Journal of Applied Microbiology, 2023, 134, 1–8
https://doi.org/10.1093/jambio/lxad094
Advance access publication date: 29 April 2023
Research Article

JOURNAL OF APPLIED MICROBIOLOGY
OXFORD UNIVERSITY PRESS

Fecal excretion of *Campylobacter jejuni* by young dairy calves and the relationship with neonatal immunity and personality traits

Delphine Rapp[1,*], Karin E. Schütz[2], Colleen Ross[1], Mhairi A. Sutherland[2], Melissa N. Hempstead[2], Rina Hannaford[3], Vanessa M. Cave[4], Gale Brightwell[1,5]

[1]Food System Integrity Team, AgResearch Ltd, Hopkirk Research Institute, Massey University, Tennent Drive, Palmerston North 4442, New Zealand
[2]Animal Behaviour & Welfare Team, AgResearch Ltd, Private Bag 3123, Hamilton 3240, New Zealand
[3]Data Science Team, AgResearch Ltd, Grasslands Research Centre, Tennent Drive, Palmerston North 4410, New Zealand
[4]Data Science Team, AgResearch Ltd, Ruakura Research Centre, Private Bag 3123, Hamilton 3240, New Zealand
[5]New Zealand Food Safety Science & Research Centre, Hopkirk Research Institute, Massey University, Tennent Drive, Palmerston North 4442, New Zealand

*Corresponding author. Food System Integrity, AgResearch Ltd Hopkirk Research Institute corner University Avenue & Library Road, Massey University Palmerston North 4442. Tel: +64 6 35 8648; E-mail: Delphine.Rapp@agresearch.co.nz

Abstract

Aims: Zoonotic pathogens in bovine herds are major concerns for human and animal health, but their monitoring in animals can be challenging in the absence of clinical signs. Our objective was to determine the association between fecal excretion of *Campylobacter jejuni*, neonatal immunity, and personality traits of calves.

Methods and results: Forty-eight dairy calves were reared in three indoor pens from birth to 4 weeks of life. Microbial analyses of the fecal samples collected weekly revealed that the proportion of calves naturally contaminated with *C. jejuni* in each pen reached 70% after 3 weeks of life. High ($>16\ g\ l^{-1}$) levels of IgG levels in the serum of neonatal calves were negatively ($P = .04$) associated with fecal detection of *C. jejuni* over the trial period. Calves that spent more time interacting with a novel object tended to be positive ($P = .058$) for *C. jejuni*.

Conclusions: Overall, the findings indicate that the immunity of neonatal dairy animals and possibly the animal's behavior may contribute to the fecal shedding of *C. jejuni*.

Significance and impact of study

Immunity and personality traits of dairy animals should be considered in future risk assessment and on-farm management of zoonotic bacteria.

Keywords: zoonosis, *Campylobacter jejuni*, dairy calves, disease management, animal exploration traits, immunity

Introduction

Campylobacter jejuni (*C. jejuni*) is a major bacterial cause of gastroenteritis worldwide (WHO 2020), with notification rates as high as 19.5 and 60.6 cases of human campylobacteriosis per 100 000 population reported in 2019 in the USA and in the European Union (EU), respectively (Tack et al. 2020, EFSA-ECDC 2021). Campylobacter infections can also cause severe neurological and auto-immune diseases (WHO 2020), and in some countries, the number of hospitalizations due to severe campylobacteriosis has risen significantly during the last decade (Baker et al. 2021).

Poultry and cattle are recognized as the major animal sources of infection for humans (Fitzenberger et al. 2010, Baker et al. 2021). Ingestion of contaminated food, consumption of raw milk or unpasteurized dairy products, ingestion of recreational or drinking water supply contaminated with animal feces, or contact with infected animals have been commonly associated with human campylobacteriosis (Liao et al. 2019, Davys et al. 2020, Gilpin et al. 2020, Mughini-Gras et al. 2021). In cattle, *C. jejuni* is usually not considered a pathogenic agent and is commonly excreted without clinical symptoms (Nielsen 2002, Häkkinen and Hänninen 2009); however, *C. jejuni* has been found in feces of diarrheic calves (Busato et al. 1999) and may play a role as preceding or synergistic infection agent (Klein et al. 2013). Hypervirulent *C. jejuni* can also cause abortion in sheep or cattle (Tang et al. 2017, Weis et al. 2017), emphasizing that on-farm control of *C. jejuni* would benefit both animal and public health.

Several risk factors associated with *C. jejuni* shedding by dairy cattle have been reported and include feed management, herd management, or environmental factors (Busato et al. 1999, Ellis-Iversen et al. 2009). Most of the studies investigating risk factors were population-based and provided limited information on individual animals. The few longitudinal studies that documented the excretion of *C. jejuni* by individual dairy animals have repeatedly shown an animal effect. Ross et al. (2008) and Hakkinen and Hänninen (2009) used a 3-month-sampling regime to monitor individual dairy animals over a 1-year period; they reported that

Received: October 2, 2022. **Revised:** April 19, 2023. **Accepted:** April 27, 2023

the majority of the studied animals were intermittently positive for *C. jejuni* and that ~10% of the cows in a herd excreted *C. jejuni* at every sampling. When a fortnightly sampling regime was used, a similar observation was reported (Rapp et al. 2012). However, the underlying factors explaining the difference in *C. jejuni* excretion among animals in the same herd have yet to be fully understood. Determining these factors could help developing effective strategies that control the presence and spread of this zoonotic pathogen in dairy herds.

