Diagnosis of investigative pathology in the genital tract of buffaloes raised extensively in the State of Amapá, Amazon, Brazil*

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ABSTRACT. Ribeiro H.F.L., Mourão F.R.P., Monteiro F.J.C., Rolim Filho S.T. & Vale W.G. **Diagnosis of investigative pathology in the genital tract of buffaloes raised extensively in the state of Amapá, Amazon, Brazil.** [Diagnóstico investigativo de patologias no trato genital de búfalas criadas extensivamente, no estado do Amapá, Amazônia, Brasil.] *Revista Brasileira de Medicina Veterinária, 38(4):358-364, 2016.* Programa de Pós-Graduação em Ciência Animal, Universidade Federal do Pará, Rua Augusto Corrêa 1, Campus Universitário do Guamá, Belém, PA 66075-110, Brazil. E-mail: wm.vale@hotmail.com

The investigation of the 1000 genital tracts was conducted at slaughterhouse in the municipality of Santana and Macapá, State of Amapá and consisted of macroscopic and microscopic examinations of the ovaries, uterine tubes, uterus and cervix. The observations of each genitalia were documented with written records in detail, all changes diagnosed genitalia were classified as non-pregnant and pregnant female, with or without pathologies, respectively pathological fragments collected and fixed in 10% buffered formalin for histopathological examinations. Of the total investigated 661 (66.10%) genital system were pregnant and 339 (33.90%) were not pregnant. Genital tracts of the 1000/ 840 (84.00%) were normal, and of these, 575 (57.50%) were pregnant 265 (26.5%) were not pregnant. Among the 661 tracts genital were pregnant cow buffalo, 412 (62.3%) were in the right horn and 249 (37.7%) in the left horn. 160 genital tracts (16.0%) had pathology, while 86 (8.6%) were pregnant and 74 (7.4%) were not pregnant. Among the 160 (16.00%) diagnosed pathologies, the most prevalent were 114 (11.4%) in the fallopian tube, followed by 30 (3.0%) in the ovary and 16(1.6%) in utero or cervix. The pathologies were most prevalent fallopian tube adhesions, ovarian dystrophy and endometritis.

KEY WORDS. Amapá, amazon, pathology, genital tract, buffaloes.

RESUMO. A investigação nos 1000 tratos genitais foi realizada em dois matadouros no Município de Macapá, Estado do Amapá e consistiu em exames macroscópicos e microscópicos dos ovários, tubas uterina, útero e cérvice. As observações de cada genitália foram documentadas em fichas especiais, com anotações detalhadas de todas as alterações diagnosticadas, com as genitálias foram classificadas em: gestante e não gestante, com ou sem patologias, respectivamente e coletados fragmentos patológicos e fixados em formol tamponado a 10%, para a realização de exames histopatológicos. Do total investigado 661 (66,10%) tratos genitais estavam gestantes e 339 (33,90%) não gestantes. Dos

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1000 tratos genitais, 840 (84,00%) apresentavam-se normais, e, destes, 575 (57,50%) gestantes, e as demais 265 (26,5%) não gestantes. Entre os 661 tratos genitais gestantes, 412 (62,3%) encontravam-se no corno direito e 249 (37,7%) no corno esquerdo. 160 tratos genitais (16,0%) apresentavam patologias, sendo que 86 (8,6%) estavam gestantes e 74 (7,4%) não gestantes. Dentre as 160 (16,00%) patologias diagnosticadas, as mais prevalentes foram as 114 (11,4%) na tuba uterina, seguida pelas 30 (3,0%) no ovário e 16 (1,6%) no útero ou cérvice. As alterações de maior prevalência foram as aderências da trompa uterina, a distrofia ovariana e as endometrites.

PALAVRAS-CHAVE. Amapá, Amazônia, patologia, trato genital, búfalas.

INTRODUCTION

In spite of recognized natural vocation state of Amapá for livestock and the growing development of this activity primarily related to buffalo production tracts, the production and productivity in this species, are still insufficient to meet regional demand for meat and milk. It is believed that such problems are linked to the inadequate management of the production tracts, which in most farms is extensive, based solely on the use of native pastures, without fences, practically absent of sanitary and breeding controls, with negative inputs on the livestock husbandry (Paiva et al. 1986). According to the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA), 2015, the buffalo and bovine populations in the state of Amapá was 178.811 and 109.422 being the state of Amapá the only unit of the Brazilian Federation which has a population with more buffalo than cattle, that certainly seems to occur due the environmental conditions in the regions used for rearing large farm animals.

