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Identification of enteropathogens from buffalo calves with and without diarrhoea in the Ribeira Valley, State of São Paulo, Brazil

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Identificação de enteropatógenos em bezerros búfalos, com e sem diarréia, do Vale do Ribeira, Estado de São Paulo, Brasil

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SUMMARY

Faecal samples of 106 buffalo calves aged 3 to 45 days were collected sequentially, once a week, during six weeks for parasitologic examination. For bacteriologic and virologic exams, faecal samples were collected from all diarrhoeic and the same amount on non-diarrhoeic calves. Blood samples from calves were collected at 3 to 10 days of age for serum IgG determination. *Eimeria* spp, *Strongyloides papillosus* and *Toxocara vitulorum* were the most common parasites. *E. coli*, *Enterobacter cloacae* and *Klebsiella pneumoniae* were the enteropathogens most frequently found (alone or in association with other microrganisms). Heat-stable (STa) enterotoxin was detected in 5 out of 34 samples of *E. coli* isolated from diarrhoeic calves. Among the antimicrobials tested susceptibilities to norphloxacin, chloramphenicol and gentamycin were the most common. None of the samples had characteristic rotavirus RNA bands in PAGE-electrophoresis. No association was observed between low levels of IgG and diarrhoea.

UNITERMS: Diarrhoea; Buffalo calves; Immunoglobulins.

INTRODUCTION

Diarrhoea is a well-known clinical sign in neonatal animals. Its aetiology is complex involving management, environmental, nutritional and physiological variations and various infectious and parasitic agents[1].

In young buffalo calves, diarrhoea was considered the most important cause of economic losses in Egypt[2] and Italy[3]. In Brazil, diarrhoea in buffalo calves has been investigated[4,5,6]; however, the studies were restricted to parasitic or viral agents of diarrhoea separately.

E. coli ETEC, *Salmonella* sp, *Clostridium* sp, *Eimeria* sp, *Cryptosporidium* sp, *Toxocara vitulorum* and rotavirus are described as important agents causing diarrhoea (either separately or in combination) in buffalo calves[2,7,8].

Studies worldwide have shown that 10 to 40% of bovine calves fail to attain adequate colostrum-derived serum IgG concentrations. Hypogammaglobulinaemia is strongly correlated with increasing morbidity and mortality[9] predisposing the animals to different enteropathogens[10,11]. In buffaloes, approximately 20 mg ml^{-1} is considered the minimum level to confer protection against infections in new-born calves[12].

The aim of this study was to investigate the relationships between potential agents of diarrhoea, passive immunity and diarrhoea occurrence in young buffaloes from Ribeira Valley, State of São Paulo, Brazil.

MATERIAL AND METHOD

Study Design

Faecal samples of 52 male and 54 female (total of 106), Murrah and crossbreed buffalo calves (*Bubalus bubalis*) from 3 to 45 days of age at the beginning and end of study, were collected sequentially, once a week, during six weeks for parasitologic examination. For bacteriologic and virologic exams, faecal samples were collected from all diarrhoeic and the same amount on non-diarrhoeic calves. Blood samples from calves were collected at 3 to 10 days of age for serum

IgG determination. The study was conducted from March to July 1996 in Ribeira Valley, State of São Paulo, Brazil.

Selection of farms, animals and faecal sampling

Ribeira Valley is located in the South of São Paulo State and is characterized by a humid tropical climate and a wet and low-fertility soil.

Four medium-scale dairy farms (range 50-150 animals per farm) were used. They were all similar in general management of calves, animal installations, hygiene and previous history of diarrhoea.

An average of 26 calves per farm (range 11 to 42) were used in the study. Buffalo calves born in the four farms during the typical calving season, from March to July (the rainy and early dry season in the region), were identified and included in the study.

All faecal samples were collected and inspected by the same group of investigators, including veterinarians and technicians.

Diarrhoea was defined as an abnormally loose consistency of faeces, and was classified according to the clinical signs present in the calves (anorexia, depression, weakness); observations including the colour and consistency of faeces.

For parasitic and virologic agents, faecal samples were collected directly from the rectum using plastic hand gloves, identified with farm name and the animal's number and age. For bacteriologic examinations faecal samples were collected directly from the rectum using swabs. Samples were taken to the laboratory immediately after collection and preserved at 4°C (for parasitologic and bacteriologic examination) and -70°C (for virologic examination) until examination.

The samples for parasitologic examination were collected from 106 calves from the first week of age and sequentially during the following five weeks and classified as diarrhoeic and non-diarrhoeic. For bacteriologic and virologic exams, the samples were collected on each farm, from all diarrhoeic animals (48 buffalo calves). When diarrhoea was identified, the same amount of non-diarrhoeic animals (same farm, age and sex) was sampled (55 buffalo calves).