Animal immunity has been associated with susceptibility to enteric agents, as shown with enteric agents affecting the health of dairy animals (Lefkaditis et al. 2020). After birth, calves can be exposed to high environmental levels of harmful enteric pathogens, which, if ingested, can lead to colonization of the intestinal membrane within the first 3 weeks of life, and to adverse consequences on calf health and growth (Meganck et al. 2014). Neonatal calves can be protected against fecal pathogens through ingestion of colostrum, the first milk produced by cows after calving, and which contains various immunoglobulins in concentrations higher than in normal milk (Hulbert and Moisa 2016). The success of passive transfer of immunoglobulins from dam to calf can be determined through measurements of the immunoglobulins IgG in the blood of neonatal calves, and IgG concentration of 10 $g\,l^{-1}$ has been proposed as a cut-off value defining success or failure of passive transfer of immunity (Besser et al. 1985, Wittum and Perino 1995). Recently, the USDA National Animal Health Monitoring System has proposed refined standards for grading serum IgG levels, in which serum IgG level of 16 $g\,l^{-1}$ was indicative of a "fair" passive transfer of immunity (Lombard et al. 2020).

Another animal factor that affects the animal's response to environmental challenges or stressors is the animal's temperament or personality, which can be assessed by measuring an animal's behavioral responsiveness to humans or objects using avoidance or approach tests (Boissy 1995). According to the literature, animals can be divided into sub-groups depending on their behavioral responses to stressors, e.g. calm or temperamental (Curley et al. 2008), more or less active (Beausoleil et al. 2008), and high or low avoidance (Steimer and Driscoll 2005). An early study revealed that beef animals that are agitated during loading onto transport trucks have twice the risk of having an enteropathogen-positive slaughter hide compared with cattle that are calm (Dewell et al. 2008), suggesting that an animal's behavioral response to an environmental challenge or stressor may impact the shedding enteric bacteria.

The aim of this study was to identify individual animal factors underlying *C. jejuni* shedding by investigating possible associations between *C. jejuni* excretion, calf immunological measures, and calf personality. A cohort of 48 dairy calves was monitored during the first month of life. Our hypothesis was that calves' neonatal immunity and personality contribute to the risk of calves being contaminated with *C. jejuni*.

Materials and methods

Project design

The study was initially designed to evaluate the impact of hygienic and biosecurity measures on the excretion of *C. jejuni* by a cohort of 48 dairy calves. It was conducted using three pens, each representing a different combination of environmental and hygienic rearing conditions (described below).

Animals, housing, and experimental design

The study was undertaken at the Massey University Research Farm in Palmerston North, New Zealand, between July and August 2020. All procedures involving animals were approved by the AgResearch (Grasslands) Animal Ethics Committee (AE application number 15024) under the New Zealand Animal Welfare Act 1999. Forty-eight (Friesian x Jersey breed) female dairy calves were used in the study. All the calves were born on straw bedding in a covered calving area. Calves were separated from their dam within a maximum of 12 h after birth as per the common practices of New Zealand dairy operations. They were transferred directly to their allocated pen in the nearby (<500 m) calf-rearing facility, which consisted of a barn with a solid roof and three side walls. The barn was divided into three pens (pen 1: 13.2 × 4.1 m; pens 2 and 3: 13.2 × 4.5 m) that were separated by clear plastic sheeting. All the pens were initially bedded with a layer of straw (~15 cm deep). New bedding material was regularly added during the experiment. Bedding was categorized as dry in 72%, 76%, and 69% of the daily observations in pen 1, 2, and 3, respectively, using a paper towel technique (Canadian Dairy Research Portal 2011). Pens 1 and 3 were covered with orchard bird netting (1 cm^2 square holes) to exclude birds that are a potential source of introduction of *C. jejuni*; both of these pens were also connected to the town water supply instead of to the roof-collected rainwater tanks (pen 2); the sides of pens 1 and 3 were checked daily for the presence of visible fecal contamination, which was cleaned with hot soapy water and surface disinfecting wipes (Mediwipes, Sulco, Auckland, and New Zealand).

Female dairy calves born during the trial period were allocated to the three pens that were each filled to capacity (space allowance: 2.4 m^2/calf) within 6–8 d. The first 23 calves born were allocated to pen 1. Pen 2 and pen 3 were then filled simultaneously until there were 25 calves/pen. The first 16 calves introduced in each pen were monitored for *Campylobacter* spp., i.e. 16 calves/pen. Each calf was identified by a unique farm identification number as well as individually marked using tail paint (Tell-tail paint, GEA FIL, New Zealand) to help with identification from videos. All calves stayed in their allocated pen for the entire duration of the trial.

Calves were individually bottle-fed 4 l of colostrum within 12 h of birth and were fed 5.2 l in two daily feeds for 2–4 d using a bucket with five soft rubber nipples cleaned daily with soap and warm water. Colostrum was harvested from cows that had calved that day and pooled; the quality of each colostrum pool was checked by refractometry by the farm staff as part of the farm routine practices for calf rearing (data not presented). Calves that refused the bottle twice were given their first colostrum meal through an esophageal tube; this was required for 4 calves. Calves were transitioned to an automatic feeder (Holm and Laue, Westerrönfeld, Germany) and were fed up to 5.2 l in two feeds per day between day 4 and day 10, and 6 l in two feeds per day after day 10. All the calves were offered hay and calf starter (20% CP, 40%–42% starch, 15%–16% NDF; Calf Max 20% pellets, SealesWinslow Ltd, Morrinsville, New Zealand) before they were 1 and 3 weeks old, respectively. All calves had free access to fresh water sup-

plied in water troughs placed in each pen and replenished daily. As part of the farm routine practices, calves of ∼2 weeks of age (16 ± 1.8 d of age, mean ± SD) were disbudded by a veterinarian. Disbudding was performed on fully sedated (Xylazine, Phoenix Pharm Distributers Ltd, Auckland, New Zealand) calves using an electric cautery iron, and calves were given a local anesthetic (Nopaine, Phoenix Pharm Distributers Ltd) and a non-steroidal anti-inflammatory drug (Meloxicam Metacam20; Boehringer Ingelheim NZ Ltd, Auckland, New Zealand). Calves were vaccinated by sub-cutaneous injection against 10 clostridial species (Covexin® 10, MSD Animal Health, Upper Hutt Wellington, New Zealand) on the day of disbudding.

