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Distribution of Mast Cells in the Respiratory Tract of the Pig at Three Stages of Development

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Abstract: The current study provides quantitative information about Mast Cells (MC) in the respiratory tract of clinically healthy pigs, at three different stages of growth. The presence of MC was quantified in the tonsils, the right cranial lung lobe, the right caudal lung lobe, the left cranial lung lobe and the left caudal lung lobe, as well as in the traqueobronchial lymph nodes, in newborn, weaned and adult pigs. Five clinically healthy animals were employed for each stage, all serologically negative to *Mycoplasma hyopneumoniae*, PRRS, Aujeszky and *Actinobacillus pleuropneumoniae*. The extracted samples were processed by inclusion in paraffin and dyed with toluidine blue. Among recently born pigs, the greatest quantity of MC was observed in the tracheobronchial lymph nodes (1.44±1.84) and the least quantity in the right cranial lung lobe (0.16±0.04). Among weaned pigs, the greatest quantity was observed in the tracheobronchial lymph nodes (11.76±8.8 **p<0.001) and in the right cranial lung lobe right cranial lung lobe (6.8±4.7 *p<0.05). In tonsils of adult pigs, weighing 100 kg, was a greater amount of MC (7.96±3.01 *p <0.05). These data obtained from healthy animals will permit us to carry out future comparative studies in order to understand immunological phenomena in respiratory diseases.

Key words: Pig, respiratory tract, mastocytes, mast cells, distribution

INTRODUCTION

The epithelial surfaces of the respiratory tract represent an important component of the mucous immune system, which is continually exposed to inhaled antigens (Peeters *et al*., 2005; Pier *et al*., 2004; Bytautiene *et al*., 2004), for example environmental contaminants, allergens and microorganisms. These induce the activation of inflammatory cells (mastocytes, macrophages, eosinophyles, lymphocytes, dendritic cells, basophilous, neutrophils and platelets (Levine, 1995; Moran *et al*., 2006), whose purpose is to liberate chemical mediators causing physiopathological changes (Barnes, 1998; Villasenor *et al*., 1999). Local blood flow increases, causing bronchial constriction, plasmatic exudation, mucous secretion, neural effects, contraction of the smooth muscle, as well as attraction and activation of other inflammatory cells.

The mastocytes (mastung) or mast cells are derived from pluripotent hematopoietic stem cells, present in bone marrow and fetal liver; these cells give rise to lymphocytes, erythrocytes, megariocytes, neutrophils, eosinophyles, basophilous and monocytes (Takemoto *et al*., 2008; Brown *et al*., 2008).

All the MC are derived from the progenitors $CD34^+$, $C\text{-}Kit^+$, Ly-1, $CD14^-$ and $CD17^-$; these precursors instigate the differentiation of the mastocyte, when it enters the tissue and here, it is particularly influenced by the micro atmosphere and by phenotypical characteristics (Brown *et al*., 2008; Migliaccio *et al*., 2003; Okayama and Kawakami, 2006; Sur *et al*., 2007).

Cytoplasmic granules are usually manifested and these are metachromatic in terms of their interaction with coloring because of their high heparinic acid content; this is made evident when they are dyed with basic aniline such as toluidine blue (Tizard, 2002; Brown *et al*., 2008;

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Buckley and Walls, 2008). MC are usually found in the thymus, spleen, heart, skin and the mucous present in the respiratory organs, gastrointestinal tract and genitor-urinary system, as well as near the blood vessels and lymphatic vessels (areas where, infection may enter) (Abraham and Malaviya, 1997; Okayama *et al*., 2004; Metz *et al*., 2008; Femenia *et al*., 2005).

MC consists of effective immunological cells, crucial for inflammatory response because of their capacity for recruiting neutrophils and for promoting the proliferation of epithelial cells as well as the secretion of mucous from the mucous membrane. They also, stimulate angiogenesis and the proliferation of bronchial smooth muscle, by liberating IL-4, which attracts lymphocyte T cooperators; resulting in an extraordinary capacity for moderating innate and adaptive responses to infections (Abraham *et al*., 1997).

