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Molecular Investigation and Risk Assessment of Neonatal Calf Diarrhea in Morocco: Emphasis on Rotavirus and Coronavirus Infections

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ABSTRACT

Neonatal calf diarrhea represents a major health and economic burden in Moroccan cattle farming and remains a leading cause of calf mortality. It is commonly associated with infectious agents such as rotavirus, bovine coronavirus, *Escherichia coli*, and *Cryptosporidium parvum*, and is often aggravated by dehydration and electrolyte imbalances. The present study aimed to update the epidemiological profile of viral pathogens involved in neonatal calf diarrhea in Morocco, with particular emphasis on rotavirus group A (RVA) and bovine coronavirus (BCoV). A total of 889 newborn calves from 20 farms located in five Moroccan regions (Casablanca-Settat, Rabat-Salé-Kenitra, Souss-Massa, Fes-Meknes, and Beni Mellal-Khenifra) were included in this cross-sectional observational study. Among them, 130 rectal swabs were collected from diarrheic calves between November 2022 and March 2023 and analyzed using reverse transcription quantitative PCR (RT-qPCR). The results indicated that 34% of diarrheic calves tested positive for RVA and 62% for BCoV, with a co-infection rate of 24%. Infection rates were influenced by several factors, including calf age, geographic region, dam vaccination status, calving rank, production system, and farm size. RVA infection peaked in calves aged 8–14 days, whereas BCoV was most prevalent in calves aged 21–29 days. Notably, RVA positivity was higher in calves born to vaccinated dams (40%) compared to those from unvaccinated dams (20%), while BCoV infection was more frequently detected in calves from heifers than from multiparous cows. Higher infection rates in calves were also observed in intensive farming systems. Overall, these findings confirmed the major role of rotavirus and bovine coronavirus in neonatal calf diarrhea in Morocco and suggest that the limited effectiveness of vaccination may be influenced by herd management, colostrum transfer, and biosecurity practices. The potential mismatch between vaccine and circulating strains highlights the need for continuous molecular surveillance.

Keywords: Bovine coronavirus, Calf, Neonatal diarrhea, Rotavirus A, Risk factor

INTRODUCTION

Neonatal calf diarrhea syndrome is a major health problem for the cattle industry worldwide (Tajik et al., 2012; Guise, 2023). Diarrhea in newborn calves causes significant financial losses for breeders due to poor growth, decreased milk production in the first lactation, higher veterinary costs, and an increased risk of death before weaning (Abuelo et al., 2021). This syndrome has been linked to sickness behaviors in calves, such as prolonged lying periods and decreased milk intake, which indicate compromised health and welfare in cattle (Sutherland et al., 2018; Goharshahi et al., 2021). Neonatal diarrhea is one of the most severe diseases impacting calves less than one month of age worldwide (Schild et al., 2020), leading to high morbidity and mortality due to dehydration, acidosis, and electrolyte imbalance (Foster and Smith, 2009).

From an etiological perspective, infectious diarrhea in infants and animals was attributed solely to bacteria and protozoa until the development of advanced methods, such as electron microscopy and molecular biology (Mebus et al., 1969). In 1969, the first evidence of a viral cause of diarrhea in veterinary medicine was reported, following the electron-microscopic detection of a reovirus-like particle in the feces of a diarrheic calf. This pathogen was subsequently

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identified as rotavirus, and four years later, its role as an etiological agent in severe pediatric diarrhea was confirmed (Bishop et al., 1973; Brugere and Tessier, 2010). Over time, coronaviruses were established as additional enteric pathogens contributing to neonatal diarrhea in cattle worldwide, either independently or in combination with rotavirus (Erdoğan et al., 2003).

