

SCIENTIFIC COMMUNICATION

MIXED INFECTIONS BY BOVINE CORONAVIRUS, ROTAVIRUS
AND *CRYPTOSPORIDIUM PARVUM* IN AN OUTBREAK
OF NEONATAL DIARRHEA IN BEEF CATTLE

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ABSTRACT

This article describes the role of bovine coronavirus, rotavirus and *Cryptosporidium parvum* in an outbreak of beef calf diarrhea in Presidente Epitácio, São Paulo State. Two out of 9 fecal samples were positive to BCoV, 6 to *C. parvum* and 6 to rotavirus, with 4 rotavirus-*C. parvum* co-infections, 1 BCoV-*C. parvum* co-infection and 1 rotavirus-BCoV co-infection. These results show the need for a detailed survey of the etiology of diarrheas, aiming to evaluate the agents spread in a flock, allowing the design of prophylactic measures against gastroenteritis outbreaks.

KEY WORDS: Cattle, coronavirus, *Cryptosporidium parvum*, diarrhea, mixed infections, rotavirus.

RESUMO

INFECÇÃO MISTA DE CORONAVÍRUS BOVINO, ROTAVÍRUS E *CRYPTOSPORIDIUM PARVUM* EM UM SURTO DE DIARRÉIA EM BEZERROS. Estudou-se a participação de coronavírus bovino (BCoV) rotavírus e *Cryptosporidium parvum* em um surto de diarréia em bezerros de corte no Município de Presidente Epitácio, Estado de São Paulo. Das 9 amostras colhidas, 2 foram positivas para BCoV, 6 para *C. parvum* e 6 para rotavírus, sendo 4 co-infecções por rotavírus e *C. parvum*, 1 co-infecção por BCoV e *C. parvum* e 1 co-infecção por rotavírus e coronavírus. Estes resultados reiteram a necessidade de que se pesquise de um modo abrangente a etiologia de diarréias, com o intuito de se avaliarem os agentes circulantes no rebanho, o que possibilita uma abordagem profilática mais exata para impedir o aparecimento de surtos epidêmicos de gastroenterites.

PALAVRAS-CHAVE: Bovinos, coronavírus, *Cryptosporidium parvum*, diarréia, infecções mistas, rotavírus.

In newborn cattle, rotavirus, coronavirus, enterotoxigenic *Escherichia coli*, *Salmonella* sp and *Cryptosporidium parvum* are the pathogens most frequently involved in cases of infectious diarrhea (SNODGRASS et al., 1986), with a higher importance attributed to rotavirus and coronavirus, found in about 60% of the cases (JEREZ, 1997).

Regarding Brazilian beef cattle, other authors have already reported the occurrence of rotavirus in calves (BUZINARO et al., 2003) and anti-rotavirus antibodies in multi-aged individuals (BRANDÃO et al., 2002), but little is known about the occurrence of co-infections with other enteric pathogens, mainly because the extensive breeding system used in Brazil does not allow a close follow up of the herd as it occurs for dairy cattle.

The aim of this study was to search for BCoV, rotavirus, *Cryptosporidium* sp., *Eimeria* sp., *Giardia* sp.

and *Strongyloidea* in fecal samples of beef calves during an outbreak of diarrhea.

During an outbreak of diarrhea in a beef cattle farm located in Presidente Epitácio, São Paulo State, in December 2000, fecal samples were collected from nine diseased calves directly from the rectum and stored under refrigeration (4-10° C). The reported attack rate of diarrhea among calves between 10-to-60-day old calves was 30 to 40%.

The nine samples were prepared as 20% (v/v) suspensions in phosphate buffer saline solution 0.01M/BSA 0.1% pH 7.2 (PBS) and clarified by centrifugation (12,000 xg/30min at 4° C). A RT-PCR assay targeted to amplify a 136-bp fragment of group II coronaviruses RNA-dependent RNA-polymerase gene (*RdRp*) was applied as described by BRANDÃO et al. (2005). Bovine coronavirus Kakegawa strain

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(AKASHI et al., 1980) was used as positive and PBS as negative controls, respectively.

Eight of the nine samples were searched for rotavirus 11-segmented RNA in PAGE (polyacrylamide gel electrophoresis) according to HERRING et al. (1982); the lacking sample had not enough volume to be tested. These samples were prepared as 20% suspensions (v/v) in TRIS (base) 0.1M pH 7.3 and clarified by centrifugation (12,000 xg/30min at 4° C). Total RNA was extracted with phenol/ chlorophormium, precipitated with ethanol and resolved in 3.5%/7.5% discontinuous polyacrylamide gel under 20 mA for 2 hours and stained with silver. The NCDV rotavirus strain (MEBUS et al., 1969) was included as positive and TRIS (base) 0.1M pH7.3 as negative controls, respectively.

Oocysts of *Cryptosporidium* sp. and *Eimeria* sp. and cysts of *Giardia* sp. and *Strongyloidea* eggs were searched in 20% suspensions of the nine fecal samples in PBS (v/v) with the sucrose flotation test (specific gravity 1.205) with an optical microscope as described by OGASSAWARA; BENASSI (1980).

From the nine fecal samples collected, two were found positive to BCoV and six to *Cryptosporidium parvum*. Eight samples were tested for rotavirus and six were positive, with a typical Group A rotavirus electropherotype. Four rotavirus-*C. parvum*, one BCoV-*C. parvum* and one rotavirus-BCoV co-infections were observed.

BCoV is an epitheliotropic virus that replicates in the villi of the absorptive cells of small gut and in non-differentiated cells from the crypts in the colon, leading to cell desquamation and villi shortening, acting therefore as a primary enteric pathogen (PENZAERT et al., 1994). Rotaviruses present a similar pathogenesis and have already been assigned a role as a major primary etiologic agent of neonatal calf diarrhea (SNODGRASS et al., 1986).

Cryptosporidium can be found both in small and large guts in calves and the frequency of infection in suckling calves may reach 100% (ROSALES et al., 1998).

Taken into account, these results allow one to assume that all the three pathogens, i.e., BCoV, rotavirus and *C. parvum*, have been responsible for the outbreak studied, acting in a synergistic way, and are important pathogens for calves in the surveyed area. Though no studies on the economic impact of neonatal calf diarrhea have been carried out in Brazil, it is estimated that in the USA the yearly loss due to this disease is of US\$ 95 millions (HOUSE, 1978), what allows one to depict the consequences the lack of control measures against diarrhea may lead to in cattle breeding.

These results reinforce the need for a broad approach when the aim is the etiology of beef cattle diarrhea, allowing the design of more exact prophylactic measures against new outbreaks based on a continuous surveillance.

Further studies are ongoing in order to elucidate the molecular characteristics of the BCoV and rotavirus strain detected.

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