# Epidemiological, Antigenic and Pathogenic Profile of Bovine Diarrhea in a Brazilian Cattle Population

J.A. Ambrosim[1] F.S. Almeida[1] E.C. Rigobelo[2]
A.F.P. Castro[3] R.P. Schocken-Iturrino[4]
J.L. Quintana[4] F.A. Avila[4]



***Key words***

Cattle – Newborn animal – Diarrhea – Epidemiology – Antibiotics – Resistance to chemicals – Brazil.

***Summary***

Fecal samples collected from 266 calves of dairy breeds in the northern region of the State of São Paulo were examined to study the frequency of various enteropathogens and to determine the resistance of isolated strains to various antimicrobial agents. A total of 127 *Escherichia coli* strains were isolated, 60 of them enterotoxigenic, as well as 23 *Enterobacter cloacae* strains, 18 *Klebsiella pneumoniae* strains, 15 *Citrobacter freundii* strains, 7 *Salmonella* strains of different serotypes, and one *Pseudomonas aeruginosa* strain. Thirty-six preparations positive for *Cryptosporidium* sp. were also identified, among which four were classified as *Cryptosporidium parvum*. The association of two or more agents was detected in 27 samples of diarrheic feces. Among the antibiotics tested, those against which *Escherichia coli* and *Salmonella* sp. presented a greater resistance were lincomycin and penicillin G.

## ■ INTRODUCTION

Neonatal calf diarrhea is a serious problem caused by several agents and factors. The causative agent may proliferate in the intestinal tract alone or in combination with another organism (26, 30). Among the various microorganisms involved, enterotoxigenic *Escherichia coli* seems to be the most frequent (16, 39).

The first observations of the production of enterotoxins were reported by Smith and Halls (38) who verified that alive *Escherichia coli* cultures or their filtrates of strains isolated from domestic animals can dilate the tied intestinal loops of pigs and rabbits, with fluid accumulation in the intestinal lumen.

There are two types of enterotoxins basically differing from one another in terms of heat resistance, antigenicity and origin. They are the LT (LT-I and LT-II) thermolabile enterotoxins and the ST (STa and STb) thermostable enterotoxins. LT-II was first described in *Escherichia coli* strains isolated from buffaloes and two antigenic variants (LT-IIa and LT-IIb) have already been described (18). The STa enterotoxin detected by the suckling mouse test (10) is of low molecular weight and mediated by a plasmid. This toxin presents variations detected in *Escherichia coli* strains isolated from swine (STap) and humans (STah).

Studies conducted in recent years also showed the increasingly frequent involvement of *Salmonella*, rotaviruses and *Cryptosporidium* (1, 22, 28).

A small number of serotypes have been observed in calf salmonellosis, with a predominance of *Salmonella dublin* and *Salmonella typhimurium*, followed by *Salmonella montevideo*, *Salmonella anatum* and *Salmonella newport* (1, 9). Rodents or birds (7) have been cited as sources of *Salmonella* on chicken farms and the microorganism is transferred to cattle farms by the consumption of chicken bedding by cattle, but the major contamination occurs because of the introduction of animals in the facilities (34).

The National Animal Health Inspection Service (NAHMS) estimated that bovine salmonellosis is responsible for annual losses of

1. Veterinary Medical, University Estadual Paulista, Jaboticabal-SP, Brazil

2. Microbiology Program, Jaboticabal, University of São Paulo, São Paulo, Brasil

3. Department of Microbiology, Biological Sciences Institute, USP, São Paulo-SP, Brazil

4. Department of Veterinary Pathology, Faculty of Agrarian and Veterinary Sciences, UNESP, Rodovia Paulo Donato Castelane, Km 5, Jaboticabal-SP, Brazil

E-mail: favila@fcav.unesp.br

100 million dollars in the United States (21). In Brazil there is no literature data about losses caused by salmonellosis in calves.

Andresen *et al.* (2) conducted a study on salmonellosis on two large dairy cattle farms in Peru for more than 30 months and showed the existence of three different syndromes: septicemia, pneumonia and/or enteritis. They reported that the *Salmonella* serotypes isolated were probably introduced by some animals imported from the United States between 1987 and 1991.