At the molecular level, rotavirus has a double-stranded RNA genome and is classified within the family *Reoviridae*, subfamily *Sedoreovirinae*, and genus rotavirus. The genus rotavirus is divided into nine officially recognized species, designated rotavirus A-J, based on their antigenic properties and genomic characteristics. Among these species, rotavirus A (RVA) is the most prevalent species among both humans and animals and is a leading cause of neonatal diarrhea in calves (Matthijnssens et al., 2011). Rotavirus A is consistently identified as the most common and epidemiologically important viral agent associated with neonatal calf diarrhea worldwide, especially in calves during their first weeks of life (Brunauer et al., 2021). Rotaviruses infect and destroy the villous epithelial cells of the small intestine, leading to nutrient malabsorption and diarrhea. In older calves and adult cows, infection is often subclinical; however, viral shedding increases significantly around calving, likely due to physiological stress and temporary immunosuppression. This shedding contaminates the environment and increases the risk of transmission to newborn calves, which are highly susceptible to infection. Once infected, diarrheic calves excrete large amounts of virus, becoming the primary source of viral transmission within the herd and sustaining the infection cycle on the farm (Naylor, 2009).

On the other hand, bovine coronavirus (BCoV) is a single-stranded RNA virus classified within the family *Coronaviridae*, subfamily Orthocoronavirinae, genus Betacoronavirus, subgenus Embecovirus, and species Betacoronavirus 1. The genus Betacoronavirus includes five subgenera and 14 species, among which are closely related bovine-like coronavirus (BCoV-like) variants found in wild ruminants (Decaro and Lorusso, 2020).

In calves, BCoV infect and destroy villous epithelial cells of the small intestine, causing villous atrophy. Furthermore, coronaviruses infect the epithelial cells of the large intestine, potentially resulting in colitis-associated signs, such as difficulty or increased exertion during defecation (straining). Similar to rotavirus, asymptomatic adult bovines may shed the BCoV and serve as an initial source of infection for neonatal calves. Once an outbreak occurs, calves that are clinically affected serve as the main sources of virus transmission (Naylor, 2009).

In Morocco, neonatal diarrhea has been identified as a primary cause of mortality within cattle farms since the initial epidemiological studies conducted in the 1980s. It has been associated with morbidity rates of up to 70% and mortality ranging from 10% to 15%. Approximately 70% of cases are attributed to RVA, *Escherichia coli*, and *Cryptosporidium parvum* (Fassi-Fihri et al., 1988; Ebode, 1989; Chhaibi, 2008). More recent investigations using rapid immunochromatographic tests reported a 10% prevalence of diarrhea in calves, with RVA detected in 37.2% of cases and BCoV in 25% (Zouagui et al., 2017). Despite the implementation of vaccination programs and improved hygiene practices, neonatal calf diarrhea has remained a persistent and significant challenge for cattle farms across Morocco from the 1980s to recent years (Fassi-Fihri et al., 1988; Ebode, 1989; El-Haous, 1990; Chhaibi, 2008; Zouagui et al., 2017). This sustained burden reflects ongoing concerns among breeders and field veterinarians, particularly regarding the continued occurrence of the disease despite control measures (Zouagui et al., 2017).

Therefore, the present study aimed to understand the mechanisms of neonatal calf diarrhea transmission and to identify key risk factors in Morocco, in order to support the development of more effective prevention strategies. To achieve this, reverse transcription quantitative PCR (RT-qPCR) was employed to update epidemiological data, focusing on the positivity rates and distribution of RVA and BCoV according to geographic region, production system, calf age, sex, dam vaccination status, and calving rank.

MATERIALS AND METHODS

Ethical approval

The Ethics Committee authorized this study concerning Animal Sciences, Animal Health, and Veterinary Public Health at the Hassan II Agronomic and Veterinary Institute in Rabat, Morocco, in accordance with approval code CESASPV 2025 A10.

Study time and location

The present cross-sectional study was conducted between November 2022 and March 2023 and involved 20 cattle farms distributed across five major livestock-producing regions of Morocco. These regions encompass Casablanca-Settat, Rabat-Salé-Kenitra, Souss-Massa, Fes-Meknes, and Beni Mellal-Khenifra (Figure 1).

