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COMPARATIVE STUDY OF HEALTHY PIGS AND WITH INCREASED ON ARTICULAR VOLUME AND/OR LAMENESS: MICROBIOLOGY, MOLECULAR AND PATHOLOGICAL ASPECTS

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Abstract- The aim was to evaluate growing/finishing phase pigs presenting increased joint volume and/or lameness through microbiological, molecular and pathological aspects in Mato Grosso State, Brazil. Macroscopic and microscopic examination of joints from 43 that presented an increase of joint volume and/or lameness and 15 without signs. Macroscopically, the major changes were: arthritis 39.53%, hypertrophy of the villi of the synovial membrane 37.21% and muscle abscess, ligaments and peri-articular tendons 32.56%. In the bone extremities, the erosion was the main alteration in the articular surface 44.19%. Osteochondrosis 88.37%, osteomyelitis, 27.90%, osteochondritis dissecans 13.95%. From the tissues submitted to bacterial isolation and PCR, 72.09% were positive for at least one infectious agent. 67.44% were positive for bacterial isolation, 41.86%, infectious arthritis and 25.58% arthritis contaminant. On the molecular evaluation of the 43 samples, only eight 18.60% were positive for at least on infectious agent. The degenerative cause was present in all pigs, infection was an element of increasing severity, mainly because of the association with typical agents of articulation and contaminants.
Key words - Osteochondrosis, osteochondritis dissecans, synovitis, infectious, arthritis, osteomyelitis

Introduction

In recent years much have been studied about locomotor system diseases in pigs, yet is still subject of much discussion [1, 2, 3]. The prevalence in swine matrices is high, most of them presenting lameness, musclular fragility, impaired moviment, reproductive failure, low milk production, sometimes being euthanized for welfare matters or still to cause carcass condemnation in slaughterhouse. This higher frequency is associated by many authors to longevity [1, 4, 5, 6]. The pathogenesis of joint pathologies is related to infectious processes and/or degenerative, however, is considered multifactorial. The main risk factors involve the management conditions, buildings, nutritional, endocrine and circulatory disorders, rapid muscle development and/or biomechanical stress [7, 8]. Infectious processes are characterized by inflammatory reaction, usually acute, causing increase on articular volume in joint swelling and abnormal gait [9]. Some organisms have a predilection for the articular components and bone extremities, suggesting a likely affinity for synovial membranes and/or persistence of joint infection even with control of systemic infection [9,10].

The degenerative process of the articular cartilage and/or epiphyseal plate of long bones are called dyschondroplasia, and this processes is evaluated according to the stage and severity of the alterations, usually starting with osteochondrosis and may vary as osteochondritis dissecans, epiphysiolysis and/or osteoarthritis [9, 11]. The aim of this study was to evaluate swine in growing/finishing phase with increase of volume articular and/or lameness through microbiological, molecular and pathological aspects compared with healthy animals on farms in Mato Grosso, Brazil.

Material and methods

The study was realized on farms complete cycle, classified as Intensive Swine Production in the period from June 2007 to June 2009, at Mato Grosso State, Brazil. On each farm was realized examination of herd in the growing/finishing phase (70-150 days of life), regardless of sex and/or genetic background. It consisted in the observation of the pigs gait accordingly to the [8] methodology. The forelimbs and hind limbs were examined, animals presenting an increase of articular volume and/or lameness were selected. Only animals that did not received any treatment with antibiotic substances and/or anti-inflammatory were selected to participate to the study. After the animals were stunned by electrocution and euthanized by bleeding [12]. The survey was conducted, always starting from the bigger increase of volume regardless if it was forelimb or hind limb, when more them one joint was affected. The examination began with the dissection of the articular region standing out from the capsule and accessing the joint space. Once the joint was opened, fluid or semi-solid samples were collected to the microbiology and molecular biology examination. The samples were kept in ice until arrived at microbiology laboratory were bacterial isolation was performed, using the method of [10], and molecular identification by the polymerase chain reaction [PCR] of the main agents responsible for joint infection in pigs: *Streptococcus suis* [13], *Streptococcus suis* type 2 [5], *Erysipelothrix rhusiopathiae* [14]; *Mycoplasma hyosynoviae* [15]; *Haemophillus parasuis* [16] and *Brucella* sp [17].