Calf diarrhea caused by coccids of the genus *Cryptosporidium* mainly occurs in the age range of 5 to 10 days. Two species have been shown to infect calves: *C. parvum* occurring in animals younger than three weeks and *C. muris* occurring in older calves (4). Bovine cryptosporidiosis caused by *Cryptosporidium parvum* normally affects very young animals or immunologically deficient animals. Older calves are not usually affected by a severe or prolonged disease (3), and infection of adult cattle has been reported to be asymptomatic (35).

The genus *Cryptosporidium* has been detected both in animals with diarrhea and in animals with normal feces (14, 27, 37, 40), with the latter, therefore, representing asymptomatic carriers in the herd. In Brazil, Ortolani (37) correlated *Cryptosporidium* sp. infections with precarious conditions of hygiene and high population density in calf rearing.

The resistance factors to antimicrobial drugs (R) are mostly of a plasmid nature. In the late fifties and early sixties, Japanese investigators revealed the existence of R plasmids. Since then, several others, such as Nakano *et al.* (33), detected their presence in the pathogenic enterobacteria *Salmonella*, *Escherichia coli* and other microorganisms of man and animals. R plasmids are responsible for the acquired resistance to various antibiotics and chemotherapeutic agents and for the onset of multiresistant strains. The indiscriminate use of these therapeutic agents has been largely responsible for the dissemination of R factors. Prolonged or repeated treatments exert selection pressure on the bacterial flora, giving origin to populations with high resistance indices. In view of these facts, an evaluation of the current situation is warranted, with comparison to previous studies conducted in this region and others.

In the northern region of the State of São Paulo, calf diarrhea causes enormous losses to breeders and its etiology deserves up-to-date and extensive studies. Thus, the objectives of the present investigation were to study the frequency of the different enteropathogens in clinically healthy and diarrheic calves of dairy breeds, and to determine the resistance of *Salmonella* sp. and enterotoxigenic *Escherichia coli* strains to different antimicrobial agents.

## ■ MATERIALS AND METHODS

### *Fecal samples*

A total of 266 fecal samples were collected using rectal swabs or fecal collection directly from the rectum of 1 to 90-day-old calves. Ten percent of these samples were from clinically healthy animals and the others from animals with diarrhea. The samples were collected on 15 dairy farms, which reared purebred and half-bred animals in the northern region of the State of São Paulo.

### *Isolation and biochemical identification*

The fecal samples were seeded onto plates containing MacConkey agar, blood agar and eosin-methylene blue (EMB) agar, and incubated at 37°C for 24-48 h. The colonies on MacConkey agar and blood agar media with culture characteristics similar to those of *Escherichia coli* were identified as belonging to the species under study on the basis of lactose fermentation and indole production tests, methyl red and Voges Proskauer reactions, citrate utilization, urease production, and sulfhydric acid gas ($H_2S$) production. The tests were read after 24-72 h of incubation at 37°C (11).

The non-lactose fermenting colonies that grew on MacConkey agar and EMB agar were submitted to biochemical identification using a larger number of media containing carbohydrates and amino acids and other tests. The isolated and biochemically identified *Salmonella* sp. strains were submitted to serotyping at the Adolfo Lutz Institute, São Paulo, SP.

### *Adherence antigen*

For the detection of the K99 adherence antigen, the isolated *Escherichia coli* strains were cultured on Minca medium (17). Five colonies from each plate were individually tested against anti-K99, anti-F165 and anti-A14 antisera by the agglutination test on slides.

### *Detection of STa enterotoxin*

The isolated *Escherichia coli* strains were cultured in BHI broth in a water bath with shaking at 150-200 rpm at 36°C for 18 h and then centrifuged at 2600 *g* for 15 min. One drop of 2% Evans blue solution was added to each supernatant. Suckling mice 3-4 days of age were orally inoculated with 0.1 ml of the previous preparation (3 mice) and kept at room temperature for 3-4 h. A positive control mouse was always used. After this time, the mice were sacrificed by excess ether inhalation, their intestines (I) removed and weighed, and the carcasses (C) weighed separately. The ratio of the two weights (I/C) was then determined. An I/C ratio higher than 0.085 was considered positive and an I/C ratio below 0.085 was considered negative (10, 12).