On the basis of this, in Amapá State this activity has become prominent in developing Araguari river area, mainly in marine tidal Alluvial Plain, where are located the largest cattle ranches using buffalo for beef production due the natural grasslands and extensive farming system. Throughout Araguari river and its tributaries in the fields are also planted artificial pastures, dominating species of Brachiaria sp. with a good development (Convênio SUDAM/OEA 1990). The region of animal's origin is formed by natural fields of native grasslands and marshes, localized on a broad floodplain area that suffers from periodic flooding, providing suitable environment for buffalo grazing in extensive conditions. Since many decades, interest on buffalo production has increased in Brazil due recognition of their exceptional husbandry characteristics as a producer of meat, milk and draft, and also because present a performance by far superior to other domestic species, when reared in extensive lowland conditions or poor quality soils, predominant in the Amazon region (Ribeiro & Vale 2005).

That being so, the knowledge about the diseases that affects the genital tract of buffaloes is essential in a management focused on results, then, to get higher reproductive efficiency, taking in account that studies should start with a basic work, such as identifying pathologies of female genital tract, that impair fertility and cause low reproductive performance in this species (Ribeiro et al. 1990). Furthermore, the knowledge of the herds reproductive status are important facts and basic for understand of inheritance characteristics and to apply suitable animal-breeding and other practices that can promote a rapid improvement of tropical livestock like artificial insemination and embryo transfer.

Thus, the objective of the present study was to investigate the prevalence of diseases of the genital tract of female buffaloes raised in Amapá state and slaughtered in two abattoirs in Macapá city, Amapá state, Brazil.

MATERIAL AND METHODS

The study was conducted at two abattoirs localized in the municipalities of Santana and the Fazendinha in the Macapá city, State of Amapá. A total of n=1000 genital tracts collected just after slaughter and were examined grossly for the investigation of status of reproductive tracts and macroscopic pathologies. The genital tracts were removed and closely inspected and palpated before any incision, there after the vagina and uterus were opened up to the utero-tubal junction examined. The gravid uteri were examined to determine the approximate age of the foetus and other external features and gestation ages were classified as early, mid and late. Then the material was put into refrigerator boxes, until a gross exam being conducted. The examination consisted of overview of the external features of the genital tracts followed thorough an investigation of the genital tract, beginning with the cervix, through the uterus, oviducts and ovaries ending.

The observations of each genitalia were documented in notes identifying numbered where they were written in detail all changes diagnosed. The searched genitalia were classified as non-pregnant and pregnant female buffalo, with or without amendment, respectively.

With the aid of a digital camera with 5.1 megapixels (Sony P93, Tokyo, Japan), higher expression records were documented in photos and reproductive fragments were collected in test tubes with 10% buffered formalin for histopathological examinations in the Laboratory of Pathology at the Federal Rural University of Amazon, in Belém, Pará, Brazil. For variables related to prevalence of diseases in every part of the genital system, was performed simple statistical analysis (Pimentel Gomes, 2000).

RESULTS AND DISCUSSION

From the total investigated reproductive tracts, n=661 (66.1%) were pregnant and n=339 (33.9%) were non pregnant. Among a total n=840 (84.0%) were found normal, and among these, n=575 (57.5%) pregnant and n=265 (26.5%) were non pregnant whereas n=160 genital tracts (16.0%) showed some form of pathology, of which n=86 (8.6%) were pregnant and n=74 (7.4%) were non pregnant, Table 1. Within this sample, it was also noted a high percentage of genital tracts pregnant 66.1% in both slaughtered samples. Even though this study indicate that the number of pregnant females were superior to that reported by Ohashi et al. (1984) which found 56.9% of pregnant genital tract in a study conducted in the state of Pará. Above all, it should be figured out that in this survey among 661 pregnant genital tracts, 236 (35.7%) belonged to females pregnant between six to nine months of pregnancy. Therefore, according to the Federal Law # 1283 of December 18th, 1950, Title VII, Chapter I, Article 113 of the Rules of the Industrial and Sanitary Inspection of Animal Products, determines that it should be avoided slaughter females in an advanced state of pregnancy (more than two thirds of the normal time of pregnancy) (Brasil 1950), which seems to be not observed at regional level. Over the n=74 (7.4%) of non-pregnant female buffaloes showing change in the genital tract were composed by pathologies of irreversible damage. Smith et al. (1973) found in a survey that in 5.238 (55.0%) non-pregnant animals, 1.644 (17.2%) had one or more abnormalities in the ovary or in different segments of the genital tract. In spite of this, in the present survey, pregnant female buffaloes showing some kind of pathologies in the genital tract totaled up 86 (8.6%) cases, revealing a prevalence much higher than that reported by Ribeiro et al. (1987), which found only 12 (1.9%). On the other hand, Vale et al. (1981) in a study involving 612 genital tract of pregnant female buffaloes obtained in a slaughterhouse, reported that among 355 of the females pregnant (58.0%), only 23 (3.7%) did not show any form of abnormalities.

