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SHORT COMMUNICATIONS

Influence of individual or group housing of newborn calves on rotavirus and coronavirus infection during the first 2 months of life

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Abstract
Bovine rotavirus A (RVA) and bovine coronavirus (CoV) are the two main viral enteropathogens associated with neonatal calf diarrhea. The aim of the present work was to study the impact of group and individual housing systems in the epidemiology of RVA and CoV infection. Eleven calves reared in individual housing (FA) and nine calves in group housing (FB) were monitored during the first 7 weeks of life. Stool and serum samples were screened for RVA and CoV antigens by ELISA. IgG1 antibodies (Ab) to both antigens were also measured. From the 160 fecal samples collected, the proportion of positive samples to RVA and CoV was significantly higher in FB (23.6%) than in FA (9%) ($p = 0.03$). The geometric mean of colostral IgG1 Ab titers to CoV and RVA in FA (IgG1 anti-CoV 1024 and anti-RVA 1782.9) was lower than in FB (IgG1 anti-CoV 10,321.2 and anti-RVA 4096) at birth. Calves less than 2 weeks of life from FB had a higher risk of being infected by RVA (OR = 4.9; $p = 0.01$) and CoV (OR = 17.15; $p = 0.01$) than calves from FA. The obtained results showed that there was higher RVA and CoV shedding in group-housed calves than in individual-housed animals.

Keywords Bovine coronavirus · Bovine rotavirus · Dairy calf · Group housing · Individual housing · Longitudinal study

Introduction

Bovine rotavirus A (RVA) and bovine coronavirus (CoV) are the two main viral enteropathogens associated with neonatal calf diarrhea (Afshari Safavi et al. 2012), and they are widely distributed throughout the world (Ghosh et al. 2008). They are well-recognized etiologic agents of the neonatal calf diarrhea syndrome together with other pathogens. Risk factors related to the surrounding environment and management practices can have a direct effect on the incidence and the epidemiology of those viral diarrheas. One of these factors is the housing system for rearing calves (Marcé et al. 2010). Cross-sectional surveys reported that systems using individual housing minimize enteric pathogen infections (Barrington et al. 2002) and decrease the risk of diarrhea (Gulliksen et al. 2009; Curtis et al. 2016).

Concerning to RVA and CoV infections in calves, there are just a few longitudinal studies, and most of them were performed in dairy farms with individual housing systems (McNulty and Logan 1983; Heckert et al. 1990; Coura et al. 2014; Bok et al. 2017). Thus, the true impact of the housing systems with regard to transmission and infection rates of bovine RVA and CoV in calves was not fully explored.

The objective of this study was to determine the impact of the calves' individual or group housing systems in the epidemiology of RVA and CoV in calves from dairy farms through a longitudinal study.

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Materials and methods

Geographic area and characteristics of farms studied

Lerma Valley is located in Salta province from Argentina, between 1100 and 1450 m above sea level, which absolute maximum temperatures stand out in November, reaching up to 40.9 °C, while July records absolute minimums of − 8.1 °C (Belmonte 2009; Martinez 2015). There are a total of 51 dairy farms with approximately 6500 Holstein cows in milking each (Suarez and Martinez 2015). The number of lactating cows in farm A (FA) and in farm B (FB) was 180 and 370 and an average of 23 and 25 of milk/cow/day, respectively. Both farms used a vaccine to prevent diarrheas with two doses 60 and 30 days prior to delivery as a general protocol.

Study design and data collection

The longitudinal study was performed on two dairy farms in 2015 between May and July. In FA, 11 calves were studied from birth until 7 weeks of life. The calves stayed with the dam 48–72 h after calving and then were housed tied in individual stakes until 2 months of life. They were fed with 4 l of raw milk (non-pasteurized, at a temperature of 37 °C) per day and calf starter ad libitum (Santa Silvina). In FB, 9 calves were studied from birth until 7 weeks of life. Calves were separated from their mothers after calving, and they were fed with 4 l of frozen-thaw colostrum before the first 12 h of life. They were housed in groups of 10 animals until 2 months of life, with a straw bed and a roof covering 50% of the pen, and they were fed with milk replacer (4 l per calf per day, at a temperature of 37 °C) and calf starter ad libitum (Santa Silvina).

To evaluate neonatal diarrhea in calves, samples were scored as non-liquid and liquid feces. This last one was defined as feces without consistency and complete dispersion in the floor or the container.