### *Serologic identification of* Escherichia coli *strains*

The isolated *Escherichia coli* strains were identified serologically using OK antiserum produced in rabbits and the agglutination test on slides, against the following serotypes: Myers N° 483 (O9:K35:K99), Myers N° 490 (O101:K30:K99), Myers N° 505 (O101:K28:K99), Myers N° 524 (O8:K85:K99), Myers N° 559 (O9K25:K99), and Myers N° Wi-1 (O20:K?:K99). The strains were first screened against a serum pool containing antibodies against the strains listed above and identification was then performed with individual sera (6, 19).

### *Detection of* Cryptosporidium

Smears of the fecal samples used for bacteriologic examination were performed on microscope slides and stained by the Ziehl Neelsen method modified by Henrisksen and Pohlenz (20) to determine the presence of *Cryptosporidium* oocysts. Sixty-four of the total fecal samples collected were sent to the parasitology sector for detection and classification of *Cryptosporidium*. The centrifugal fluctuation technique in a saturated sucrose solution was thus used for parasite identification (15). One gram of feces was homogenized and mixed with a saturated sucrose solution (density: 1.2 g/cm$^3$) and the preparation was centrifuged at 1000 *g* for a period of 10 min. One drop was then collected from the centrifuge tube and placed between a slide and coverslip with the aid of a platinum loop for visualization under the microscope at 100x first and at then at 400x (Figure 1).

### *Test of sensitivity to antibiotics and chemotherapeutic agents*

A total of 127 *Escherichia coli* strains and 7 *Salmonella* strains were tested by the method of Bauer *et al.* (8) against the following

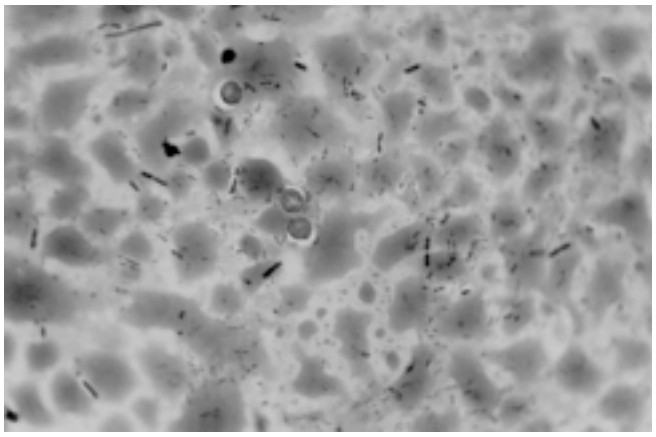

***Figure 1:*** Cryptosporidium *sp. on a slide smear of diarrheic calf feces stained by the method of Henrisksen and Pohlenz (1981,* Acta vet. Scan.*). Magnification 400x.*

antimicrobial agents: nalidixic acid, ampicillin, cephalotin, chloramphenicol, erythromycin, streptomycin, gentamycin, kanamycin, lincomycin, penicillin G, novobiocin, neomycin, tetracycline, nitrofurantoin, and trimethoprim-sulfadiazine.

## ■ RESULTS

From the 266 fecal samples of calves with or without diarrhea collected directly from the rectum on 15 farms in the northern region of the State of São Paulo, the authors isolated from diarrheic feces 112 *Escherichia coli* strains, 20 *Enterobacter cloacae* strains, 16 *Klebsiella pneumoniae* strains, 14 *Citrobacter freundii* strains, 7 *Salmonella* strains, and one *Pseudomonas aeruginosa* strain. In addition, 34 preparations were positive for *Cryptosporidium*, of which 4 were classified as *Cryptosporidium parvum* (Table I).