Campylobacter jejuni detection

Fecal samples were collected from each calf within 1 d after arrival in the pen, and then once weekly for 4 consecutive weeks, i.e. from day 1 to 30 of age. Fecal sample collection was by direct retrieval after digital rectal stimulation. Fecal samples were transported to the laboratory in insulated containers and analyzed for *Campylobacter* spp. within 6 h of collection.

A 0.1 g aliquot of each calf's homogenized fecal sample was inoculated into 10 ml of pre-warmed Exeter Broth (ExBr) prepared as previously described (Rapp et al. 2012) and incubated at 42°C for 48 h. After incubation, 20 μl of the enriched ExBr was transferred to an mCCDA plate (Fort Richard Laboratories Ltd, Auckland, New Zealand) and incubated in a microaerophilic atmosphere at 42°C for 24 h. All microbial growth collected from mCCDA plates was resuspended in 1 ml of milli-Q water, from which total DNA was extracted using the PrepSEQ Rapid Spin Sample Preparation Kit (Applied Biosystems™ by Thermo Fisher Scientific, Life Technologies Corporation, Austin, USA). The presence of *C. jejuni* in every extracted DNA was confirmed using the TaqMan *Campylobacter* Multiplex Assay Beads (Applied Biosystems™) in an ABI 7500 Fast qPCR instrument (Applied Biosystems™). All the molecular assays were carried out according to the manufacturer's instructions.

Animal measurements and observations

Blood samples for evaluation of immunocompetence were collected from each calf at 2 d of age by jugular venipuncture and into vacuum collection tubes containing heparin (Becton Dickinson, Franklin Lakes, NJ). Blood was centrifuged, and the plasma was stored at –20°C. Total IgG titer in sera was determined by enzyme-linked immunosorbent assay using the IgG ELISA Development Kit (Mabtech AB, Sweden) following the manufacturer's instructions.

The calves' health was assessed daily by health checks carried out by farm and research personnel. Calves were scored at least twice weekly based on general appearance, coat condition, ocular and nasal discharges, umbilicus condition, cleanliness of the rear end, and gut fill, as described previously by Lowe et al. (2019).

The calves' responsiveness to humans was evaluated using a voluntary approach test as previously described by Schütz et al. (2012). Briefly, calves in groups of four were moved into a test arena pen (∼3 × 2.5 m) set up using portable gates within the home pen, and the behavior of the calves was recorded using two overhead video cameras (DS-2CD2332-I, Hikvision, Hangzhou, China) mounted at a height of ∼2 m in opposite corners of each home pen. A person unfamiliar to the calves entered the test arena pen and stood motionless for 1 min in the middle of the pen. One trained observer analyzed the video recordings using a video management system (Nx Witness, v4.1, Network Optix, Burbank, CA). It was recorded whether the calf approached the person by touching any part of the person with its muzzle or if the muzzle was within a head's length away from the person or not. On the same day as the voluntary approach, calves' willingness to approach a novel object was assessed using the same settings as described for the voluntary approach test but using an object (one orange traffic cone) that was new to the calves. It was placed in the middle of the test arena pen, in which it was left for the entire duration of the test which was 15 min. The total number of interactions and total duration of interactions with the object were determined from the video recordings. All the behavioral testing was undertaken one day prior to a fecal sampling, and because of the different days of enrolment, calves tested were 14 ± 1.7 d old (range: 12–17 d), 8 ± 2.0 (range: 5–11 d), and 8 ± 1.3 (range: 7–11 d) in pen 1, 2, and 3, respectively. One trained observer watched all the video recordings. For the recordings of voluntary approach and novel object tests, the intra-observer reliability was measured by rescoring a subset of the videos. Intra-observer reliability on 48 calves was 100% for the approach test (48 calves) measured as percentage agreement. The intra-observer reliability (done on 16 calves) for the number of interactions and total duration of interactions with a novel object was 0.97 and 1.0, respectively, as measured by correlation analysis.

Pen environmental conditions

Air temperature (AT) and relative humidity (RH) inside the calf rearing facility were measured continuously during the trial period using two data loggers (tinytag TGP-4500; Gemini Data Loggers Ltd, Chichester, UK). Both the loggers were positioned on walls at ∼1.5 m above the surface of the bedding, out of reach of the calves. AT and RH recorded at 1 min interval during the study averaged throughout the trial period to 10.8°C ± 4.3°C and 78.1% ± 14.8%, respectively.

Data analysis

Graphical and statistical analyses were conducted using the software R version 4.1.2 (R Core Team 2021) and Genstat® version 22 (VSNi, Hemel Hempstead, UK).

Occurrence of *C. jejuni*. The relationship between the presence/absence of *C. jejuni* in calf feces was visualized for each pen over the trial period. The impact of pen and time on *C. jejuni* presence was investigated using a generalized linear mixed model (GLMM) with a binomial distribution for the response variable (i.e. presence/absence of *C. jejuni)*, allowing for dispersion, and a logit link function. Sampling occasion and pen were included as fixed terms. To account for repeated measures, calf ID was incorporated as a random term. The significance of the fixed terms was tested using a random permutation test (99 permutations) with calf ID included as a blocking factor.