The importance of these cells has been studied by Abraham *et al*. (1997) focusing on infections caused by respiratory pathogens such as *Klebsiella pneumonie* and *Bordetella pertusis*, where three types of cells participate: the first increasing mucous secretions, causing the proliferation of epithelial cells and inducing bronchoconstriction; the second participating in phagocytosis and in the recruitment of neutrophils by means of secretion of TNF α (Suzuki and Verma, 2008) and the third stimulating Th_1 and Th_2 responses, as well as causing lung patophysiology.

In the study, the presence of the MC in the respiratory tract of pigs is scarce. A few reports exist concerning their distribution in areas such as; the uterine cervix of pigs, mucous and sub mucous of the intestine, tongue, urethra, skin, nerves and autonomous ganglions, as well as in the arteries and renal vein of the pig (Prado *et al*., 1999; Xu *et al*., 1993; Vodenicharov *et al*., 2005; Vodenicharov, 2008). There have also been studies describing distribution in human beings and other species (Villasenor *et al*., 1999; Iwamura *et al*., 2002; Peeters *et al*., 2005; Stefanov *et al*., 2007; Dimitrov *et al*., 2007; Kleinschmidt *et al*., 2008; Sanderson *et al*., 1985). Thus, the aim of this study was to establish the distribution and number of mastocytes in the tonsils, the 4 lung regions and the tracheobronchial lymph nodes, among newborn, weaned and clinically healthy, adult pigs (100 kg), using staining with toluidine blue.

MATERIALS AND METHODS

Animals: Fifteen clinically healthy Yorkshire pigs were used, 5 newborn, 5 weaned and 5 adult (100 kg), serologically negative for *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae* and *Pasteurella multocida* from a farm free of respiratory diseases.

Necropsy, collection and processing of samples: Pigs were sedated, anesthetized and sacrificed by bleeding. All the experiments were conducted in compliance with the Mexican regulations for animal care and maintenance (NOM-062-200-1999) (Chianini *et al*., 2001).

Aseptically obtained from the respiratory tract samples were taken of tonsils, right cranial lung lobe, right caudal lung lobe, left cranial lung lobe, left caudal lung lobe, lymph nodes tracheobronchiales without apparent pathological changes. A sample of each tissue was fixed immediately by immersion in 4% paraformaldehyde for 24 h at 4°C for observation of mast cells and 10% buffered formalin to seek changes in the microscopic structure of tissues sampled (Cruz *et al*., 2008). The samples were processed by the method of inclusion in paraffin and routine were cut with a microtome (Leica RM820) 3 and 5 μm thick, which were stained with hematoxylin and eosin and cut to 3-5 μm were stained with 1% toluidine blue (Sigma).

Detection of mast cells: The cells were identified by the metacromacia of their granules (intense pink colour) through an optical microscope at 40×. As a positive control staining was considered matacromacia chondroitin sulfate, a fundamental substance bronchial Cartilage's (Joseph *et al*., 2003; Sheehan Hrapchaek, 1980).

Counting and statistical analysis: Positive cells were counted in at least 10 randomly selected fields of each lung sample, using the Image-Pro Express (Media Cybernetics version 4.01) at 400× and the mean and standard error were obtained from each animal. The results were analyzed using the nonparametric Turkey test, using the program. GraphPad Software version 4.

RESULTS AND DISCUSSION

There is little information in the bibliography referring to the distribution of MC among pigs and thus, this study aims to analyze the population of these cells in specific regions of the porcine respiratory tract, by staining with toluidine blue.

No apparent pathological changes were observed with the macroscopic observation of the respiratory tract. Nor were any microscopic abnormalities observed when samples dyed with H-E were evaluated. When samples dyed with toluidine blue were quantified, it was found that there was a similar average number of mastocytes among newborn and weaned pigs, with the latter manifesting certain variations at this stage of growth. The average

Table 1: Number of Mast Cells (MC) per microscopic field in tonsils, lungs regions, tracheal and lymph nodes of pigs at different stages (n = 5)

Anatomic site	Newborn	Weaning	Adult 100 kg
Tonsils	0.86±1.06	1.16±1.53	7.96±3.01*
Right cranial lung lobe	0.16±0.047	6.80±4.7*	4.26±1.92
Right caudal lung lobe	0.64±0.85	0.63±0.91	3.54±1.41
Left cranial lung lobe	0.64±1.00	0.73±0.90	3.15±1.60
Left caudal lung lobe	0.55±0.42	0.53±1.37	3.26±1.32
Traqueobronchial lymph nodes	1.44±1.84	11.76±8.8**	4.81±3.92

Statistically significant difference *:p<0.05; **: p<0.001

quantity of MC in adult pigs is shown in Table 1, where an increase in this cellular population is notable. When considering the anatomical samples presented for newborn pigs, a greater number of MC were found in the tracheobronchial lymph-nodes (1.44±1.84), than in the right cranial lung lobe (0.16±0.047).