Fecal and serum samples and diagnosis procedures

Fecal and blood samples were taken once a week during the first 7 weeks of life. The first collection was on the day of birth after administration of colostrum. Aliquots of fecal samples were diluted 10% (w/v) in phosphate saline buffer (pH 7) and conserved at − 20 °C and − 70 °C until the analysis of bovine RVA and CoV, respectively. Polyclonal indirect enzyme-linked immune sorbent assay (iELISA) was performed for RVA antigen detection (Garaicoechea et al. 2006; Badaracco et al. 2013) and a monoclonal iELISA for CoV antigen detection (Smith et al. 1996). Serum samples were conserved at − 20 °C until the analysis. The IgG1 antibody (Ab) titers to RVA and CoV were measured using a double sandwich ELISA (Fernandez et al. 1996; Parreño et al. 2004; Bok et al. 2017).

Data analysis

Every sample was considered as an individual event. Descriptive analysis was conducted with the central tendency, and dispersion statistics and pathogen-specific and diarrhea prevalence were calculated. Odds ratio (OR) and confidence interval (CI 95%) were calculated to estimate if there was a relationship between RVA and CoV shedding and the housing system. Fisher's exact test and Pearson Chi-squared ($\chi 2$) test were used to establishing the degree of association. Geometric mean IgG1 antibody (Ab) titers (GMTs) to RVA and CoV determined by ELISA were log10-transformed before the statistical analysis. The IgG1 Ab titers to RVA and CoV were analyzed by a general linear mixed statistical model (GLMM) using the glm function (Epicalc packages 2.15.1.0) where the best-fit model was the one with the smallest Akaike information criterion (AIC), and the analysis was performed with RStudio 1.2.1335 (Bok et al. 2017). Statistical differences between the area under the curve (AUC) of diarrhea were calculated with the receiver operator characteristic (ROC) curve with the software Prism 5 version 5.01. Statistical significance was assessed at $p < 0.05$ for all comparisons.

Results

The proportion of calves infected at least once with RVA and CoV was 63.3% (7/11) and 100% (9/9) in FA and FB, respectively. In total, 160 fecal samples from 20 calves were collected. The proportion of samples positive to RVA and CoV is higher in FB (23.6%) than in FA (9%) for both antigens, being this difference statistically significant (Fisher exact test, $p = 0.03$) (Table 1). Only one calf from FA is co-infected with both viruses (calf #11, on day 35, Tables 2 and 3).

The distribution of RVA and CoV infections during the first 7 weeks of life in calves from FA (individual housing) and FB (group housing) is presented in Fig. 1a and b. In calves from FA, RVA shedding was registered between 21 and 42 days of life, while CoV shedding was detected only in two calves on day 2 and day 35, respectively. In calves from FB, both RVA and CoV antigens were mostly detected in the first 2 weeks of life. The individual housing was a protector

Table 1 Fecal samples positive to RVA and CoV

Farm	% of positive fecal samples		
	CoV	RVA	Total
A	2.2% (2/88)	9% (8/88)	9% (8/88)
B	5.5% (4/72)	18% (13/72)	23.6% (17/72)
Fisher exact test p value	0.4	0.1	0.03

Table 2 RVA IgG1 Ab titers measured at birth (0 days) and then every 7 days

Farm	Calf	0 days	7 days	14 days	21 days	28 days	35 days	42 days	49 days	SC
A	1	256	1024	1024	256	256	256	256	1024	No
	2	1024	1024	1024	1024	1024	4096/RVA	256	1024	No
	3	1024	1024	1024	1024	1024	1024/RVA	1024	256	No
	4	4096	4096	4096	1024/RVA	4096	4096/RVA	1024	4096	No
	5	4096	64	64	64	64	64	1024	1024	Yes
	6	256	256	256	1024	256	256/RVA	1024/RVA	1024	No
	7	4096	4096	1024	1024	256	1024	1024	1024	No
	8	4096	4096	4096	4096	1024	1024	1024	1024	No
	9	4096	4096	4096	1024	1024	256	256	1024	No
	10	1024	1024	1024	4096	4096/RVA	1024	1024	4096	No
	11	4096	4096	4096	4096	1024	1024/RVA	1024	1024	No
	GM	1782.9	1317.5	1494.5	1024	701.6	701.6	701.6	1161.5	
B	12	4096	1024/RVA	256/RVA	256	1024	1024	1024	256	Yes
	13	1024	1024/RVA	256/RVA	256	256	256	4026	256	Yes
	14	16,384	4096	4096	4024	1024	1024	1024	1024	No
	15	4096	1024/RVA	1024	4024	1024	1024	1024	1024/RVA	No
	16	1024	16,384	256/RVA	256	4096	4096	16,384	4096/RVA	Yes
	17	16,384	1024	1024	4024	256/RVA	256	256	256	No
	18	4096	4096	4096	1024	256/RVA	256	1024	16,384	Yes
	19	4096	16,384/RVA	4096/RVA	256	4096	1024	256	1024	Yes
	20	4096	4096	4096/RVA	4096	4096	1024	1024	1024	No
	GM	4096	3444.3	1896.1	1385.2	1024	752.5	1021.8	1024	