Novel object/person tests. The responses from the two behavioral trials were visualized along with the pathogen status of the animals as measured on the previous day. Two separate binomial generalized linear regression models with logit link functions were fitted to assess the potential effect of

an animal's test response [e.g. total interaction time (in second) for the novel object test; and whether an animal approached or not for the Novel Person test] on *C. jejuni* detection (presence/absence) in the calf feces. Each model comprised of additive effects of pen, calf age on the test day, and the behavioral test response. For the novel object test, eight calves in pen 1 were excluded because of a video malfunction. For the novel person test, one calf was excluded as it exited the test area before the end of the test period.

Passive transfer of immunity. Another aspect that was considered was whether *C. jejuni* present in the animal or not can be explained by the neonatal IgG levels after accounting for any effects of calf age and pen. This was assessed visually and using a binomial GLMM with a logit link, where the response was whether or not *C. jejuni* was present, and the explanatory variables were pen, age, and a factor indicating whether IgG levels were $>16\ g\,l^{-1}$ or not. To account for repeated measures, calf ID was incorporated as a random term. Permutation tests (99 permutations) were used to assess the significance of explanatory variables by dropping each one in turn from the full model.

Results

Fecal detection of *Campylobacter jejuni*

In each pen, *C. jejuni* was first detected in the feces of 6 calves that were between 30 h and 48 h old (pen 1: 1 calf; pen 2: 4 calves; pen 3: 1 calf), with no detection in calves that were younger than 30 h. The number of calves excreting *C. jejuni* changed significantly ($P = .01$) over time. There was no evidence of an overall pen effect ($P = 1.00$) but there was evidence of an interaction between time and pen ($P = .02$) (Fig. 1). *C. jejuni* was detected in >70% of the calves that were 3 or 4 weeks old. On the week of the personality tests, 25 (52%) calves were detected positive for *C. jejuni* (pen 1: 9; pen 2: 7; and pen 3: 9 calves).

Passive transfer of immunity

The concentration of IgG in the plasma of the 48 neonatal calves ranged from 6 to 40 $g\,l^{-1}$ (median:13 $g\,l^{-1}$), with no evidence of a difference among pens (pen 1: 14.8 ± 4.21; pen 2: 13.0 ± 3.91; and pen 3: $13.6 \pm 7.82\ g\,l^{-1}$). Age was found to be a useful predictor of *C. jejuni* status ($P = .01$). Calves with neonatal IgG plasma levels $\leq 16\ g\,l^{-1}$ were more likely to excrete *C. jejuni* during their first month of life than calves with IgG levels $>16\ g\,l^{-1}$ ($P = .04$) (Fig. 2).

Novel object and human approach tests

All the calves interacted with the novel object within 15 min of the object being introduced into the pen. The total amount of time the calves spent interacting with a novel object was associated ($P = .058$) with the presence/absence of *C. jejuni* in calf feces; calves that interacted for longer period of time with the novel object tended to be positive for *C. jejuni* (Fig. 3). There was no statistical evidence of a pen effect ($P = .598$) or of age of calf on the day of the test ($P = .884$). The extra uncertainty for interaction with an object in the first pen was likely due to the smaller number of animals observed in that pen.

The proportion of calves that approached an unfamiliar person is shown in Fig. 4. A large majority (42/48 calves) of the calves approached the unfamiliar person within the 1-min period, and as a result there was no statistical evidence that the calf response to a novel person ($P = .112$), the pen in which the calves were kept ($P = .632$), or the age of the calves on the day of the test ($P = .744$) contributed to the presence/absence of *C. jejuni* in the feces.

Health scores

The calves were examined daily for signs of illness or injury as part of the farm routine monitoring; they were also assessed as per previously published procedures (Lowe et al. 2019) for an average of 11 times (range 9–12) during the study period, amounting to 505 individual health scores. No calves were recorded as having a severe clinical score, and only few health issues (<3% total observations) were recorded during the study. The most common health issues were minimal nasal discharge, minimal ocular discharge, or slightly swollen navels with normal temperature, reported once or twice for 20 calves, and at three or more occasions for 2 calves. The number of days for which health issues were recorded was similar between calves that had IgG levels $\leq 16\ g\,l^{-1}$ (0.7 ± 0.77 d) and calves that had IgG levels $>16\ g\,l^{-1}$ (0.7 ± 1.88 d).

Discussion

This study investigated the association between excretion of *C. jejuni*, a bacterial agent that can be spread among animals without clinical symptoms, immunocompetence, and personality traits of young (<1 month) dairy calves. Seventy percent of calves were excreting *C. jejuni* at 3 weeks of age. High ($>16\ g\,l^{-1}$) neonatal IgG levels were negatively associated with the presence of *C. jejuni*. Our study did not demonstrate a strong effect of the calf personality on fecal excretion of *C. jejuni*, possibly due to a low number of animals in each pen; however, there was a weak tendency that calves that interacted more with a novel object were *C. jejuni* positive.

Previous observations reported that calves are campylobacter free at birth but gradually become contaminated with *C. jejuni* during the first 3 to 4 months after birth (Nielsen 2002, Hansson et al. 2021). The occurrence of *C. jejuni* in the young calves in our study increased rapidly during the trial period as the large majority of the 48 calves excreted *C. jejuni* when they were ~1 month of age. While it is possible that large group-housing facilitated the fecal-oral transmission of the bacteria among animals housed in the same pen, our study confirmed that *C. jejuni* can successfully colonize the gastrointestinal tract of young calves (Terzolo et al. 1987). It also showed that a small proportion (4/48) of calves appeared to be more resistant to colonization or re-infection by the enteric bacteria, as previously observed in calves and in adult animals (Rapp et al. 2012, Hansson et al. 2021).