Two calves died during the period of study and the dates were not included in the evaluation of the results.

Parasitological Techniques

For coccidia and *Giardia* diagnosis, the water-ether centrifugal sedimentation technique[13] followed by saccharose (specific gravity = 1,203 g cm^{-3}) flotation was used[14]. The first technique was used to remove the excess of fat present in the faeces of new-born animals. *Cryptosporidium* oocysts were observed and measured microscopically (400 x) for species diagnosis[15]. *Eimeria* spp - positive samples were incubated at 26°C for 10 days in potassium dichromate for sporulation and identification[16].

Bacteriological Techniques

Faecal samples were cultured in defibrinated sheep blood-agar, MacConkey agar and tetrathionate base[17]. The microorganisms isolated were identified on the basis of cultural, morphological and biochemical characteristics[18]. The agents isolated from diarrhoeic animals were submitted to antimicrobial susceptibility test[19] using ampicillin, cephalexin, chloramphenicol, enrofloxacin, furazolidone, gentamycin, neomycin, norphloxacin, tetracycline and trimethoprim-sulfamethoxazole. The tested drugs were commercially available and extensively employed in the region of the study.

E. coli isolated from diarrhoeic and non-diarrhoeic samples were submitted to heat-stable (STa) enterotoxin using the infant mice-test[20] and heat-labile (LT) by radial immune hemolysis test[21,22].

Virological Techniques

These samples were submitted to polyacrylamide gel electrophoresis (PAGE) examination, and were considered positive for rotavirus if they exhibited characteristic patterns when stained with silver[23,24].

IgG Quantification

Serum samples collected from the 3rd to the 10th day of age were submitted to radial gel diffusion[25,26] using a commercial kit (IgG NL RID Plate, Bind a Raid™).

Statistical methods

Statistical analyses were made with the stratified Mantel-Haenszel Test[27] using Epi Info (version 6) Statistics Program[28], with alpha=0.05 (two-tailed). This compared the isolation or detection rates of the different enteropathogens with diarrhoeic and non-diarrhoeic calves, controlling for the effect of farms.

RESULTS

Fig. 1 shows the prevalence and incidence of diarrhoea during the six first weeks of age. Forty-eight calves showed diarrhoea and, from these, 10 presented enteric signs in 2 or 3 different weeks over the study (totalling 62 diarrhoeic samples).

The occurrences of the more common enteropathogens identified in buffalo calves with and without diarrhoea are in Tab. 1. From the 106 animals examined during the study from parasitic origin, we identified: *Eimeria* spp, *Strongyloides papillosus*, *Toxocara vitulorum*, *Cryptosporidium parvum*, *Giardia* spp and Strongyloidea. The occurrences of these agents

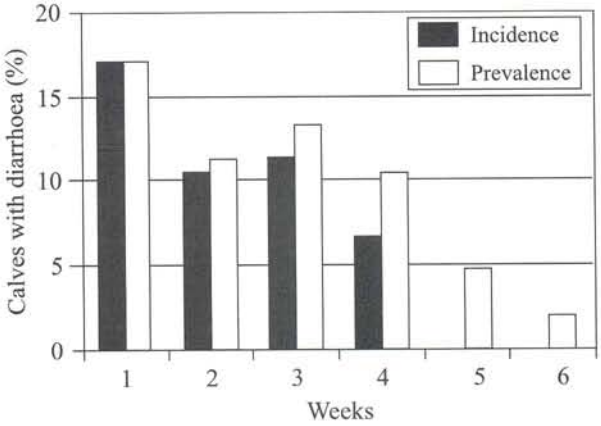


Figure 1
Prevalence and incidence of diarrhoea in buffalo calves during the six first weeks of age (Brazil, 1996).