RVA ELISA virus shedding. *SC* seroconversion. *GM* geometrics mean

factor for infection with RVA in calves less than 2 weeks of life (OR = 0.09; IC 95% = 0.005–1.647; $p = 0.04$) in FA, while calves less than 2 weeks of life from FB showed higher risk of becoming infected by RVA (OR = 4.9; IC 95% = 1.362–17.84; $p = 0.01$) and CoV (OR = 17.15; IC 95% = 0.888–331; $p = 0.01$) than older animals in that farm.

The proportion of samples with diarrhea was not significantly different between farms (FA 18/88; FB 11/72; Fisher exact test, $p = 0.4$). In FA 63% (7/11) and FB 77% (7/9) of calves had at least one episode of diarrhea, with 2.5 and 1.3 diarrhea cases per day, respectively. The distribution of diarrhea during the first 7 weeks in FA and FB animals is presented in Fig. 2. In FA, at 7 days of life, the prevalence of calves with diarrhea reached the maximum, and in general terms, it remained constant until the end of the study. In FB, since the first days of life (day 0), we found sick calves with a maximum prevalence at 7 and 21 days of life, where it starts declining until the end of the study. The AUC of diarrhea was not significantly different among groups (ROC curve, $p = 0.2$). There was not a significant association between virus shedding and diarrhea.

Results of anti-RVA and anti-CoV IgG1 Ab titers in calf sera from FA and FB are presented in Tables 2 and 3 and Fig. 1c and d. Geometric mean of colostral IgG1 Ab titers to CoV and RVA at 0 days in FA was 1024 and 1782.9, respectively, while in FB was 10, 321.2 and 4096 for CoV and RVA, respectively. The IgG1 Ab titers to CoV were statistically different between both farms (GLMM, $p = 0.004$, AIC 46.1), while no significant differences were observed in the IgG1 Ab titers to RVA at this time point (0 days of life). The area under the curve (AUC) of the IgG1 Ab titers anti-CoV and anti-RVA in both farms showed a significant difference only in the profile of IgG1 to CoV (ROC curve, $p = 0.03$).

Finally, 27.7% (3/11) of calves (#1, #8, and #10) from FA showed anti-CoV IgG1 seroconversion even when CoV shedding was not detected with the methodology used in the samples taken weekly. Regarding anti-RVA IgG1 Ab response, only 9% (1/11) of calves (#5) show seroconversion without RVA detection in feces (Tables 2 and 3). On the other hand, 44.4% (4/9) of calves (#14, #17, #18, and #19) from FB showed anti-CoV IgG1 seroconversion, and three of them shed CoV in feces detectable by ELISA. Anti-RVA IgG1 seroconversion is observed in 55.5% (5/9) of the calves (#12, #13, #16, #18, and #19) from FB, where four of them shed RVA detectable by the ELISA assay used in this study (Tables 2 and 3).