To better understand the factors driving animal differences in pathogen colonization, we investigated whether calf immunocompetence can affect the risk of contamination with *C. jejuni*. Adequate transfer of immunity from the dams to the calves has been repeatedly shown as reducing the risk of diarrheal diseases or mortality in calves (Lefkaditis et al. 2020) but its impact on other enteric bacteria not associated with calf disease has not yet been fully established. In the current study, we found that the occurrence of *C. jejuni*

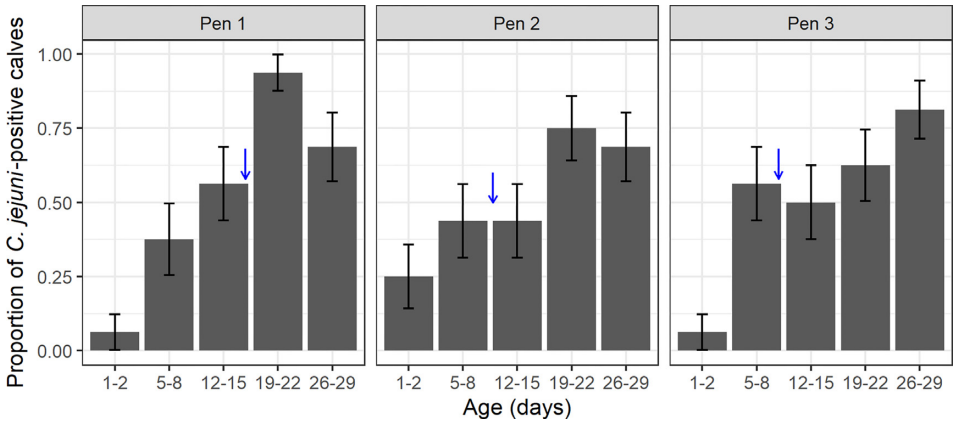

Figure 1. Proportion of calves excreting *C. jejuni* in the three groups of calves ($n = 16$ calves/pen) at different ages (in days) during the study. Time of the novel object and human approach tests is indicated by an arrow. Error bars represent the standard errors of the proportions.

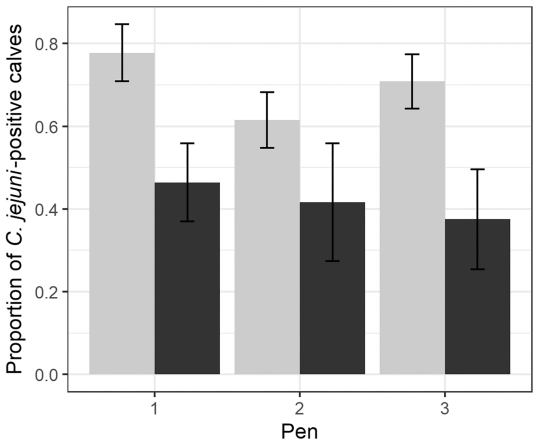

Figure 2. Proportion of calves excreting *C. jejuni* according to neonatal concentration of plasma IgG (≤16 g l^{-1} in gray; >16 g l^{-1} in black) in each pen ($n = 16$ calves/pen). Error bars represent the standard errors of the proportions.

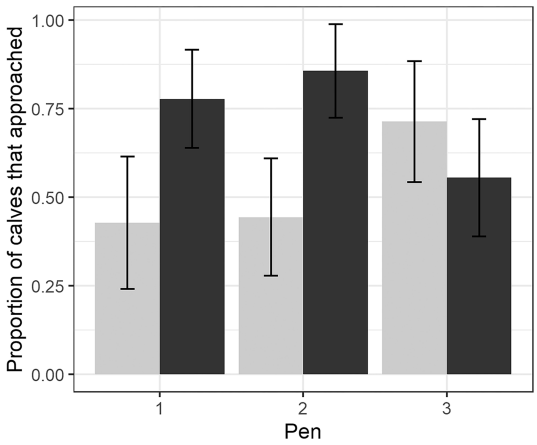

Figure 4. Proportion of *C. jejuni*-negative (in gray) and *C. jejuni*-positive (in black) calves that approached an unfamiliar person in each pen. The response was recorded for sixteen calves in each pen for a 1-min period. Error bars represent the standard errors of the proportions.

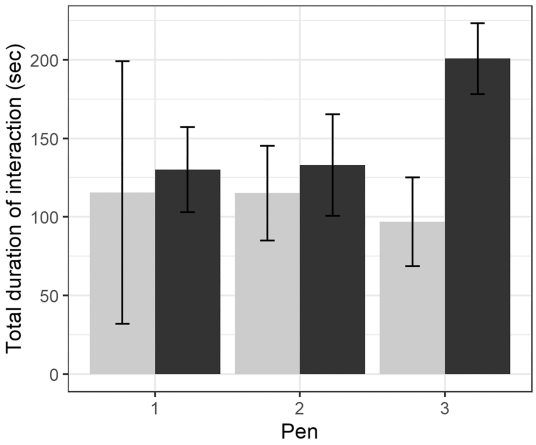

Figure 3. Total time (in second) the *C. jejuni*-negative (in gray) and *C. jejuni*-positive (in black) calves in each pen spent interacting with a novel object. The approach response was tested in the calf home pen for 15 min. Sixteen calves were tested in each pen, except for pen 1 with data available for only 8 calves. Error bars represent the standard errors of the observed means.

was negatively associated with plasma IgG levels in young calves, adding to previous evidence that higher concentration of IgG can be associated with lower odds of zoonotic bacteria shedding by 3-week-old calves (Stenkamp-Strahm et al. 2018). Our findings also confirmed that low neonatal calf immunocompetence increases the risk of *C. jejuni* excretion (Terzolo et al. 1987, Busato et al. 1999) and highlight the importance of adequate quantities of high-quality colostrum to protect against infection with *C. jejuni* during the first 4 weeks of life. Adequate transfer of immunity promotes the development of intestinal organs (Hammon et al. 2020) and possibly the establishment of a protective gut microbiota (Chuang et al. 2022). Further studies investigating the mechanisms associated with IgG protection against *C. jejuni* are warranted.