Table I also lists the bacteria isolated from non-diarrheic animals, i.e. 4 enterotoxigenic *Escherichia coli* strains, 11 non-enterotoxigenic *Escherichia coli* strains, 3 *Enterobacter cloacae* strains, 2 *Klebsiella pneumoniae* strains, 1 *Klebsiella oxytoca* strain, 1 *Citrobacter freundii* strain, 1 *Pseudomonas aeruginosa* strain, and 1 *Providencia stuartii* strain. Two preparations were positive for *Cryptosporidium* sp.

Of the 127 *Escherichia coli* strains examined by the suckling mouse test, 60 were positive and 67 negative. Enterotoxic activity was determined by the I/C ratio (Table II). The results of the serum agglutination test on slides of the *Escherichia coli* strains isolated against anti-OK sera are presented in Table III. The distribution of virulence factors among the *Escherichia coli* strains isolated is shown in Table IV. Sixty of the 127 strains analyzed produced enterotoxin STa and carried the adherence antigen K99. Two $K99^+$ strains and five $A14^+$ strains were not enterotoxigenic.

Two or more agents were isolated from 27 diarrheic samples, with the major associations being *Enterobacter cloacae* and *Klebsiella pneumoniae* (21.2%), *Citrobacter freundii* and *Klebsiella pneumoniae* (18.5%), and enterotoxigenic *Escherichia coli* and *Cryptosporidium parvum* (14.8%) (Table V).

The antibiograms of 27 *Escherichia coli* strains and 7 *Salmonella* strains in the presence of different antibiotics and chemotherapeutic agents showed that the highest percentage of resistance occurred for lincomycin, penicillin G and novobiocin.

Figure 1 shows stained *Cryptosporidium* sp. cells on a slide with a smear of diarrheic calf feces observed under light microscope at 400x magnification.

**Table I**

Number, percentage and types of agents isolated from 240 diarrheic fecal samples and from 26 normal fecal samples of calves

| Agents | Positive samples | | | |
|---|---|---|---|---|
| | Diarrheic | | Normal | |
| | Num. | % | Num. | % |
| Enterotoxigenic *Escherichia coli* | 56 | 23,3 | 4 | 15,4 |
| Non-enterotoxigenic *Escherichia coli* | 56 | 23,3 | 11 | 42,3 |
| *Cryptosporidium* sp. | 34 | 14,2 | 2 | 7,7 |
| *Cryptosporidium parvum* | 4 | 1,5 | - | - |
| *Enterobacter cloacae* | 20 | 8,3 | 3 | 11,5 |
| *Klebsiella pneumoniae* | 16 | 6,7 | 2 | 7,7 |
| *Klebsiella oxytoca* | - | - | 1 | 3,8 |
| *Citrobacter freundii* | 14 | 5,8 | 1 | 3,8 |
| *Salmonella dublin* | 3 | 1,1 | - | - |
| *Salmonella enterica* spp. | 2 | 0,7 | - | - |
| Rough enteric variety | | | | |
| *Salmonella agona* | 1 | 0,4 | - | - |
| *Salmonella* I 4, 5, 12: i: | 1 | 0,4 | - | - |
| *Pseudomonas aeruginosa* | 1 | 0,4 | 1 | 3,8 |
| *Providencia stuartii* | - | - | 1 | 3,8 |
| Others | 32 | 13,3 | - | - |

**Table II**

Intestinal/carcass weight ratio obtained by testing 127 enterotoxin preparations in suckling mice

| Intestinal weight/ carcass weight | Num. of preparations | Interpretation |
|---|---|---|
| > 0,085 (X = 0,097) | 60 | Enterotoxic |
| < 0,085 (X = 0,080) | 67 | Non-enterotoxic |

**Table III**

Number, percentage and antigenic types of *E. coli* isolated from calves with and without diarrhea in the northern region of the State of São Paulo

| Antigenic types | | | Num. of serotypes/ total ETEC* num. | % |
|---|---|---|---|---|
| 0 | K | K99 | | |
| 101 | 28 | + | 19/60 | 31.6 |
| 9 | 25 | + | 18/60 | 30.0 |
| 20 | ? | + | 9/60 | 15.0 |
| 8 | 85 | + | 7/60 | 11.6 |
| 9 | 35 | + | 4/60 | 6.6 |
| 101 | 30 | + | 3/60 | 5.0 |