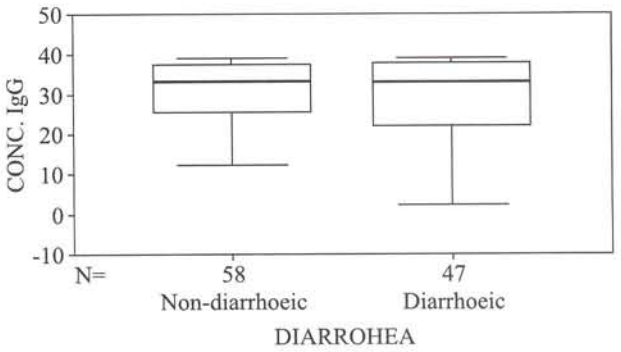
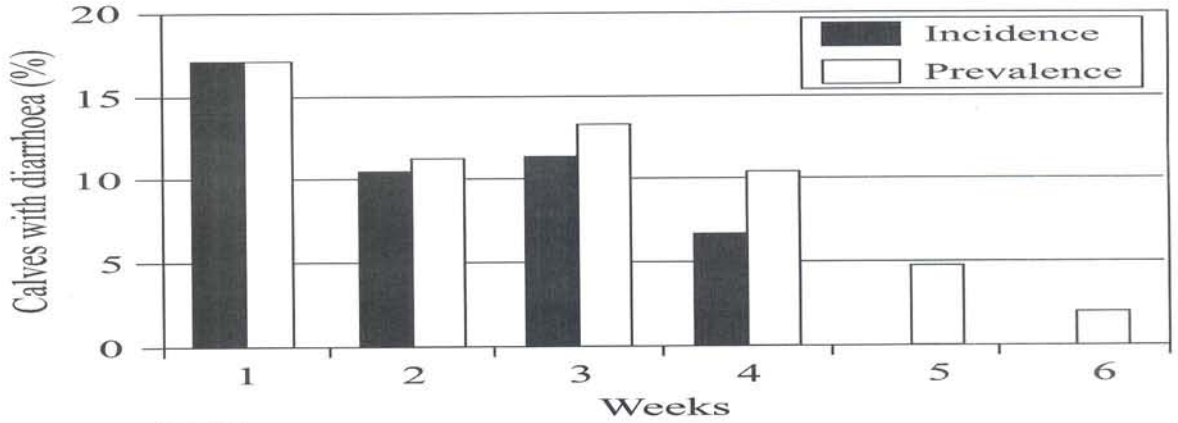


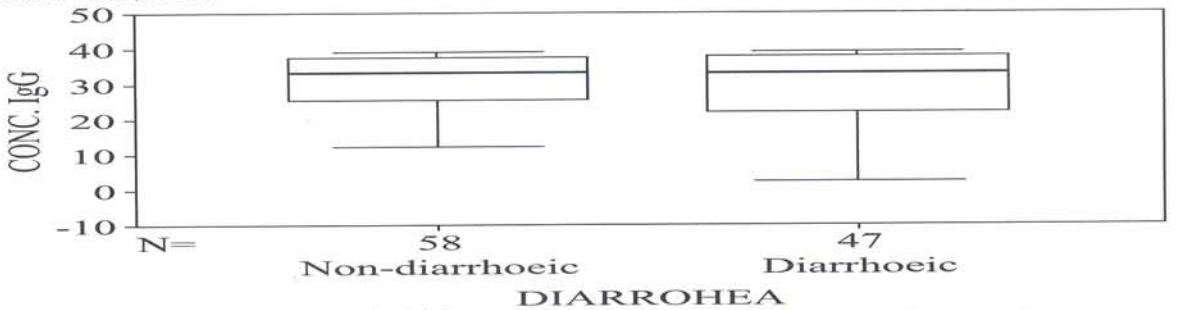
Figure 2
Distribution of IgG concentration in the diarrhoeic and non-diarrhoeic buffalo calves (Brazil, 1996).

Table 1
Occurrence of the more common enteropathogens identified in buffalo calves with and without diarrhoea in Brazil, 1996.



[a] Recurrent cases are not included.

Table 2
Results of antimicrobial susceptibility test of more common microorganisms isolated (pure or associated) from buffalo calves with diarrhoea in Brazil, 1996[a].



[a] Recurrent species of the same animal were not included;
[b] trimet-sulfam.= trimethoprim-sulfamethoxazole.

in the diarrhoeic and non-diarrhoeic calves were not significantly different (Tab. 1).

Eimeria was the most frequent genera identified, present in all animals (with and without diarrhoea) at least in one sample during the study and with its highest prevalence at age 3 weeks. The species identified were: *E. bovis, E. zuernii, E. auburnensis, E. canadensis, E. cilindrica* and *E. subspherica*.

S. papillosus and *T. vitulorum* were identified from the 1st and 2nd week of age, respectively. The highest prevalence was observed at the fourth week of age (with 50% and 25%, respectively, for *S. papillosus* and *T. vitulorum*) and remained with high prevalence until the last observation.

Strongyloidea eggs were found in 5 calves between the 2nd and the 3rd week, only one of which was identified as diarrhoeic. It was not possible to do faecal cultures for identification of the nematode genera, due to the low amounts of faecal material collected from the new-born animals.

The genera *Giardia* was found in 2 diarrhoeic and 3 non-diarrhoeic calves, from the 3rd week and highest prevalence occurred at age 6 weeks.

Cryptosporidium parvum was identified in 4 calves with enteric signs and in 6 calves without diarrhoea. It was identified in samples taken between the 3rd and the 6th week of age, and most frequently in the 6th week.

