper describes the frequency and severity of pneumonia, anatomopathological and immunohistochemical findings associated with SIA infection in pig in Mozambique. Pneumonia is the most frequent lung lesion detected in slaughter pigs [13]. The frequency of pneumonia observed in this study (8.3%) is lower than observed in similar studies in France (50.8%) [13], Spain (55.7%) [14] and New Zealand (63.4%) [15]. This difference of prevalence is possibly due to the fact that in Mozambique, swine farming is mainly extensive and semi-intensive, with majority of farms with a

low animal density. In the present study, the highest frequency of pneumonia was observed in pigs from a farm in Namaacha 68% (26/38) that makes the rearing of pigs in a semi-intensive breeding system. Farms with a greatest number of pigs are most likely susceptible to be affected by respiratory disease [8].
Pulmonary consolidation areas usually presented cranioventral pattern and mainly involved cranial, lobes. The main differentials diagnoses for this pattern of lesions in pigs include SIA, *Mycoplasma hyopneumoniae*, PCV2 and *Pasteurellamultocida*infection [14, 16].

Necrotizing bronchiolitis was one of the most frequent histologic lesion observed. This lesion has been described in pigs infected by H1N1, H1N2, H3N2 and H1N1pdm2009 SIA subtypes [1,17]. As described in other studies [10,17] SIA antigens were detected in epithelial cells of bronchi and bronquioles and / or alveolar macrophages. In 11 lung samples, IAV nucleoprotein was also detected in the epithelium of bronchial glands as previously described in pigs[18] and humans [19] infected by H1N1pdm2009.

Three pigs with histopathological lesions suggestive of SIA presented a negative result on IHQ probably because they had a sub-acute to chronic infection. Demonstration of SIA antigens by IHA occurs mainly in the acute phase of the disease (one to five days after infection) and the amount of antigens in the lung is reduced from the seventh day after infection [1,4,16].

SIA antigens were detected in pigs with 5 to 120 kg live weight demonstrating that this virus can infect pigs of different age groups, as described by Watanabe et al. (2012) and Rajão et al. (2013). Pigs infected by SIA usually present co-infection with other virus (eg. PRRSV, PCV2) and bacterias (eg. *Pasteurella multocida, Mycoplasma hyopneumoniae*) that cause respiratory diseases [20, 18]. In this study, lung samples were not submitted to bacterial culture or for PRRSV antigens or genome. It is not ruled out that there was coinfection between SIA and bacterial agents, since, several lungs had suppurative bronchopneumonia suggestive of bacterial infection. PRRSV has not yet been described in Mozambique.

SIA antigens were detected in pigs coming from several districts suggesting that this virus is widespread in the swine population in Mozambique. In this country, human infection by pandemic influenza A / H1N1 / 2009 virus was reported [21], however, there is no data reporting transmission of this virus between humans and pigs. In Africa, the pandemic influenza A / H1N1 / 2009 virus has been detected in pigs in Cameroon [22], Togo [23] and Ghana [24]. The results of this work demonstrate that SIA is a cause of pneumonia in pigs in Mozambique. Further studies should be developed to determine the true prevalence of SIA on the pig population and characterize SIA subtypes circulating in Mozambique.

Acknowledgments
Staff of the Division of Pathology, Veterinary Faculty, Eduardo Mondlane University for their work on the preparation of histologic samples. CNPq-TWAS Fellowships Programme 2012 and the NationalResearch Fund (FNI) of Mozambique for financial support.