In studies on hygiene practices and management of calves pre-weaning, it was found that the environmental exposure of calves to feces in the calving or rearing environment or the use of contaminated feeding equipment could potentially affect contamination with fecal pathogens and calf diarrhea (Duse et al. 2015, Stenkamp-Strahm et al. 2018). In our study, we expected calves in the same pen to have a similar environmental

exposure to fecal contaminants, but predicted that calves that explore their environment more would have a greater risk of being contaminated with *C. jejuni*. We used the human approach and novel object tests to evaluate the animal personality and exploration traits potentially governing asymptomatic excretion of *C. jejuni*. Our findings revealed that calves that interacted longer with a novel object tended to be *C. jejuni* positive, suggesting an association between calf behavior and contamination by *C. jejuni*. This is in agreement with the study by Tamminen et al. (2020), in which individual differences in calf behavioral traits were linked to excretion of another enteric zoonotic bacteria, i.e. *Escherichia coli* O157: H7. The behavior of animals in the voluntary approach tests is an indication of fear-related responses and has commonly been used in welfare research (Forkman et al. 2007, Lecorps et al. 2018). Fear of novelty is balanced by the individual's motivation to explore and is one of the traits describing calf personality (Wood-Gush and Vestergaard 1989, Koolhaas and Van Reenen 2016). However, the response to an unfamiliar person or object can be affected by other factors, including the way calves are handled prior to the test (Schütz et al. 2012), disease (Cramer and Ollivett 2020), or in the early days of recovering after sickness (Cramer and Stanton 2015). In this study, the calves were overall healthy and were cared for by experienced staff who treated the animals in a gentle and calm manner. We therefore speculate that the calves' responses to the novel object were more a reflection of the animals' motivation to explore rather than level of fearfulness or sickness, and that exploration may be positively associated with contamination with *C. jejuni*; however, more research is needed to confirm this. While our study showed a possible association between calf personality and excretion of *C. jejuni*, the causality was not established. Future longitudinal studies investigating multiple behavioral measures of calves on different farms are recommended to establish the exact relationship between excretion of zoonotic bacteria and calf personality.

Overall, our results showed that calves are natural amplifiers of *C. jejuni* and excrete it as early as the first week of life, which may have implications for human health. Calves infected with *C. jejuni* may be a direct source of infection to humans, particularly to calf rearers and veterinarians who are likely to be exposed to contamination with *C. jejuni* and other fecal bacteria (Graveland et al. 2010). Moreover, *C. jejuni* has developed resistance to multiple antimicrobial treatments and now represents one of today's serious threats to global health (CDC 2019). In the context of a One Health approach, methods leveraging animal health and welfare to reduce the health risk to humans and the need for either veterinary or human treatment could help in controlling the spread of enteric zoonotic bacteria that carry antimicrobial resistance markers.

In conclusion, the excretion of *C. jejuni* by asymptomatic calves was associated with immunocompetence markers and possibly with animal behavioral traits related to exploration. Promoting management practices ensuring appropriate passive transfer of immunity, which is already recommended for improving calf health and productivity, may reduce excretion of zoonotic bacteria such as *C. jejuni*. Our study moreover indicates that further assessment of the relationship between animal immunity, animal personality traits, management practices, and excretion of zoonotic bacteria by dairy cattle could provide additional valuable insights for holistic management of human health risks in dairy herds.

Acknowledgements

The authors would like to acknowledge the Massey Farm Dairy N°4 manager and personnel for their co-operation and veterinary advice; G.L., A.G., T.W., A.R., A.N., C.T., and N.H. for their technical assistance. Financial support was provided by the New Zealand Ministry of Business, Innovation & Employment through the AgResearch Ltd Strategic Science Investment Fund (SSIF) and the New Zealand Bioeconomy in the Digital Age (NZBIDA) platform. The authors confirm that there is no conflict of interest regarding the publication of this article.

Conflict of interest statement

No conflict of interest declared.

Author contributions

Delphine Rapp (Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing), Karin E. Schütz (Data curation, Investigation, Writing – review & editing), Colleen Ross (Conceptualization, Data curation, Investigation, Writing – review & editing), Mhairi A. Sutherland (Conceptualization, Writing – review & editing), Melissa N. Hempstead (Investigation, Methodology, Writing – review & editing), Rina Hannaford (Formal analysis, Writing – review & editing), Vanessa M. Cave (Formal analysis, Writing – review & editing), and Gale Brightwell (Funding acquisition, Writing – review & editing).

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

References

Baker M, Grout L, Wilson N. Update on the campylobacter epidemic from chicken meat in New Zealand: the urgent need for an upgraded regulatory response. *Epidemiol Infect* 2021;**149**:E30. https://doi.org/10.1017/S095026882000299X.

Beausoleil NJ, Blache D, Stafford KJ *et al*. Exploring the basis of divergent selection for 'temperament' in domestic sheep. *Appl Anim Behav Sci* 2008;**109**:261–74. https://doi.org/10.1016/j.applanim.2007.03.013.

Besser TE, Garmedia AE, McGuire TC *et al*. Effect of colostral immunoglobulin G1 and immunoglobulin M concentrations on immunoglobulin absorption in calves. *J Dairy Sci* 1985;**68**:2033–7. https://doi.org/10.3168/jds.S0022-0302(85)81065-1.