Eighty-eight species of microorganisms were isolated from 48 diarrhoeic animals, in pure culture or in associations. From these, *E. coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Citrobacter* spp were the most frequent (Tab. 1), following by other agents less frequently identified as: *Enterococcus* spp, *Hafnia alvei*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Enterobacter aglomerans*, *Enterobacter aerogenes* and *Shigella* spp.

Of the 48 diarrhoeic animals, 20 result in single species isolations (pure culture) and 28 resulted in mixed species isolation. *E. coli* (55.0%) and *E. cloacae* (30.0%) were the most frequent species in pure culture and the most common association in mixed isolations (28.6%).

The microorganisms identified in the 55 non-diarrhoeic calves are summarised in Tab. 1. One hundred and eighteen species of microorganisms were identified (in pure culture or in association), involving mainly *E. coli*, *E. cloacae* and *K. pneumoniae*. Other agents less frequently observed were: *Edwardsiella tarda*, *E. aerogenes*, *E. aglomerans*, *Enterococcus* spp, *H. alvei*, *K. oxytoca*, *Providencia stuartii*, *P. aeruginosa*, *Shigella sonei* and *Shigella* spp.

E. coli, *E. cloacae* and *K. pneumoniae* were, respectively, the more common agents identified in the diarrhoeic and non-diarrhoeic calves (Tab. 1); however the occurrence was statistically higher only for *E. coli* ETEC (limit of confidence interval), *Citrobacter* spp and *E. cloacae* ($p < 0.05$).

STa enterotoxin was detected only in the diarrhoeic calves, in 5 (14.7%) of the 34 *E. coli*; LT enterotoxin was not identified in any calf.

Among the antimicrobials tested, norphloxacin, chloramphenicol and gentamycin presented the highest sensitivity against the most frequent bacteria identified in diarrhoeic calves (Tab. 2).

Several associations of agents from parasitic and bacterial origin in the diarrhoeic animals were observed. The most frequent associations were: *Eimeria* spp, *S. papillosus*, *T. vitulorum* and *E. coli* (12.5%); *Eimeria* spp, *S. papillosus*, *T. vitulorum* and *E. cloacae* (10.4%).

Only in two diarrhoeic animals bacteria were not isolated. From these, one presented only parasites and the other no enteropathogen was identified.

Rotavirus was not identified in any diarrhoeic or non-diarrhoeic animal.

No association was observed between low IgG levels and the occurrence of diarrhoea in the present study. The values ranged from 0 to 39 mg ml^{-1} (Fig. 2). From the 105 calves, 21 presented IgG levels lower than 20 mg ml^{-1}, and from these 53.4% (11 calves) and 46.6% (10 calves) were, respectively, diarrhoeic and non-diarrhoeic.

DISCUSSION AND CONCLUSIONS

The new-born buffaloes presented diarrhoea mainly over the first four weeks of age. Ram Mohan *et al.*[29] and El-Garhi *et al.*[2], in India and Egypt, also observed the highest occurrence of diarrhoea during this period.

The occurrence of parasitic agents in both diarrhoeic and non-diarrhoeic calves, in despite of having no statistic significance in diarrhoeic animals ($p > 0.05$), demonstrated the importance of all calves as potential source of infection.

Eimeria spp was the pathogen from parasitic origin more frequently observed in the four farms and the species described were the same found by Rebouças *et al.*[4], in the same region, and by Lau[30] in the North of Brazil. Galiero; Consalvo[7] also described the same species in Italy. It is important to emphasise the presence of *E. zurnii* and *E. bovis*, due to the pathogenic potential of these species in young calves. The high occurrence of *Eimeria* spp observed in this study was, probably, due the environmental and management characteristics, which promote the maintenance and dissemination of the agent among the young calves in the region.

S. papillosus was the most prevalent nematode of young buffaloes occurring from the first week on. *T. vitulorum* was also common during the period of study. The results agree with those of Fujii *et al.*[31] and Buzetti[32] from Brazil and Patnaik; Pande[33] from India. Control of these nematodes is accomplished using anthelmintics in the first weeks of age, because the infection is mainly acquired from colostrum and milk[34]. However, the 4 study farms had been deworming calves with fenbendazole (Panacur, Hoechst Roussel™) and ivermectin (Ivomec, Merck Sharp Dohme™) during the first month of age, and the drugs used or frequencies of use are not currently prevently prevalent infections.

C. parvum also presented the same tendency found in buffalo calves in other countries[3,35], with oocysts in faeces between the 3rd and 6th weeks of age. Dubey *et al.*[35] in India and Canestri-Trotti; Quesada[36] in Italy associated the presence of *Cryptosporidium* mainly in diarrhoeic animals; however, in the present study, six animals without signs of diarrhoea presented oocysts in faeces, suggesting that non-symptomatic

calves are potential sources of infection.