Moreover, as can be seen in Table 2, among 160 (16.0%) of female genital tracts diagnosed with abnormalities, the most prevalent 114 (11.4%) were observed in the oviducts, followed by 30 (3.0%)

in the ovary and 16 (1.6%) in the uterus or cervix. Thus, it seems be obvious that the results here obtained were much lower than that found by Sharma et al. (1968) who showed an incidence of 40.9% cases of different pathological conditions in 1.518 genital analyzed somewhere in India.

In the present study the frequency of changes in the genital tract showed no difference between the seasons, in spite of Bhattacharya et al. (1971) in India, correlated the findings with different seasons and observed that in the warmer months there was a higher incidence of disorders, while in the colder months these percentages decreased significantly.

Ovarian abnormalities

Ovarian dystrophy. In the present investigation, among ovarian pathologies, the most prevalent was the ovarian dystrophy, 16.0 (1.6%) cases, Figure 1A. Further on, Smith et al. (1973) in a study of 500 female genital tract of buffaloes, observed 59.0 (11.8%) cases of ovarian dystrophy whereas Rama Rao and Rajya (1976), in a study involving 7.500 female genital tract of buffaloes, found 7.4% of ovarian hereditary pathologies, Table 2.

Notwithstanding Narasimha Rao and Sreemannarayana (1982) stated that the occurrence of dystrophic ovaries were the most prevalent and important changes occurring in the ovary of buffalo, the present study is in accordance with the statement of these authors and corroborates the findings of Ribeiro et al. (1987) which investigated 629 genitalia of buffaloes and concluded that ovarian dystrophy is the pathology of highest incidence in buffalo females raised in state of Pará, with 52.3% cases.

These findings were later confirmed by Ribeiro et al. (1990) when it was concluded that the reproductive efficiency of female buffaloes may be limited during the postpartum period by the presence of ovarian dystrophy, either the author's highlights food deficiency as a predisposing factor, which is accentuated in the time of flood of Amazon river when food is scarce. Such assumption was confirmed in the present study throughout the use of histopathological examination, which revealed a reduced presence primordial follicles, high number of primary atretic follicles as well as follicular cysts of type 1 to 3, showing also flattened wall at the final stage of evolution while in the cortex there was a further development of fibrosis and absence of corpus luteum and albicans Figure 1B.

Follicular cyst. In this study it was found only one (0.1%) cases of follicular cyst, totaling 0.6% of the total changes in the ovary, lower the percentage

found by Smith et al. (1973) which studied 500 genital tracts of buffaloes and found that 17 cases (3.4%) of ovarian cysts and was also lower than reported by Rama Rao and Rajya (1976) and Narasimha Rao and Sreemannarayana (1982), who reported 0.3% and 0.5% cases respectively.

However our result was similar to that found by Ribeiro & Vale (1988), noted that only one case (0.16%). Histopathology showed the presence of primordial follicles and various tertiary atretic follicles.

Oviduct abnormalities

Adhesions. The occurrence of adhesions in the different segments of the oviduct were frequently observed and the focal adhesions with n=70 (8.3%)



Figure 1. A- Ovarian dystrophy; B- Photomicrograph of ovarian stroma with no growth of tertiary follicles. The arrow indicate atretic follicles. Obj 40x, H.E; C- Focal adhesion between the ovary and oviduct. Arrow; D- Hydrosalpingx uni-lateral right on the right ovary and oviducts; E- Uterus with diffuse adhesions between right horn, mesometrium and ovary, forming a large tube-ovarian cyst and hydrosalpinge; F- Postpartum metritis infection affecting the whole uterus.

Table 1. Gestational status, numbers and percentages of changes from n=1000 female genital systems originating from slaughterhouses, the municipalities of Santana and Macapá, Amapá state, Brazil.

Reproductive status	Normal	Abnormal	Total
Pregnant	575 (57,5%)	86 (8,6%)	661(66,1%)
Nonpregnant	265 (26,5%)	74 (7,4%)	339 (33,9%)
Total	840 (84,00%)	160 (16,00%)	1000(100,00%)

Table 2. Number and percentage of abnormalities of the genital tract of n=160 female buffaloes among n=1000 investigated in two slaughter- houses in the municipalities of Santana and Macapá, in Amapá state, Brazil.