Boissy A. Fear and fearfulness in animals. *Q Rev Biol* 1995;**70**:165–91. https://doi.org/10.1086/418981.

Busato A, Hofer D, Lentze T *et al*. Prevalence and infection risks of zoonotic enteropathogenic bacteria in Swiss cow-calf farms. *Vet Microbiol* 1999;**69**:251–63. https://doi.org/10.1016/S0378-1135(99)00119-4.

Canadian Dairy Research Portal. *Producer Resources. Stall Wetness*. 2011. https://www.dairyresearch.ca/animal-comfort-tool.php. (30 April 2020, date last accessed).

CDC. Antibiotic resistance threats in the United States. 2019. https://www.cdc.gov/DrugResistance/Biggest-Threats.html. (30 April 2022, date last accessed).

Chuang S-T, Chen C-T, Hsieh J-C *et al*. Development of next-generation probiotics by investigating the interrelationships between gastrointestinal microbiota and diarrhea in preruminant Hol-

stein calves. *Animals* 2022;**12**:695. https://doi.org/10.3390/ani12060695.
Cramer C, Ollivett T. Behavior assessment and applications for BRD diagnosis: preweaned dairy calves. *Anim Health Res Rev* 2020;**21**:188–91. https://doi.org/10.1017/S1466252320000213.
Cramer MC, Stanton AL. Associations between health status and the probability of approaching a novel object or stationary human in preweaned group-housed dairy calves. *J Dairy Sci* 2015;**98**:7298–308. https://doi.org/10.3168/jds.2015-9534.
Curley KO, Jr, Neuendorff DA, Lewis AW *et al*. Functional characteristics of the bovine hypothalamic-pituitary-adrenal axis vary with temperament. *Horm Behav* 2008;**53**:20–27. https://doi.org/10.1016/j.yhbeh.2007.08.005.
Davys G, Marshall J, Fayaz A *et al*. Campylobacteriosis associated with the consumption of unpasteurised milk: findings from a sentinel surveillance site. *Epidemiol Infect* 2020;**148**:E16. https://doi.org/10.1017/S0950268819002292.
Dewell GA, Simpson CA, Dewell RD *et al*. Risk associated with transportation and lairage on hide contamination with *Salmonella enterica* in finished beef cattle at slaughter. *J Food Prot* 2008;**71**:2228–32. https://doi.org/10.4315/0362-028X-71.11.2228.
Duse A, Persson Waller K, Emanuelson U *et al*. Risk factors for quinolone-resistant *Escherichia coli* in feces from preweaned dairy calves and postpartum dairy cows. *J Dairy Sci* 2015;**98**:6387–98. https://doi.org/10.3168/jds.2015-9453.
EFSA-CDC (European Food Safety Authority & European Centre for Disease Prevention and Control). The European Union one health 2019 zoonoses report. *EFSA J* 2021;**19**:e06406. https://doi.org/10.2903/j.efsa.(2021)6406.
Ellis-Iversen J, Cook AJC, Smith RP *et al*. Temporal patterns and risk factors for *Escherichia coli* O157 and *Campylobacter* spp. in young cattle. *J Food Prot* 2009;**72**:490–6. https://doi.org/10.4315/0362-028X-72.3.490.
Fitzenberger J, Uphoff H, Gawrich S *et al*. Urban–rural differences of age- and species-specific campylobacteriosis incidence, Hesse, Germany, July 2005–June 2006. *Euro Surveill* 2010;**15**:19693. https://doi.org/10.2807/ese.15.42.19693-en.
Forkman B, Boissy A, Meunier-Salaün M-C *et al*. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. *Physiol Behav* 2007;**92**:340–74, https://doi.org/10.1016/j.physbeh.2007.03.016.
Gilpin BJ, Walker T, Paine S *et al*. A large scale waterborne campylobacteriosis outbreak, Havelock North, New Zealand. *J Infect* 2020;**81**:390–5. https://doi.org/10.1016/j.jinf.(2020)06.065.
Graveland H, Wagenaar JA, Heesterbeek H *et al*. Methicillin resistant *Staphylococcus aureus* ST398 in veal calf farming: human MRSA carriage related with animal antimicrobial usage and farm hygiene. *PLoS One* 2010;**5**:e10990. https://doi.org/10.1371/journal.pone.0010990.
Hakkinen M, Hänninen ML. Shedding of *Campylobacter* spp. in Finnish cattle on dairy farms. *J Appl Microbiol* 2009;**107**:898–905. https://doi.org/10.1111/j.1365-2672.2009.04269.x.
Hammon HM, Liermann W, Frieten D *et al*. Review: importance of colostrum supply and milk feeding intensity on gastrointestinal and systemic development in calves, *Animal* 2020;**14**:s133–43. https://doi.org/10.1017/S1751731119003148.
Hansson I, Tamminen L-M, Frosth S *et al*. Occurrence of *Campylobacter* spp. in Swedish calves, common sequence types and antibiotic resistance patterns. *J Appl Microbiol* 2021;**130**:2111–22. https://doi.org/10.1111/jam.14914.
Hulbert LE, Moisá SJ. Stress, immunity, and the management of calves. *J Dairy Sci* 2016;**99**:3199–216. https://doi.org/10.3168/jds.2015-10198.
Klein D, Alispahic M, Sofka D *et al*. Prevalence and risk factors for shedding of thermophilic *Campylobacter* in calves with and without diarrhea in Austrian dairy herds. *J Dairy Sci* 2013;**96**:1203–10. https://doi.org/10.3168/jds.2012-5987.
Koolhaas JM, Van Reenen CG. Animal behavior and well-being symposium, interaction between coping style/personality, stress, and welfare, relevance for domestic farm animals. *J Anim Sci* 2016;**94**:2284–96. https://doi.org/10.2527/jas.2015-0125.
Lecorps B, Weary DM, von Keyserlingk MAG. Pessimism and fearfulness in dairy calves. *Sci Rep* 2018;**8**:1421. https://doi.org/10.1038/s41598-017-17214-3.
Lefkaditis M, Mpairamoglou R, Sossidou A *et al*. Importance of colostrum IgG antibodies level for prevention of infection with *Cryptosporidium parvum* in neonatal dairy calves. *Prev Vet Med* 2020;**176**:104904. https://doi.org/10.1016/j.prevetmed.(2020)104904.
Liao SJ, Marshall J, Hazelton ML *et al*. Extending statistical models for source attribution of zoonotic diseases, a study of campylobacteriosis. *J R Soc Interface* 2019;**16**:20180534. https://doi.org/10.1098/rsif.2018.0534.
Lombard J, Urie N, Garry F *et al*. Consensus recommendations on calf- and herd-level passive immunity in dairy calves in the United States. *J Dairy Sci* 2020;**103**:7611–24. https://doi.org/10.3168/jds.2019-17955.
Lowe GL, Sutherland MA, Waas JR *et al*. Physiological and behavioral responses as indicators for early disease detection in dairy calves. *J Dairy Sci* 2019;**102**:5389–402. https://doi.org/10.3168/jds.2018-15701.
Meganck V, Hoflack G, Opsomer G. Advances in prevention and therapy of neonatal dairy calf diarrhoea, a systematical review with emphasis on colostrum management and fluid therapy. *Acta Vet Scand* 2014;**56**:75. https://doi.org/10.1186/s13028-014-0075-x.
Mughini-Gras M, Pijnacker R, Coipan C *et al*. Sources and transmission routes of campylobacteriosis, a combined analysis of genome and exposure data. *J Infect* 2021;**82**:216–26. https://doi.org/10.1016/j.jinf.(2020)09.039.
Nielsen EM. Occurrence and strain diversity of thermophilic campylobacters in cattle of different age groups in dairy herds. *Lett Appl Microbiol* 2002;**35**:85–89. https://doi.org/10.1046/j.1472-765X.2002.01143.x.
R Core Team. *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria*. 2021. https://www.R-project.org/ (17 April 2023, date last accessed).
Rapp D, Ross CM, Pleydell EJ *et al*. Differences in the fecal concentrations and genetic diversities of *Campylobacter jejuni* populations among individual cows in two dairy herds. *Appl Environ Microbiol* 2012;**78**:7564–71. https://doi.org/10.1128/AEM.01783-12.
Ross CM, Donnison AM, Clark DA. Effect of using a stand-off pad on *Campylobacter jejuni* strain diversity in a herd of dairy cows. *Lett Appl Microbiol* 2008;**47**:192–6. https://doi.org/10.1111/j.1472-765X.(2008)02397.x.
Schütz KE, Hawke M, Waas JR *et al*. Effects of human handling during early rearing on the behaviour of dairy calves. *Anim Welf* 2012;**21**:19–26. https://doi.org/10.7120/09627281279912941 1.
Steimer T, Driscoll P. Inter-individual vs line/strain differences in psychogenetically selected Roman High-(RHA) and Low-(RLA) avoidance rats, neuroendocrine and behavioural aspects. *Neurosci Biobehav Rev* 2005;**29**:99–112. https://doi.org/10.1016/j.neubiorev.2004.07.002.
Stenkamp-Strahm C, Lombard JE, Magnuson RJ *et al*. Preweaned heifer management on US dairy operations: part IV. Factors associated with the presence of *Escherichia coli* O157 in preweaned dairy heifers. *J Dairy Sci* 2018;**101**:9214–28. https://doi.org/10.3168/jds.2018-14659.
Tack DM, Ray L, Griffin PM *et al*. Preliminary incidence and trends of infections with pathogens transmitted commonly through food—Foodborne Diseases Active Surveillance Network, 10 US Sites, 2016–(2019). *MMWR Morb Mortal Wkly Rep* 2020;**69**:509–14. https://doi.org/10.15585/mmwr.mm6917a1.
Tamminen LM, Hranac CR, Dicksved J *et al*. Socially engaged calves are more likely to be colonised by VTEC O157:H7 than individuals