For bacterial evaluations, *E. coli* was the most prevalent agent observed in diarrhoeic calves, and often was isolated alone. Amrousi *et al.*[37] and Ismail *et al.*[38], in Egypt, and Bali *et al.*[39], in India, also identified a high prevalence of this microorganism in new-born buffalo calves. However this high isolation rate is to be expected, as the organism belongs to the normal enteric microflora[40].

STa enterotoxin was present in 14.7% of *E. coli* isolates from diarrhoeic animals. Using the same technique (infant mice) in buffalo calves, Joon; Kaura[40], in India, found only one out of twenty (5.0%) faecal sample suspected of STa toxin production; while Ahmed; Afzal[41], in Pakistan, using the intestine loop ligated technique, found one faecal sample positive (5.5%) and other suspected (5.5%) of enterotoxigenic *E. coli* in sixteen diarrhoeic buffalo calves.

From the 34 samples of *E. coli* isolated from the calves with diarrhoea and 55 non-diarrhoeic animals, the LT enterotoxin was not detected, confirming the observations of Yano *et al.*[22], Acres[42] and Gyles[43] that this type of toxin is, normally, associated with *E. coli* from human and porcine origins.

E. coli, E. coli ETEC, *E. cloacae, K. pneumoniae* and *Citrobacter* spp were the more common microorganisms observed in diarrhoeic calves. Despite the highest occurrence in diarrhoeic animals (Tab. 1), these microorganisms were also presented in the non-diarrhoeic animals (with exception of *E. coli* ETEC and *Citrobacter* genera), reflecting the presence of these agents in the enteric microflora of the buffalo calves in the studied region.

Amrousi *et al.*[37] and Ismail *et al.*[38] in Egypt and Verma; Karla[44] in India, in studies with new-born buffaloes found similar results to the present observations with highest occurrence of *E. coli* and *Klebsiella* sp associated at low frequency of *K. oxytoca, Shigella* sp and *Pseudomonas* sp in diarrhoeic animals. In the same studies, they also found low occurrence of *E. aerogenes* and *K. oxytoca* in the non-diarrhoeic calves.

Salmonella spp was not isolate in the study; however, in previous reports, it was considered one of the most important agents causing diarrhoea in buffalo calves[7,45]. One of the reasons for the absence of this agent is likely the age of the animals during the study period, since Amrousi *et al.*[37] and Ismail *et al.*[38] observed *Salmonella*, in buffalo calves older than 4 weeks.

Norphloxacin had the highest sensitivity among all antimicrobials we tested followed by chloramphenicol and the gentamycin and high resistance to cephalexin and neomycin. Nicolas *et al.*[46] and Ismail *et al.*[38] also found similar results.

Using the PAGE-electrophoresis technique, no RNA segments characteristic of group A rotavirus were identified. However, studies in other countries identified rotavirus as one of the most important agents causing diarrhoea in young buffaloes[47,48,49]. In the same region of this study, Rácz *et al.*[5] identified the agent in only one of 112 buffaloes examined, and this animal was 90 days of age. Factors as management, age of the animals and season of the year could be responsible for the low occurrence of rotavirus in the region.

The values found for IgG levels in buffalo serum were similar to those found by other authors[12,50]. Levels lower than 20 mg ml^{-1} were not associated with increased risk for diarrhoea. However, in new-born animals other classes of immunoglobulins (IgM and IgA) may play a greater role in enteric defense[51].

Among the pathogens observed in this study, *C. parvum*, *Giardia* spp, *E. coli* (enterotoxigenic), *Shigella* spp and *P. aeruginosa* deserve special attention due to the zoonotic character of these agents.

The more frequent enteropathogens, from bacterial and parasitic origin, observed in new-born buffalo calves with diarrhoea in the present study involving *E. coli*, *E. cloacae*, *K. pneumoniae*, *Citrobacter* spp, *Eimeria* spp, *S. papillosus*, *T. vitulorum* and *C. parvum.* Lau[52,53] in the North region of Brazil and Galiero; Consalvo[7], in Italy, also demonstrated the etiologic complexity of diarrhoea in neonatal buffaloes, confirming the need to study diarrhoea as a syndrome and not based on the evaluation of specific agents isolated.