* Enterotoxigenic *Escherichia coli*

**Table IV**

Virulence factors in 67 of the 127 *Escherichia coli* strains isolated from calves with diarrhea

| Enterotoxin | Num. of positive strains /total num. of strains | Colonization factors | | | |
|---|---|---|---|---|---|
| | | K99 | F41 | F165 | A14 |
| Sta* | 60/127 | 60 | 0 | 0 | 0 |
| N-ECET** | 07/127 | 2 | 0 | 0 | 5 |
| Total | 67/127 | 62 | 0 | 0 | 5 |

* Thermostable enterotoxins

** Non-enterotoxigenic *Escherichia coli*

**Table V**

Distribution of the agents isolated in association from 27 calf fecal samples in the northern region of the State of São Paulo

| Agents | Positive samples (Num.) | % |
|---|---|---|
| *Enterobacter cloacae* + *Klebsiella pneumoniae* | 6 | 21,2 |
| *Citrobacter freundii* + *K. pneumoniae* | 5 | 18,5 |
| *Escherichia coli* + *Cryptosporidium parvum* | 4 | 14,8 |
| *Ent. cloacae* + *Cit. freundii* | 3 | 11,1 |
| *Esc. coli* + *Ent. cloacae* | 2 | 7,4 |
| *Salmonella dublin* + *Esc. coli* | 1 | 3,7 |
| *Ent. cloacae* + *K. pneumoniae* + *Pseudomonas aeruginosa* | 1 | 3,7 |
| *Esc. coli* + *Cit. freundii* + *K. pneumoniae* | 1 | 3,7 |
| *S. dublin* + *Cryptosporidium* sp. | 1 | 3,7 |
| *Salmonella* I 4.5.12:i: + *Cryptosporidium* sp. | 1 | 3,7 |
| *S. enterica* + *Cryptosporidium* sp. | 1 | 3,7 |
| *S. agona* + *Cryptosporidium* sp. | 1 | 3,7 |

## ■ DISCUSSION

Diarrhea is the major cause of death among calves throughout the world. It is caused by various agents such as bacteria, protozoa and viruses, with Enterobacteriaceae and *Cryptosporidium* sp. the main bacteria and protozoa, respectively (1, 2, 16, 20, 26).

For instance, in the United States, Howie (21) reported monitoring data for the US Department of Agriculture concerning the occurrence of *Salmonella* and *E. coli* O157 on 96 dairy farms. On the basis of the isolates obtained, the most common *Salmonella* serotypes detected were *S. montevideo* (21%), *S. cerro* (13.4%), *S. kentucky* (8.5%), *S. menhaden* (7.7%), *S. anatum* (6.2%), *S. meleagridis* and *S. muenster* (4.7%).

Andresen *et al.* (2) conducted a study on salmonellosis on two large dairy cattle farms in Peru for more than 30 months and showed the existence of three different syndromes: septicemia, pneumonia and/or enteritis. They reported that the *Salmonella* serotypes isolated were probably introduced by some animals imported from the United States between 1987 and 1991. Similarly, there are many reports on the importance of cryptosporidiosis in other countries (22, 27, 41) and enteroxigenic *E. coli* (8, 13, 16, 23).

Conversely, in Brazil there are only a few studies on the agents that cause diarrhea in young calves. Also most of the researches are either very specific to one or two agents without determining serogroups/serotypes or any other characteristics such as the virulence factors of the microorganisms involved (14, 15, 25). Other studies are limited to very restricted regions, besides involving only some groups of diarrheogenic bacteria.