showing signs of poor welfare. *Sci Rep* 2020;**10**:6320. https://doi.org/10.1038/s41598-020-63186-2.

Tang Y, Meinersmann RJ, Sahin O *et al*. Wide but variable distribution of a hypervirulent *Campylobacter jejuni* clone in beef and dairy cattle in the United States. *Appl Environ Microbiol* 2017;**83**:e01425–17. : https://doi.org/10.1128/AEM.01425-17.

Terzolo HR, Lawson GHK, Angus KW *et al*. Enteric campylobacter infection in gnotobiotic calves and lambs. *Res Vet Sci* 1987;**43**:72–77. https,//doi.org/10.1016/S0034-5288(18)30745-8.

Weis AM, Clothier KA, Huang BC *et al*. Draft genome sequence of multidrug-resistant abortive *Campylobacter jejuni* from Northern California. *Genome Announc* 2017;**5**:15. https://doi.org/10.1128/genomeA.00171-17.

Wittum TE, Perino LJ. Passive immune status at postpartum hour 24 and long-term health and performance of calves. *Am J Vet Res* 1995;**56**:1149–54.

Wood-Gush DGM, Vestergaard K. Exploratory behavior and the welfare of intensively kept animals. *J Agric Ethics* 1989;**2**:161–9. https://doi.org/10.1007/BF01826929.

World Health Organization. *Campylobacter*. 2020. https://www.who.int/news-room/fact-sheets/detail/campylobacter. (30 April 2022, date last accessed).