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RESUMO

Foram avaliados semanalmente, por um período de seis semanas, os exames parasitológicos de amostras fecais, diarréicas e não-diarréicas, de 106 bezerros búfalos, com três a 45 dias de idade, provenientes do Vale do Ribeira-SP. Para os exames bacteriológicos e virológicos, foram colhidas amostras fecais de todos os búfalos diarréicos, e igual amostragem de animais sem diarréia. Amostras de sangue foram colhidas dos búfalos neonatos, entre o terceiro e décimo dias de idade, para determinação dos níveis de imunoglobulina G (IgG). Nos exames parasitológicos, verificou-se a maior ocorrência de *Eimeria* spp, *Strongyloides papillosus* e *Toxocara vitulorum*. Dentre os agentes bacterianos, observou-se maior freqüência da *Escherichia coli* (*E. coli*), *Enterobacter cloacae* e *Klebsiella pneumoniae* (isolados ou em associação). Detectou-se a enterotoxina STa em cinco das 34 amostras de *E. coli* isoladas de búfalos com diarréia. A norfloxacina, cloranfenicol e gentamicina foram os antimicrobianos mais efetivos frente aos microrganismos isolados. Nenhuma das amostras apresentou bandeamento característico para rotavírus, a partir da técnica de eletroforese em gel de poliacrilamida. Não foi constatada nos animais examinados a associação entre a ocorrência de diarréia e baixos níveis de IgG sérica.

UNITERMOS: Diarréia; Bezerros de búfalos; Imunoglobulinas.

REFERENCES

1- SNODGRASS, D.R.; TERZOLO, H.R.; SHERWOOD, D.; CAMPBELL, I.; MENZIES, J.D.; SYNGE, B.A. Aetiology of diarrhoea in young calves. **Veterinary Record**, v.119, n.1, p.31-4, 1986.

2- EL-GHARI, M.M.; EL-RASHIDY, A.A.; METTIAS, K.N.; HASSEN, E.R.; HASSEN, H.M. Studies on neonatal diarrhea in buffalo calves. *In*: WORLD BUFFALO CONGRESS, 4., São Paulo, 1994. **Proceedings**. p.361-3.

3- GALIERO, G.; CONSALVO, F.; CARULLO, M. La criptosporidiosis nei vitelli bufalini: un aggiornamento. **Selezione Veterinaria**, v.35, n.5, p.449-53, 1994.

4- REBOUÇAS, M.M.; FUJII, T.U.; AMARAL, V.; SANTOS, S.M.; SPÓSITO FILHA, E.; BARCI, L.A.G.; FUJII, T. Eimeridios parasitas de búfalos (*Bubalus bubalis*) da região do Vale do Ribeira, Estado de São Paulo, Brasil. **Arquivos do Instituto Biológico**, São Paulo, v.57, n.1/2, p.1-3, 1990.

5- RÁCZ, M.L.; MUNFORD, V.; KROEFF, S.S.; KOTAIT, I. Identificação de rotavírus em amostra fecal de búfalo. *In*: REUNIÃO ANUAL DO INSTITUTO BIOLÓGICO, 5., São Paulo, 1992. **Anais**. p.29.

6- SPÓSITO FILHA, E.; FUJII, T.U.; REBOUÇAS, M.M.; AMARAL, V.; SANTOS, S.M. *Cryptosporidium* spp em amostras de fezes de búfalos (*Bubalus bubalis*) no Vale do Ribeira, Estado de São Paulo, Brasil. *In*: SEMINÁRIO BRASILEIRO DE PARASITOLOGIA VETERINÁRIA, 8., Londrina, 1993. **Anais**. p.20.

7- GALIERO, G.; CONSALVO, F. Indagine sulla presenza e diffusione delle malatie infettive e parissitarie tra vitelli bufalini in aziende da latte. **Selezione Veterinaria**, v.34, n.11, p.1055-63, 1993.

8- ROBERTS, J.A. *Toxocara vitulorum* in Ruminants. **Veterinary Bulletin**, v.63, n.6, p.545-67, 1993.

9- LIBERG, P. Colostral immunoglobulin concentrations in Swedish dairy cows. A preliminary study. *In:* WORLD BUIATRICS CONGRESS, 19., Edinburgh, 1996. **Proceedings**. p.114-5.

10- ZEMAN, D.H.; THOMSON, J.U.; FRANCIS, D.H. Diagnosis, treatment and management of enteric colibacillosis. **Veterinary Record**, v.84, n.1, p.794-802, 1989.

11- KLINGENBERG, K.V. Serum gammaglobulin levels, rotavirus excretion and neonatal enteritis in calves. *In*: WORLD BUIATRICS CONGRESS, 19., Edinburgh, 1996. **Proceedings**. p.116-7.

12- BUTLER, J.E. Bovine Immunoglobulines: An augmented review. **Veterinary Immunology and Immunopathology**, v.4, n.1/2, p.43-152, 1983.

13- FERREIRA, L.F.; MORTEO, R.E.; SILVA, J.R. Padronização de técnicas para exame parasitológico de fezes. **Jornal Brasileiro de Medicina**, v.6, p.241-57, 1962.