Madruga *et al.* (29) carried out their studies in the State of Mato Grosso do Sul (MS). Although they showed that *Salmonella* sp. and *Escherichia coli* were the main agents found among diarrheic beef calves, there was no report on the serovars that they isolated nor was there any report as to which group of diarrheogenic *E. coli* the isolates belonged. Similarly, Kuchembuck *et al.* (24), in the region of Botucatu, State of São Paulo, reported the isolation of only two *Salmonella* sp. strains, without determining the species or the serovars. Furthermore, the *E. coli* they isolated were not serogrouped and the other adhesins but K99 were not searched, as for instance F41, F165 and A14, known to be involved in diarrhea in young calves (6, 13, 31). There are references to *Cryptosporidium* sp. in the investigation carried out by Madruga *et al.* (29) in calves in the MS State. Some reports on the role of *Cryptosporidium* sp. are found in the studies carried out by Garcia and Lima (14) and Garcia *et al.* (15) in the State of Minas Gerais, located in the north-northeast of the State of São Paulo. In the State of São Paulo there is a report by Ortolani *et al.* (37) on some epidemiological aspects of cryptosporidiosis.

Therefore, the above cited researches show that a wide study covering more groups of enterobacteria, and *Cryptosporidium* sp. and *C. parvum* are missing, mainly in the State of São Paulo. The results obtained in the present research showed that enterotoxigenic *Escherichia coli* (ETEC), *Cryptosporidium* sp., *C. parvum*, and *Salmonella dublin*, *S. agona* and third isolate of *Salmonella* sp. with the antigens 1, 4, 5, 12:i were isolated. The low number of isolates found in this study may lead to the wrong idea that salmonellosis in young calves is not important. With regard to *Salmonella* isolates they were recovered only from diarrheic animals, suggesting that among young calves the carrier state may be rare. However, it is important to call the attention to the fact that in bovine salmonellosis, in contrast with other enteric infections that are limited to the intestine, with different clinical signs and symptoms, among which diarrhea is the most important, the bacterial cells from the gut may invade the blood causing septicemia (2, 40). With regard to the frequency of *Salmonella* sp. isolation in other countries, Tzipori *et al.* (40) report that about 31.9% of calves' infections with diarrhea in the United States occur at 60 days of age or more and that these infections could be attributed to *Salmonella dublin*. In the present study, the frequency of the *dublin* serotype was lower (1.1%), although the age range of the calves studied was the same.

ETEC were isolated much more frequently from diarrheic than from non-diarrheic calves. Only STa enterotoxin was searched since it is well-known that thermolabile (LT) enteroxin is not a cause of diarrhea among calves (23, 24, 26, 40). It is noteworthy that ETEC isolates were found much more frequently in diarrheic calves than in healthy ones. It is possible that non-diarrheic calves remained infected and played a role as carriers of ETEC after an outbreak of diarrhea. The other bacteria found in the present

research, i.e. *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Citrobacter freundii* and *Providencia stuartii*, were probably part of the normal gut flora of the studied calves, as it is the case in man, and did not play a role in calves' diarrhea. The same comment could be made with regard to the isolation of *Pseudomonas aeruginosa*.

It is important to emphasize that among bacteria ETEC is a major etiological agent of diarrhea in the northeast region of the State of São Paulo, a fact that can undoubtedly be applied to many parts and cities of the country, since in other countries these STa+ *E. coli* are the main cause of diarrhea among calves (23, 26, 30, 31, 40). Although there are primers for the detection of the STa gene (32), the expression of the enterotoxin can be done easily by an *in vivo* test such as the infant mouse test (IMT) (10) as was done in this study. The findings in this study showed that IMT was very efficient to detect STa+ strains.

With regard to *E. coli* serogroups the present data are important because other studies in Brazil (4, 29) do not report on ETEC serogroups. It was possible to observe that serogroups O101, O9 and O20 were more frequent. Similar findings are reported in researches carried out in other countries (16, 17, 23, 26, 39).

The findings in this study were similar to those reported by other authors in other countries and in other areas from Brazil (3, 4, 14, 15, 20, 22, 27, 35). However, in the present investigation it was possible to establish a comparison of the frequencies of the different enteropathogens found in the State of São Paulo. These findings are important to establish treatment measures or prophylactic procedures to control diarrhea-related diseases in farms of the State of São Paulo. Since STa enterotoxin is not immunogenic unless special and difficult techniques are used, a vaccine against bovine collibacillosis can be made based on the K99 antigen, the most common adhesin found in bovine STa+ ETEC.