Among the weaned pigs, a greater quantity of MC was obtained from the tracheobronchial lymph nodes (11.76±8.8) and less from the left lung caudal lobe (0.53±1.37).

Among adult pigs, a greater number of MC was obtained from the tonsils (7.96±3.01) and a smaller amount from the left lung cranial lobe (3.15±1.60).

Concerning, the quantity of mast cells present at different stages of growth; a greater quantity of MC was detected from adult pigs. The data indicate that the greatest number of MC in newborn pigs was found in the tracheobronchial lymph nodes, however on comparing the data, no statistical difference was observed between the number of MC located in the samples for the various anatomic areas (p>0.05, Turkey test). Among, the weaned pigs, it was also observed that as for the newborn pigs, the greatest amount of MC was present in the tracheobronchial lymph nodes, this being one of the anatomical locations, which manifested significant statistical variation (**p<0.001, Turkey test). Possibly, the quantity of MC in lymph nodes is determined by their function, which is to retain antigens, which may enter by crossing the lymphatic liquids, subsequently applying antigenic processing with the collaboration of macrophages and lymphocytes, which form part of their composition. Among weaned pigs, significant statistical differences were also revealed in the case of the right cranial lung lobe (*p<0.05, Turkey test).

It has been suggested that the greater quantity of MC in the right cranial lung lobe arises because a bronchus exists in pigs, which leads directly from the trachea and ventilates the right cranial lung lobe, with the air, which enters this study. Once, it has overcome the physical barriers presented by the trachea, if there are any pathogens, these will enter directly into the lungs. Anatomically, pigs display a bronchus that ventilates the right cranial lobe and directly originates in the trachea, thus, allowing the entry into this portion of air, which has been previously filtered by the barriers where pathogens are trapped.

When comparative analysis is carried out using the data obtained from adult pigs, it is apparent that the tonsils manifest significant statistical difference (*p<0.05), when compared to the various anatomical sites sampled, owing to the fact that they act as a protective barrier against pathogenic agents (Church and Young, 1983) and form part of the immune system provided by the respiratory and digestive mucous.

The number of MC obtained in the present study was similar to the data presented by Xu *et al.* (1993) taken from the organs of healthy pigs, as the quantity of MC was greater in adult pigs than among pigs of only 1 month of age. It is thought that the immune system of adult pigs has already been stimulated by a number of antigens and is thus, more mature. Wilkes *et al.* (1992) and Chen *et al.* (1990) report that the MC in the respiratory tract of rats and calves also increases with age.

Concerning, the participation of MC in porcine respiratory problems, Cruz *et al.* (2008) carried out a study, which reports that the quantity of mastocytes in the lungs of weaned pigs, experimentally infected with *Mycoplasma hyopneumoniae* is greater when compared to the number of mastocytes found in the control group (healthy pigs). These data coincide with the data reported in this study, where the number of mast cells is also less.

Generally, the quantity of mastocytes may be explained by their particular importance in the respiratory tract, as they represent an important component in the mucous immune system, as this is constantly exposed to inhaled antigens (Peeters *et al.*, 2005) and is therefore, one of the main entrances for infection. Here, the MC may represent the principal inflammatory cells, which the invasive pathogens have to face (Abraham *et al.*, 1997).

On the other hand, no reports exist referring to the kinetics of MC in the porcine respiratory tract, as the pigs grow. Thus, it was observed that at newborn, the lymph nodes represent one of the organs with the greatest number of MC, reaching a maximum quantity at weaning; whereas in the case of the tonsils, the greatest quantity are found in the adult pig.