The bones were examined macroscopically in two planes, the first it was the evaluation of all components extra-articular and articular after dissection mentioned earlier. The second was based on tranversal cross-section on proximal and distal extremities of long bones in relation to its axis using a hand saw. For the external evaluation of articular cartilage and the cut surfaces of the extremities, it was used the modified criteria of [5] as follow: Erosion - thinning of the articular cartilage; Ulceration - absence of articular cartilage, exposition of the subchondral bone, including the formation of flaps; Repair - formation of connective tissue or fibrocartilage, osteophytes, bony projections in the articular cartilage; Retraction - protrusion of the articular cartilage and subchondral bone and Normal - no changes in articular cartilage. These criteria were evaluated according to the intensity and distribution of the lesion in: mild (up to 50% of area) and severe (above 50%).

The histopatologic evaluation was performed on samples from proximal and distal bone of the same joint and associated structures. The samples were fixed in 10% buffered formalin for 24 to 48 hours. The bones were desmineralized with nitric acid to 8% for up to 96 hours. Histology sections were prepared in order to observe all the structural elements of a bone extremity: articular cartilage, growth plate and epiphyseal and metaphyseal trabecular bone, and the joint capsule, bursa and tissue attachments. On histological processing were realized two stains: hematoxylin-eosin (HE) and Masson trichrome [18], and visualization in optical microscopy. As control group it was used samples from health animals all around 130 days of age and selected from a slaughterhouse on the Federal Inspection Service without presenting increased of volume articular and/or lameness or other change locomotor activity. Tissues from control group were subjected to the same procedures of pigs with gait alterations.

Results and discussion

We evaluated 58 pigs, 43 pigs with an increase on articular volume and/or lameness, obtained from 06 commercial farms owning, approximately, a total of 20.000 pigs in the growing/finishing phase, its prevalence were not evaluated. And a group of 15 pigs, without presentation of clinical signs, selected in the pre-slaughtered in a slaughterhouse.

In the gross examination all animals from the diseased group showed firm and swollen areas in the joint region. The main changes were: arthritis (39.53%), hypertrophy of the villi of the synovial membrane (37.21%), abscesses in the muscles, ligaments and tendons peri-articulars (32.56%), cutaneous fistula in the region to articulate with pus of semi-solid consistence (30.23%), fractures (6.98%) and thickening and edema of the joint capsule (6.98%). Some of these changes were observed simultaneously in the same animal. When the cut surfaces of the bone extremities were examined, it became evident erosion of the articular surface (44.19%), repair (16.28%), ulceration (11.63%), retraction (9.30%) and without changes (18.60%). About the intensity and distribution it ranged from minor alterations (51.17%) and severe (30.23%).

All pigs had microscopic changes in the articular components, being the main find: osteochondrosis 38 (88.37%), osteomyelitis, 12 (27.90%), osteochondritis dissecans 6 (13.95%) and synovitis (6.98%). This result was obtained considering the main microscopic change in intensity and distribution.

Of the 43 samples from pigs submitted to bacterial isolation and PCR, 31 (72.09%) were positive for the presence of at least one infectious *Arcanobacterium pyogenes, Mycoplasma hyosynoviae, Haemophillus parasuis, Streptococcus suis* Type 1*, Streptococcus suis* Type 2 *and Erysipelothrix rhusiopathiae* remaining samples were considered opportunistic and responsible for causing arthritis contaminant.

Table 1 shows the morphological diagnosis, bacterial isolation and molecular diagnosis of 43 pigs. We note the following results: osteochondrosis (25.58%), osteochondrosis, arthritis infectious and per contaminant (18.60%), osteochondrosis, and arthritis per contaminant (16.28%), osteochondrosis and infectious arthritis (13.95%) osteomyelitis and infectious arthritis (6.98%), osteochondrosis, infectious arthritis, arthritis per contaminant and osteomyelitis (4.65%), osteochondrosis, infectious arthritis and osteomyelitis (4.65%), osteomyelitis and arthritis per contaminant (4.65 %), osteochondrosis, arthritis per contaminant

and osteomyelitis (2.33%) and osteochondritis and osteomyelitis (2.33%). Another significant finding is that 16.27% of the samples there was no macroscopic alterations, however bacterial growth and/or positive reaction in PCR. Moreover, in 11.63% of the samples was observed macroscopic changes without bacterial isolation/PCR.