Table 3 CoV IgG1 Ab titers measured at birth (0 days) and then every 7 days

Farm	Calf	0 days	7 days	14 days	21 days	28 days	35 days	42 days	49 days	SC
A	1	1024	4096	4096	1024	256	256	4096	4096	**Yes**
	2	1024	4046	4096	1024	256	256	256	256	No
	3	4096	16,384	4096	4096	4096	4096	1024	4096	No
	4	4096	4096	4096	4096	4096	1024	1024	1024	No
	5	4096/CoV	256	256	16	4	4	4	4	No
	6	1024	256	256	256	256	256	256	256	No
	7	4096	16,384	4096	4096	4096	4096	4096	1024	No
	8	1024	1024	1024	65,536	1024	1024	4096	256	Yes
	9	1024	4096	1024	1024	1024	1024	1024	1024	No
	10	4	4096	4096	4096	1024	1024	4096	1024	Yes
	11	1024	4096	4096	4096	4026	1024/CoV	4096	1024	No
	GM	1024	2803.3	1695.2	1695.2	700.5	545.3	902.7	545.3	
B	12	16,384/CoV	4096	1024	1024	1024	1024	1024	256	No
	13	16,384	4096	1024	1024	256	1024	1024	1024	No
	14	16,384/CoV	4096	4096	16,384	1024	16,384	4096	1024	Yes
	15	4096	4096	1024	1024	1024	4096	4096	4096	No
	16	16,384	16,384	16,384	16,384	4096	65,536	4096	4096	No
	17	4096	4096	4096/CoV	1024	1024	4	1024	1024	Yes
	18	65,536	4096	1024	1024	4096	16,384	1024	1024	Yes
	19	4096/CoV	4096	4096	16,384	16	1024	1024	1024	Yes
	20	4096	4096	4096	4096	4096	4096	4096	4096	No
	GM	10,321.2	4778.1	4778.1	3010	877.2	2211.9	2048	1717.7	

CoV ELISA virus shedding. *SC* seroconversion. *GM* geometrics mean

Discussion

The aim of the present study was to determine if housing conditions and other management practices including colostrum intake (directly from their dams or artificial feeding) of calves less than 7 weeks of life are risk factors for CoV and RVA infections and disease through a longitudinal study. In this survey, we observed in the group-housed system (FB) a higher RVA and CoV infection rates in calves. These results agree with general recommendations where calves should be reared in individual pens until 3 weeks of age, where avoiding direct contact between animals would help to minimize transmission of viral infections (Radostits 1991; Gulliksen et al. 2009; Curtis et al. 2016).

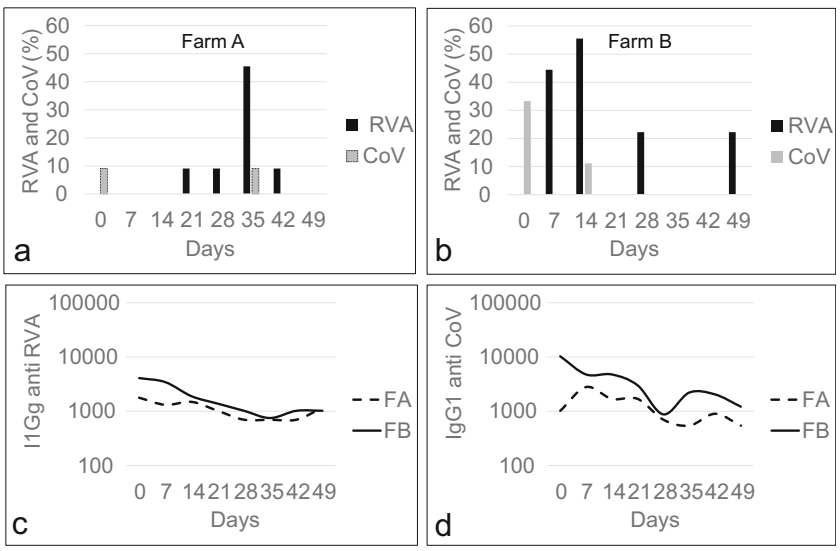

Fig. 1 **a** RVA and CoV shedding in FA (individual housing). **b** RVA and CoV shedding in FB (group housing). **c** IgG1 anti-RVA Ab titers in FA and FB. **d** IgG1 anti-CoV Ab titers in FA and FB