Concerning, the lung lobes the number of cells found within increases homogeneously, with the exception of the right cranial lung lobe, among weaned animals. It is notable that the MC reach their greatest number in this lobe, at this stage, this being where the greatest number

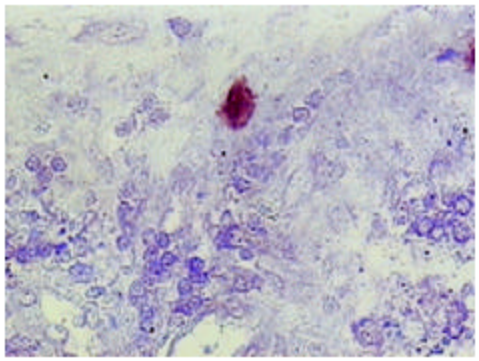

Fig. 1: Mast cell (red cell) located in the connective tissue of trabeculae interfollicular of tonsils. Toluidine blue staining 40×

of respiratory diseases occur, for example that caused by *Mycoplasma hyopneumoniae*, an infection, which begins precisely in this lobe. It would not be surprising if the macroscopic damage observed to result from this infection was caused by the stimulation of the MC together with CD8, which unleashes immunopathological processes as suggested by Cruz *et al*. (2008).

The MC in the tonsils was found to be distributed throughout the connective tissue of the trabeculae, around the blood vessels (Fig. 1). In the tracheobronchial lymph nodes of newborn pigs, MC was observed in the diffuse lymphatic tissue and the lymphatic nodules in contrast to that of weaned and adult pigs, where, it was mostly localized around the blood vessels in the connective tissue of the trabeculae.

MC was located in the alveolar septum of the lung, in the bronchial walls (location intraepithelial) in the smooth muscle and connective tissue around the bronchi and bronchioles. This distribution was similar to that reported by Xu *et al*. (1993) in pigs, Peeters *et al*. (2005) in dogs, Mair *et al*. (1988) in horses and Chen *et al*. (1990) in sheep at the level of the respiratory tract.

CONCLUSION

Among newborn pigs, the greatest quantity of MC is observed in the tracheobronchial lymph nodes and the least quantity in the right cranial lung lobe. Among adult pigs, the greatest quantity of MC is located in the tonsil and the least quantity is observed in the left caudal lung lobe.

The current study describes the distribution and quantification of the MC in the tonsils, traqueobronchial nodules and the lungs of clinically healthy pigs, observing a considerable tendency for MC to be located in the connective tissue.

These results will permit future comparisons to be conducted among pigs, which suffer from inflammatory or infectious respiratory diseases.

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REFERENCES

Abraham, S.N., K. Thankavel and R. Malaviya, 1997. Mast cell as modulators of host defense in the lung. Frontier in bioscence, 2: 78-87. DOI: 10.1172/JCI 119623.

Abraham, S.N. and R. Malaviya, 1997. Mast cells in infection and immunity. Infect. Immun. Minireview, 65 (9): 3501-3508. PMCID: PMC175499. ICID: 534944. PMID: 9284112.

Barnes, P.J., K. Fan Chung and C.P. Page, 1998. Inflammatory mediators of asthma: An update. Pharmacol. Rev., 50 (4): 515-596. PMID: 3064106.

Brown, M.A., B.A. Sayed and A. Christy, 2008. Mast cells and adaptative immune response. J. Clin. Immunol., 28: 671-676. DOI: 10.1007/s10875-008-9247-7. PMID: 18802742.

Buckley, M. and A.F. Walls, 2008. Identification of mast cells and mast cell subpopulations, methods in molecular medicine. Allergy Methods and Protocols, 138: 285-297. DOI: 10.1007/978-1-59745-366-0_24.

Bytautiene, E., Y.P. Vedernikov, G.R. Saade, R. Romero and R.E. Garfield, 2004. Degranulation of uterine mast cell modifies contractility of isolated myometrium from pregnant women. Am. J. Obstet. Gynecol., 191: 1705-1710. PMID: 15547545.

Chen, W., M.M. Alley, B.W. Manktelow and P. Slack, 1990. Mast cells in the bovine lower respiratory tract: Morphology, density and distribution. Br. Vet. J., 146 (5): 425-36. PMID: 2224488. http//www.defra.gov.uk.