The fifteen animals used as a control considering the absence of lameness and an increase of articular volume showed no lesions. However, microscopic changes found in all characterized by changes in the epiphyseal plate, characterized morphologically as osteochondrosis, in a mild degree. These 15 samples tested positive for bacterial isolates and PCR were negative for both tests.

In this work was realized a field study about the occurrence of clinical-pathological condition of increased articular volume and/or lameness in the growing/finishing pigs on farms in central Brazil. This work was motivated by numerous reports of condemnation of carcasses in slaughterhouses.

The morphological and etiological diagnosis proved to be an association between degenerative and infectious diseases. This is either by agents usually responsible for causing arthritis [19, 20] as well as other opportunistic agents, known contaminants [21].

The morphologic diagnosis of osteochondrosis recorded 88.37%, and its variant osteochondritis dissecans at 13.95%, this high rate is similar to other studies, including prevalence in farms or slaughterhouses. Nakano and Aherne [22] reported 75% lameness with boars showed severe humeral osteochondrosis; Althaus et al. [23] reported that 70% of arthritis observed in slaughterhouses processes are not infectious; Arnbjerg [24] in their study found 90% of finishing animals with osteochondrosis of radiographic evaluation, results similar to those found by [25]. However, Johnston [26] evalueted 52 joints collected from carcasses polyartropathy in slaughterhouse, and of these 21 (40.38%) had osteochondrosis. These differences should be analyzed with discretion, because as it is a complex etiology of the lesion, its multiple factors must be considered to justify their percentages.

The etiological diagnosis of articular and peri-articular components demonstrated by bacterial presence of typical agent of articular infection of 41.86%,being the most prevalent *Arcanobacterium pyogenes, Streptococcus,* Type 1 and 2, *Mycoplasma hyosynoviae* and *Haemophillus parasuis.* These results are similar to many studies [26], Thompson et al. [9, 11, 21, 27, 28, 29] examining 48 pigs with articular and peri-articular lesions, condemned at slaughterhouses founded contradicted results such as 87.5% of positive samples, and that was the main agent isolated *Arcanobacterium pyogenes* in 75% of cases. Similar to the lesion of osteochondrosis infectious arthritis is also considered multifactorial, so the differences percentage and even the type of infectious agents depend on their risk factors, geographic location and time of year.

The history of the treatment was realized on farms and the results demonstrated that infections by agents without tropism for articular region occur probably were iatrogenic action, mainly related to drilling in the articular region to drain fluid and application of drugs aimed at treating arthritis. This management practice was described in almost all farms. Many of the agents identified in this study are enterobacs, surfactants, skin and/or environmental and become opportunistic mainly by an entry in the lower limbs, mainly because of injuries hoof, or skin and even hematogenous way. These agents may be associated with other opportunistic agents or even with articular tropism as observed in this study.

Another significant finding is that 16.27% of the samples there was not macroscopic alterations, however bacterial growth and/or positive reaction in PCR. The likely explanation is a mild local inflammatory reaction not forming an exudative course, very common in pigs with immunosuppression, but it was not possible to corroborate this suspicion. Moreover, in 11.63% of the samples were observed with no gross changes bacterial isolation/PCR. In this case, animals should be being subjected to some form of antibiotic medication, probably in the feed or even parenterally. However, this question has not been confirmed by the head of the farm.

Conclusion

According to the results found that the clinical condition of an increased articular volume and/or lameness commonly observed in pigs herds was caused by a complex set of factors degenerative and infectious. The degenerative factor was present in all pigs, and infection was an element of increasing severity, mainly because of the association between typical agents of articulation and contaminants. In this study it was found a peculiar and improper practice of health management that was the drilling of the articular region with therapeutic goal.

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Author’s contribuition

ACSDF, JXDO, MDAS, MIVDS and CDSC were members responsible for data colleting and analysis., RLDS, LN and VD were responsible for performing the theoretical study, bibliographic review, manuscript writing and the supervised the process and edited the manuscript, manuscript drafting and final approval.

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Competing interest

The authors declare that there are no competing interests

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