14- OGASSAWARA, S.; CASTRO, J.M.; KASAI, N.; PENA, H.F.J.; HOGE, A.I.A.; VILLELA, B.C.B. *Cryptosporidium* tipo C muris em bovinos do Estado de São Paulo. *In*: SEMINÁRIO BRASILEIRO DE PARASITOLOGIA VETERINÁRIA, 6., Bagé, 1989. **Anais**. p.123.

15- DUBEY, J.P.; SPEER, C.A.; FAYER, R. **Cryptosporidiosis of man and animals**. Boston : CRC Press, 1990. 199p.

16- LEVINE, N.D. **Veterinary protozoology**. Ames: Iowa State University Press, 1985. p.130-232.

17- EDWARDS, P.R.; EWING, W.H. **Identification of enterobacteriaceae**. Minnesota: Burgess, 1972. p.7-47

18- KRIEG, N.R.; HOLT, J.G. **Bergey's manual of systematic bacteriology**. London: Williams and Wilkins, 1984. 984p.

19- BAUER, A.W.; KIRBY, W.M.M.; SHERRIS, J.C.; TURCK, M. Antibiotic susceptibility testing by a standardized single disk method. **American Journal of Clinical Pathology**, v.45, p.493-6, 1966.

20- DEAN, A.G.; CHING, Y.C.; WILLIAMS, R.G.; HARDEN, J.B. Test for *Escherichia coli* enterotoxin using infant mice: application in a study of diarrhea in children in Honolulu. **Journal of Infectious Diseases**, v.125, p.407-11, 1972.

21- SERAFIM, M.B.; CASTRO, A.F.P.; LEONARDO, M.B.; MONTEIRO, A.R.A. A single radial immune hemolisis (SRIH) test for the detection of thermolabile (LT) enterotoxin of *Escherichia coli*. **Journal of Clinical Microbiology**, v.14, p.473-8, 1981.

22- YANO, T.; OLIVEIRA, M.S.; FONTES, C.F.; ALMEIDA, A.C.P.; CASTRO, A.F.P. Detection of heat-labile (LT) enterotoxin of enterotoxigenic *Escherichia coli* by the radial immune hemolisis test: a modification for a clinical use. **Medical Microbiology and Immunology**, v.171, p.171-87, 1982.

23- HERRING, A.J.; INGLIS, N.F.; OJEH, C.K.; SNODGRASS, D.R.; MENZIES, J.D. Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver-stained polyacrylamide gels. **Journal of Clinical Microbiology**, v.16, n.3, p.473-7, 1982.

24- PEREIRA, H.G.; AZEVEDO, R.S.; LEITE, J.P.G. Eletroforetic study on the genome of human rotaviruses from Rio de Janeiro, São Paulo and Paraná, Brazil. **Journal of Hygiene**, v.90, p.117-26, 1983.

25- MANCINI, G.; CARBONARA, A.O.; HERMANS, F.J. Immunochemical quantitation of antigens by a single radial immunodifusion. **Immunochemical**, v.2, p.235-54, 1965.

26- FAHEY, J.L.; MCKELVEY, E.M. Quantitative determination of serum immunoglobulins in antibody-agar plates. **Journal of Immunology**, v.94, n.1, p.84-90, 1965.

27- MEAD, R.; CURNOW, R.N.; HASTED A.M. **Statistical methods in agriculture and experimental biology**. London: Chapman Hall, 1993. 415p.

28- DEAN, A.G.; DEAN, J.A.; COULOMBIER, D.; BRENDEL, K.A.; SMITH, D.C.; BURTON, A.H.; DICKER, R.C.; SULLIVAN, K.; FAGAN, R.F.; ARNER, T.G. **Epi Info, Version 6. A Word Processing, Database and Statistics Program for Epidemiology on Microcomputers**. Atlanta, Georgia: Centers for Disease Control and Prevention, 1994. 601p.

29- RAM MOHAN, S.; RAM RAO, R.S.; GAFFAR, A.A. Studies on neonatal diarrhea in buffalo calves with special reference to epizootiology and fluid therapy. **Indian Veterinary Journal**, v.67, n.11, p.1057-9, 1990.

30- LAU, H.D. Eimerídeos parasitos de búfalos no Estado do Pará. **Boletim de Pesquisa EMBRAPA-CPATU**, n.42, p.5-11, 1982.

31- FUJII, T.U.; DEL'PORTO, A.; OLIVEIRA, F. Influência do tratamento anti-helmíntico na eliminação de ovos de *Toxocara vitulorum* e *Strongyloides papillosus*, em bezerros búfalos. *In*: REUNIÃO ANUAL DO INSTITUTO BIOLÓGICO, 7., São Paulo, 1994. **Anais**. p.5.

32- BUZETTI, W.A.S. **Endo e ectoparasitoses em búfalos**. Jaboticabal : Funep, 1993. p.121-42.