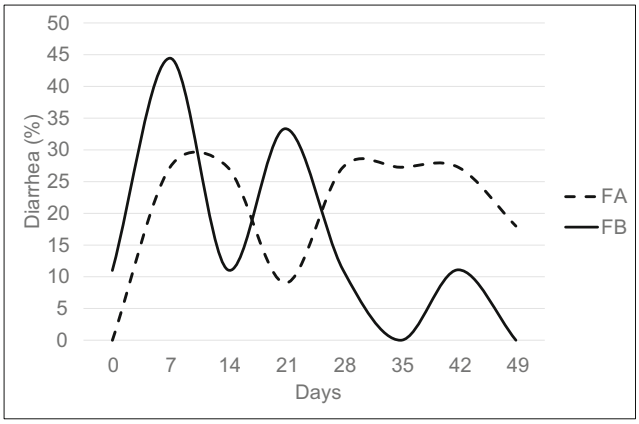

Fig. 2 Neonatal calf diarrhea and virus shedding in FA and FB

Regarding the time frame of virus shedding throughout this study, RVA and CoV were observed among 3 to 6 weeks of life in the individual-housed animals and in the first 2 weeks of life in the group-housed animals. These results are in agreement with Heckert et al. study in which calves housed in hutches or tied showed fecal and respiratory CoV shedding starting at 4 weeks of life (Heckert et al. 1990). However, our results differ from other longitudinal surveys in which RVA and CoV shedding was detected in the first 3 weeks of life in calves housed individually (McNulty and Logan 1983; Coura et al. 2014; Bok et al. 2017), while other surveys reported that RVA shedding starts at 30 days of life in most of the calves living in groups (Nagata et al. 1987). Strikingly, there was not a significant association between virus shedding and diarrhea. Probably, the reduced number of calves enrolled in the study and taking one sample per week was not enough to find this association.

Regarding the immune response of calves in different rearing systems, in group-housed calves (FB), where colostrum was artificially administered with bottles, the Ab titers (GM IgG1 Ab titer anti-CoV at day 0 = 10, 321.2 and GM IgG1 Ab titer anti-RVA at day 0 = 4096) were higher than Ab titers in individual-housed calves (GM IgG1 Ab titer anti-CoV at day 0 = 1024 and GM IgG1 Ab titer anti-RVA at day 0 = 1782.9), where colostrum intake was directly from the dam, similarly as has been reported before (Besser et al. 1991). Despite the higher titer of Abs after colostrum intake in group-housed calves compared with individual-housed calves, a higher proportion of seroconversion and virus shedding in group-housed calves (FB) was observed, confirming the hypothesis that group housing will increase the environmental contaminations with viral pathogens, where calves with high Ab titers still get infected. However, the fact that calf can get infected without developing diarrhea in the presence of high titers of circulating maternal Ab was previously studied (Parreño et al. 2004). The higher titers of Ab to CoV in dam's colostrum and calves' serum in FB could be associated with a higher circulation of this virus in that farm.

It is important to highlight that previous surveys reported optimum titers of passive IgG1 anti-RVA and anti-CoV above 16, 384 and 1024, respectively (Parreño et al. 2004; Bok et al. 2017). Considering that, in this study, 10% (2/20) and 95% (19/20) of calves had a successful passive transference of IgG1 anti-RVA and anti-CoV, respectively. These results help to explain the greater proportion of calves shedding RVA compared to calves shedding CoV in feces.

Respect the IgG1 curve in animal 5 for both viruses is really interesting since it was diagnosed with CoV infection at birth and the animal did not seroconvert for coronavirus during the time-frame of the study but do seroconverted for rotavirus at 42 days. This situation was also reported in a previous survey (Bok et al. 2017). As we can see, this happens in both RVA and CoV titers eliminating a possible error in the moment of the procedures, and this could be explained by a rapid metabolism of the passive antibodies in this particular calf and a longer immune gap than in the other calves. On the other hand, in calves 17 and 19, clearly we see how they get infected with RVA when the titers of passive maternal antibodies decreased. It is hard to explain why calf 17 did not seroconvert for IgG1. The study of IgM and IgA Ab responses in serum and feces might help to understand its behavior. Concerning the late seroconversion for CoV infection also shows a delay in the Ab response, although the lack of enough sensitivity of the ELISA for antigen detection can not be discharged. Probably it would have been better to use a more sensitivity technique like RT-PCR.

Since this was an observational study, there was no control of any of the variables that could influence the result. The trends regarding virus infection and diarrhea found throughout the study would probably be more evident (statistically significant) when comparing both rearing systems if a larger number of calves were enrolled and followed daily instead of weekly. Despite the objective was pointed to the study of RVA and CoV, it would have been useful to investigate the other pathogens involved in the microbiology of the neonatal calf diarrhea. These points will be considered in the experimental design of the future field trials.

In this study, we observe how the housing system could influence RVA and CoV infections in neonatal calves, highlighting the importance of the global approach over the study of neonatal calf diarrhea.

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Compliance with ethical standards

Animal rights This article does not contain any experimental studies with animals performed by any of the authors.

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