Chianini, F., N. Majo, J. Segales, J. Dominguez and J. Domingo, 2001. Immunohistological study of the immune system cells in paraffin-embedded tissues of conventional pigs. Vet. Immunol. Immunopathol., 82: 245-255.

Church, M.K. and K.D. Young, 1983. The characteristics of inhibition of histamine release from human lung fragments by sodium cromoglycate, salbutamol and chlorpromazine. Br. J. Pharmacol., 78: 671-679. PMID: 6189542. PMCID: PMC2044757.

Cruz, S.T., R.S.S. Martinez, F.G. Garrido, P.J.L. Tortora, E.S. Mendoza, C.A. Ciprian, B.E. Hennandez, R.A. Romero and L.M.A. Vega, 2008. *In situ* analysis of CD4, CD8 and mast cells in lung of *Mycoplasma hyopneumoniae* experimentally infected pigs. J. Anim. Vet. Adv., 7 (6): 752-758.

Dimitrov, R., A. Vodenicharov, G. Kostadinov and H. Hristov, 2007. Morphological investigations on mast cells. In: Feline pelvic urethra Bulgarian. J. Vet. Med., 10 (1): 59-62. http://www.uni-sz.bg.

Femenia, F., M. Arockb, L. Lericheb, C. Delouisc, G. Milletd, N.B. Hamoudab, M. Cotea, A. Alliota, T. Liline, A. Pintonf, N. Iannuccelig, A.L. Parodie and P. Boireaua, 2005. Establishment and characterization of continuous hematopoietic progenitor derived pig normal mast cell lines. Life Sci., 77: 808-823. DOI: 10.1016/j.lfs.2005.01.017. PMID: 15896811.

Iwamura, T., K. Shinuzu and S. Tanaka, 2002. Morphological and histochemical characteristics of mast cellsand the content of in tissue histamine in various patological parathyroids: Do mast cells participate in hormone secretion in human Parathyroids? J. Nippon Med. Sch., 69 (4): 347-354. DOI: 10.1272/jnms.69.347. PMID: 12187367.

Joseph, S., S. Das, R. Chand, R. Roopa and I.M. Thomas, 2003. omparasion of toluidine blue vs. thionin for mast cells in rat mesentery using Carnoy's fixative. J. Anat. Soc. India, 52: 166-167.

Kleinschmidt, S., A.F. Meneses, I. Nolte, M. Hewicker-Trautwein, 2008. Distribution of mast cell subtypes and immune cell populations in canine intestines: Evidence for age related decline in T-cells and macrophages and increase of IgA-positive plasma cells. Vet. Sci., 84: 41-48. DOI: 10.1016/j.rvsc.2007.03.009. PMID: 17521688.

Levine, S.J., 1995. Bronchial epithelial cell-cytokine interactions in airways ephitelium. J. Invest. Med., 43 (3): 241-249. PMID: 7614070.

Mair, T.S., C.R. Stokes and F.J. Bourne, 1988. Distribution and ultraestructure of mast cells in the equine respiratory tract. Equine. Vet. J., 20 (1): 54-58. PMID: 3366107.

Metz, M., F. Siebenhaar and M. Maurer, 2008. Mast cell functions in the innate skin immune system. Immunobiol. Rev., 213: 251-260. DOI: 10.1016/j.imbio.2007.10.017. PMID: 18406371.

Migliaccio, A.R., R.A. Rana, M. Sanchez, R. Lorenzini, L. Centurione, L. Bianchi, A.M. Vannucchi, G. Migliaccio and S.H. Orkin, 2003. GATA-1 as a Regulator of mast cell differentiation revealed by the phenotype of the GATA-1 low Mouse Mutant. J. Exp. Med., 197 (3): 281-296. DOI: 10.1084/jem.20021149. PMID: 12566412. PMCID: PMC2193836.

Moran, G., O. Araya and H. Folch, 2006. Recurrent airway obstruction in horses. Arch. Med. Vet., 38 (3): 207-217.

Okayama, Y., S. Okumura, H. Tomita, H. Katayama, K. Yuki, S. Kagaya, J. Kashiwakura and H. Saito, 2004. Human mast cell activation through FC receptors and toll-like receptors. Allergol. Int., 53: 227-233. DOI: 10.1111/j.1440-1592.2004.00338.x.