Finally, though antimicrobial drugs are not often used as treatment against bacterial agents responsible for diarrhea in calves, the resistance pattern of one isolate is an important marker to trace the sources of infection among calves from different farms or regions. Furthermore, these strains can play a role in the transference of R factors from *Salmonella* sp. or *Escherichia coli* to other bacteria, some of which can infect other animals and man (33). Still regarding multiple resistance to antimicrobial drugs the indiscriminate use of these therapeutic agents has been largely responsible for the dissemination of R factors. Prolonged or repeated treatments exert selection pressure on the bacterial flora, giving origin to populations with high resistance indices. In view of these facts, the present evaluation of the current situation is highly justified (3, 4, 14, 15, 20, 22, 27, 35) with comparison to previous studies conducted in this region and others.

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## *Résumé*

**Ambrosim J.A., Almeida F.S., Rigobelo E.C., Castro A.F.P., Schocken-Iturrino R.P., Quintana J.L., Avila F.A.** Profil épidémiologique, antigénique et pathogénique des diarrhées dans une population de bovins au Brésil

Des échantillons de fèces prélevés chez 266 veaux de race laitière, provenant de la région au nord de l'Etat de São Paulo, ont été examinés afin d'étudier la fréquence des différents agents entéropathogènes et de déterminer la résistance des souches isolées à plusieurs substances antimicrobiennes. Au total, 127 souches d'*Escherichia coli* – parmi lesquelles 60 étaient entérotoxigéniques – ont été isolées, ainsi que 23 souches d'*Enterobacter cloacae*, 18 souches de *Klebsiella pneumoniae*, 15 souches de *Citrobacter freundii*, 7 souches de *Salmonella* avec des sérotypes différents et une souche de *Pseudomonas aeruginosa*. Trente-six souches positives pour *Cryptosporidium* sp. ont aussi été identifiées, dont quatre ont été classées sous l'espèce *Cryptosporidium parvum*. L'association de deux ou plus agents a été rencontrée dans 27 échantillons de fèces diarrhéiques. Parmi les antibiotiques testés, la lincomycine et la pénicilline G ont été ceux contre lesquels *Escherichia coli* et *Salmonella* sp. ont présenté une résistance plus importante.

***Mots-clés :*** Bovin – Animal nouveau-né – Diarrhée – Epidémiologie – Antibiotique – Résistance aux produits chimiques – Brésil.

## *Resumen*

**Ambrosim J.A., Almeida F.S., Rigobelo E.C., Castro A.F.P., Schocken-Iturrino R.P., Quintana J.L., Avila F.A.** Perfil epidemiológico, antigénico y patogénico de la diarrea bovina en una población de ganado brasileño

Se examinaron muestras fecales, colectadas de 266 terneros de razas de leche en la región norte del Estado de São Paulo, con el fin de estudiar la frecuencia de varios patógenos entéricos y de determinar la resistencia de las cepas aisladas a varios agentes anti microbianos. Se aislaron un total de 127 cepas de *Escherichia coli*, 60 de ellas enterotoxigénicas, así como 23 cepas de *Enterobacter cloacae*, 18 cepas de *Klebsiella pneumoniae*, 15 cepas de *Citrobacter freundii*, 7 cepas de *Salmonella* de diferentes serotipos y una cepa de *Pseudomonas aeruginosa*. También se identificaron 36 preparaciones positivas a *Cryptosporidium* sp., entre las cuales cuatro fueron clasificadas como *Cryptosporidium parvum*. La asociación de dos o más agentes se detectó en 27 muestras de heces diarreicas. Entre los antibióticos examinados, aquellos contra los cuales *Escherichia coli* y *Salmonella* sp. presentaron una mayor resistencia fueron la lincomicina y la penicilina G.

***Palabras clave:*** Ganado bovino – Animal recien nacido – Diarrea – Epidemiología – Antibiótico – Resistencia a productos químicos – Brasil.