Type of abnormality	Number	Ov	Overall percent		
Ovary					
Ovarian dystrofy	16	1,6	10,00	53,33	
Paraovarian cyst	5	0,5	3,125	16,66	
Epithelial inclusion cyst	8	0,8	5,0	26,66	
Folicular cyst	1	0,1	0,625	3,33	
Sub-total	30	3,0	18,75		
Oviduct					
Hydrossalpingx	18	1,8	11,25	15,79	
Tuboovarian	5	0,5	3,125	4,38	
Focal adhesion	70	7,0	43,75	61,40	
Difuse adhesion	21	2,1	13,125	18,42	
Sub-total	114	11,4			
Uterus and cervix					
Endometritis I	2	0,2	1,25	12,5	
Endometritis II	5	0,5	3,125	31,25	
Endometritis III	2	0,2	1,25	12,5	
Perimetritis	4	0,4	2,5	25,00	
Uterine segmental aplasia	1	0,1	0,625	6,25	
Twin pregnancy	1	0,1	0,625	6,25	
Irregular cervix	1	0,1	0,625	6,25	
Sub-total	16	1,6			
Total	160	16,00	100,00	-	

cases, Table 2 and Figure 1C, followed by diffuse adhesions with n=21 (3.5%) cases, both types of abnormalities bilateral occurrence present in pregnant and non-pregnant buffaloes. The percentage of this abnormalities occurrence in this study is similar to the 25 (3.9%) cases found by Ribeiro & Vale (1988) but higher than six (0.9%) cases reported by Shalash (1958), as well as the data described by Narasimha Rao & Sreemannarayana (1982) who found 153 (0.7%) cases. Hence, Ohashi et al. (1984) observed an incidence of 54 (9.1%) cases, of which 11 were mild focal adhesions present in pregnant animal. This fact confirms the view Shalash (1958) which stated that this form of adherence can hinder uptake of the oocyte, and should not necessarily lead to infertility in animals.

Macroscopically the form of these middle adhesions presents itself constituted by fine grooves adhered to the ovary, easy to be removed manually seems not to interfere with the oocyte capitation by the fimbria, whereas the severe form of multi--strands constitute fibrous adhering the oviduct and the ovary ligament can certainly interfere with oocyte capitation and lead for a impairment of the fertility (Ribeiro & Vale 1988).

Hydrosalpinx. On the whole in this study were found 18 cases (1.8%) of hydrosalpinx, Table 2 and Figure 1D, below to 3.5% quoted by Ohashi et al. (1984), however, it was slightly higher than that found by Sharma et al. (1968) of 1.0% and Luktuke et al. (1973), with one (1.1%) as well as higher than those found by Narasimha Rao and Sreemannarayana (1982) and Kumar et al. (1978) that reported an incidence of (0.03%) and (0.3%) cases, respectively.

In state of Pará, Ohashi et al. (1984), reported 21 (3.3%) cases and Johari (1960) that stated the presence of bilateral hydrosalpinx in two buffalo heifers, which had been considered as repeat breeders.

Likewise, Ohashi et al. (1984) and later Ribeiro & Vale (1988) stated that this change was always accomplished by adhesions from tuba to varying degrees, mainly in the region of the infundibulum. This fact was also observed in this study. The hydrosalpinx according Ohashi et al. (1984) when unilateral not prevent pregnancy, since some animals were expected to become pregnant but significantly show reduced fertility.

Tube-ovarian cyst. In this study there were five cases (0.5%) of tube-ovarian cysts, Table 2 and Figure 1E, lower than the percentage of 1.8% reported by Ohashi et al. (1984). According to the same author, despite the occurrence of this formation is relatively rare in certain species, buffaloes is great importance for determining clinical problems related to female fertility. The changes of the oviduct has a prominent place as a cause of reproductive problems in buffaloes, a fact already noted by (Elwishy et al. 1972, Ribeiro et al. 1987).

The formation of tube-ovarian cyst is consequent to inflammation of infectious origin, which ascend to the uterus and oviducts originate directly from peri oophoritis and asymptomatic salpingitis. In bovine, in enzootic areas of the occurrence of this disturb, brucellosis has been considered one of the main causative agent (McEntee 1990).

Indeed, as the occurrence of brucellosis at regional area is high, mainly in the extensive buffalo production tracts in the region it can be inferred that this disease can be responsible for such aberrations which is in accordance with the report of Freitas et al. (1997) that considered as one of the causes for the formation of tube-ovarian cysts.