Okayama, Y. and T. Kawakami, 2006. Development, migration and survival of mast cells. Immunol. Res., 34 (2): 97-115. DOI: 10.1385/IR:34:2:97. PMID: 16760571. PMCID: PMC1490026.

Peeters, M.J., F. Day, P. Farnir, C. Moore and J. Clercx, 2005. Distribution of leucocyte subsets in the canine respiratory tract. Comp. Path., 132: 261-272. DOI: 10.1016/j.jcpa.2004.10.003. PMID: 15893984.

Pier, G.B., J.B. Lyczak, L.M. Wetzler and M.J. Ruebush, 2004. Inmunology infection and inmunity editorial ASM press. Washington, DC, pp: 742. ISBN: 1-55581-246-5.

Prado, M.R., E. Lavielle, S.R. Anzaldua and M. Perez, 1999. Differential distribution of barley cells in the cervix in pig follicular development and ovarian corpus luteum. Vet. Mex., 30 (2): 143-147.

Sanderson, I., G. Slavin and T.J. Walker-Smith, 1985. Density of mucosal mast cells in the lamina propria of the colon and terminal ileum of children. J. Clin. Pathol., 38: 771-773. PMCID: PMC499301. DOI: 10.1136/jcp.38.7.771. PMID: 4019799.

Sheehan, D.C. and B.R. Hrapchack, 1987. Theory and Practice of Histotechnology. 2nd Edn. CV Mosby, St. Louis (USA), pp: 149. ISBN: 1574770675.

Stefanov, I.S., A. Vodenicharov and R. Dimitrov, 2007. Density, shape and dimensions of mast cells in Canine anal canal. Bulg. J. Vet. Med., 10 (2): 77-82.

Sur, R., D. Cavender and R. Malaviya, 2007. Different approaches to study mast cell functions. Int. Immunopharmacol., 7: 555-567. DOI: 10.1016/j.intimp.2007.01.009. PMID: 17386403.

Suzuki, K. and I.M. Verma, 2008. Phosphorylation of SNAP-23 by IkBKinase. Regulates Mast Cell Degranulation, Cell, 134: 485-495. DOI: 10.1016/j.cell.2008.05.050. PMID: 18692471. PMCID: PMC2586340.

Takemoto, C.M., Y.N. Lee, A.G. Jegga, D. Zablocki, S. Brandal, A. Shahlaee, S. Huang, Y. Ye, S. Gowrisankar, J. Huynh and M.A. McDevitt, 2008. Mast cell transcriptional networks. Blood Cells, Molecules Dis., 41: 82-90. DOI: 10.1016/j.bcmd.2008.02.005. PMID: 18406636. PMCID: PMC2478671.

Tizard, I.R., 2002. Veterinary Immunology: An Introduction. W.B. Saunders Co., Philadelphia, USA, pp: 121-129. ISBN: 0721682189.

Villasenor, G.H., R.E. Leville, S.R. Anzaldúa Arce and M. Perez Martinez, 1999. Distribution of mast cells mamamario estrus in the bitch (*Canis familiaris*) in active and inactive periods. Vet. Mex., 30 (4): 318-320. ID: 266736.

Vodenicharov, A., 2008. Mast cells in autonomic ganglio and nerves and in small blood vessels in the renal hilus of domestic swine, Bulg. J. Vet. Med., 11 (1): 31-36.

Vodenicharov, A., R. Leiser, M. Gulubova and T. Vlaykova, 2005. Morphological and immuno-cytochemical investigations on mast cells in porcine ureter. Anat. Histol. Embryol., 34: 343-349. DOI: 10.1111/j.1439-0264.2005.00618.x. PMID: 16288 604.

Wilkes, L.K., C. McMenamin and P.G. Holt, 1992. Postnatal maturation of mast cell subpopulations in the rat respiratory tract. Immunology, 75: 535-541. PMID: 1572699. PMCID: PMC1384751.

Xu, L.R., M.M. Carr. A.P. Bland and G.A. Hall, 1993. Histochemistry and morphology of porcine mast cells. Histochemical. J., 25: 516-522. DOI: 10.1007/BF00159282. PMID: 7691781.