33- PATNAIK, M.M.; PANDE, B.P. Notes on the helmintic infestations encountered in one month old buffalo calves. **Indian Veterinary Journal**, v.40, n.3, p.128-33, 1963.

34- STARKE, W.A.; ZOCOLLER, M.C.; MACHADO, R.Z. Transmammary passage of gastrointestinal nematode larvae to buffalo calves. I. *Strongyloides papillosus*. *In*: WORLD BUFFALO CONGRESS, 4., São Paulo, 1994. **Proceedings**. p.330-2.

35- DUBEY, J.P.; FAYER, R.; RAO, J.R. Cryptosporidial oocyst in faeces of water buffalo and zebu calves in India. **Journal of Veterinary**, v.6, n.1, p.55-6, 1992.

36- CANESTRI-TROTTI, G.; QUESADA, A. Primo reperto di *Cryptosporidium* spp in bufali italiani (*Bubalus bubalis*). **Atti Della Societá Italiana Delle Scienze Veterinarie**, v.37, n.único, p.737-40, 1983.

37- AMROUSI, S.E.L.; NAFIE, E.K.; REHEWI, M.E.L.; MOTTILIB, A.A. Studies on enteritis in buffalo calves in Assiut. **Journal of Egypt Veterinary Medical Association**, v.31, n.3/4, p.219-25, 1971.

38- ISMAIL, M.; GIRGIS, S.M.; EL-JAKEE, J.; SHOKRY, S.; RIAD, E.M. Bacteriological studies on the diarrhea in newly born buffalo calves. **Veterinary Medical Journal Giza**, v.38, p.219-32, 1990.

39- BALI, M.K.; JUNEJA, I.J.; KHANA, R.N.S. A clinical note on buffalo calf mortality. **Indian Journal of Dairy Science**, v.32, n.4, p.370-2, 1979.

40- JOON, D.S.; KAURA, Y.K. Isolation and characterization of the enterobacteria from diarrhoeic and non-diarrhoeic calves. **Indian Journal of Animal Science**, v.63, n.4, p.373-83, 1993.

41- AHMED, J.; AFZAL, M. Enterotoxigenicity of *Escherichia coli* isolates from buffalo and cow calves. **Pakistain Veterinary Journal**, v.9, n.1, p.9-11, 1989.

42- ACRES, S.D. Enterotoxigenic *Escherichia coli* infections in newborn calves: a review. **Journal of Dairy Science**, v.68, n.1, p.229-56, 1983.

43- GYLES, C.L. *Escherichia coli* cytotoxins and enterotoxins. **Canadian Journal Microbiology**, v.38, p.734-46, 1992.

44- VERMA, P.C.; KARLA, D.S. Studies on buffalo (*Bus bubalis*) calf mortality, with special reference to its aetiology. **Indian Veterinary Journal**, v.52, n.8, p.605-9, 1975.

45- KAURA, Y.K. A two year surveillance (1984-1986) on the incidence of *Salmonella* serotypes in diarrhoeic calves at some of the organized animals farms. **Indian Journal of Animal Science**, v.58, n.12, p.1361-8, 1988.

46- NICOLAS, A.; GAYAUD, C.; NOEL, F. Neonatal calf diarrhoea. **Recuel Medicine Veterinaire**, v.160, p.107-10, 1984.

47- SINGH, A.; PANDEY, R. Analysis of electrophoretypes of rotavirus from diarrhoeic faeces on neonatal buffalo calves in India. **Acta Virologica**, v.32, p.156-9, 1988.

48- SUNIL-CHANDRA, N.P.; MAHALINGHAN, S. Rotavirus-associated diarrhoea in buffalo calves in Sri Lanka. **Research in Veterinary Science**, v.56, n.3, p.393-6, 1994.

49- HEGAZY, A.A.; FAHMY, L.; BAKEER, A.; GARHEY, M.L.; SELIUM, S.A. Pathological and immunopathological studies on newly born buffalo calves infected with rotavirus. I. Field study. *In*: WORLD BUIATRICS CONGRESS, 19., Edinburgh, 1996. **Proceedings**. p.125-9.

50- TIZARD, I. **Imunologia veterinária**. São Paulo: Roca, 1985. 329p.

51- KLAUS, G.G.B.; BENNET, A.; JONES, E.W. A quantitative study of the transfer of colostral immunoglobulins to the new-born calf. **Immunology**, v.16, p.293-9, 1969.

52- LAU, H.D. Principais doenças dos bezerros búfalos lactentes no Estado do Pará. **Boletim de Pesquisa EMBRAPA-CPATU**, n.83, p.5-12, 1987.

53- LAU, H.D. Suckling buffalo calves mortality in Pará State-Brazil. *In*: WORLD BUFFALO CONGRESS, 4., São Paulo, 1994. **Proceedings**. p.307-9.

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