Histopathology processed material showed that the cysts consisted of smooth muscle tissue, which

is seen cystic structure hyalinized wall, covered by pseudostratified epithelium, with some hair cells. In some areas proved atrophic and monostratified epithelium, these characteristics compatible with cystic dilation of the oviduct.

Uterus abnormalities

Uterine segmental aplasia. Abnormalities caused due to arrested development of the paramesonephric ducts was found in this study, one case (0.01%) in the form of segmental aplasia of an uterine horn, Table 2, such disturb have been reported in female buffalo (Rama Rao & Raja 1976, Vale et al. 1981, Vale et al. 1991). Although this finding is of almost negligible incidence, it is worth highlighting the involvement of this form of hereditary pathology.

Endometritis. Regarding to uterus abnormalities, uterine infection were found in a very low occurrence, nine (0.9%) cases of endometritis, result that were lower than that reported by Smith et al. (1973), studying 500 tracts genital buffaloes, observed 137 (27.4%) cases of endometritis. Ohashi et al. (1984), found 44 (7.4%) cases of uterine infection. Likewise, the findings of this research showed also to be lower than those cited by Kumar et al. (1978), who found 36 (4.3%) cases, Narasimha Rao and Kevasamurthy (1971) with 318 (30.2%) cases and Chaudhry et al. (1978) with (42.2%).

Ribeiro & Vale (1988) in a study in the lower Amazon region, state of Pará, found 32 (5.0%) females with varied types of inflammation of the endometrium. According to the authors, macroscopically the uterine horns showed up enlarged, elongated, rigid and distortion in your configuration. After cutting the structure, there was swelling, redness and intense mucopurulent secretion.

In this study uterine infection were classified in endometritis of first, second and third degrees, according to the amount of pus in the uterus, and here it was found 2 (0.2%) 5 (0.5%) 2 (0.2%) cases of 1st, 2nd and 3rd degree, respectively Figure 1F, Results which were quite lower to those reported by Ribeiro & Vale (1988) that found that 17 (2.7%), 9 (1.4%) and 6 (0.9%) cases of the 1st, 2nd and 3rd degree, respectively.

Indeed, some factors have been suggested as predisposing to an increased incidence of uterine infections in buffaloes such as the anatomical constitution of the vulvar labia of the female buffalo, the habit of this species watering in stagnant water, contaminated with faeces and poor hygienic conditions, as well as poor management and health conditions of flocks raised extensively (Ribeiro et al. 1987, Ribeiro et al. 1995).

Perimetritis. In the present study were diagnosed four cases (0.4%) of perimetritis. Ribeiro & Vale (1988) found an incidence of (0.6%) of this pathology and noted the diffuse presence of fibrin throughout the extent of uterine serosa. Inflammation of serous uterine tissue adjacent to the uterus, result in the formation of adhesions between the uterus and its ligaments, omentum, rectum, intestine and body wall.

On histopathological examination, the following characteristics showed hypertrophic uterine vessels and hyalinized endometrium with maceration and with spongy areas infiltrated by lymphocytes and haemorrhagic areas. The perimetrium showed villous excrescences organized without cellular reaction, being characterized perimetritis fibroplasic associated to a mild chronic endometritis.

Probably this pathology may be a result of tuberculosis in as much as such disease is relative common at regional level therefore, may be one reason for the prevalence of perimetritis and other forms of uterine infection. Besides, the high incidence of tuberculosis has been reported in Amazon condition by Freitas et al. (1997) which was also supported McEntee (1990) which described a correlation between tuberculosis and perimetritis which may be its primary cause.

In term of the incidence of this disease, Freitas et al. (1997) studied pathological and microbiological aspects of tuberculosis in buffaloes slaughtered for consumption in the State of Pará. The author revealed a prevalence rate of 7.7%, being 72.1% of changes localized caused by tuberculosis and 27.9% of generalized alterations.

Irregular cervix. Among the abnormalities described in the cervix of buffalo females, irregular cervix, Table 2, seems to be very rare and in the literature it was not found any report on this form of disturb.

Finally it was found a case of twin pregnancy, Table 2, although even not considered as pathology, because of its rare occurrence in buffaloes the record was made for such occurrence.

CONCLUSIONS

The diseases most prevalent were of acquired source such as: uterine horn adhesions to the ovaries, ovarian dystrophy and uterine abnormalities (endometritis and perimetritis). The findings and results such pathologies observed, reinforce the importance of gynecological examinations and health control in female buffaloes raised in extensive areas of herding buffaloes the Amapá state, Brazil.

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