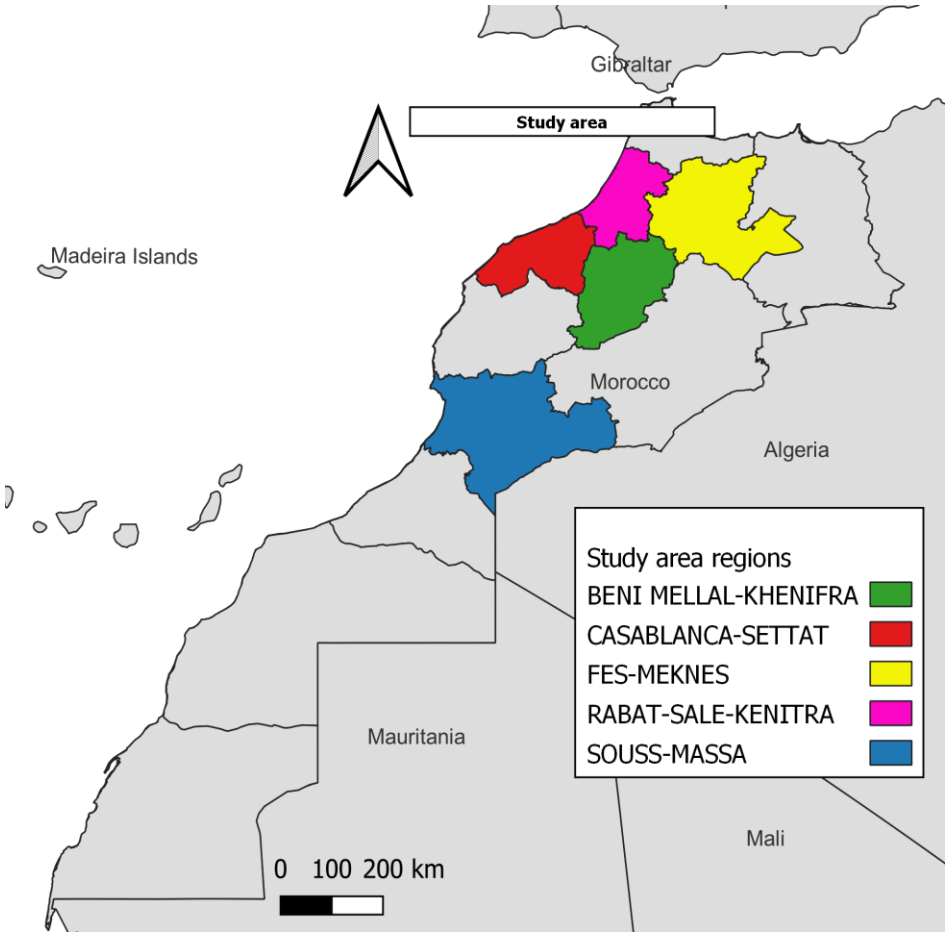


Figure 1. Geographic distribution of the five Moroccan regions involved in the study. Map produced by the author using Geographic Information System, based on administrative boundaries from the High Commission for Planning, Morocco.

Samples

A total of 889 newborn calves were clinically examined using convenience sampling. Among them, 130 animals presenting diarrhea were selected, and rectal swabs were collected between November 2022 and March 2023. Samples were immediately placed in an insulated cooler and transported at 4°C. Upon arrival at the laboratory, they were stored at -80°C until analysis. The calves included in the study were Holstein and crossbred dairy breeds, with an average birth weight ranging from 20 to 45 kg. At the time of sampling, detailed information was recorded for each individual, including the total number of calves, number of diarrheic cases, age, sex, and farming system. These data were collected through structured interviews with farm personnel and technicians. Additional information on the dams, including vaccination status and calving rank (primiparous or multiparous), was also obtained.

Molecular assays

RNA extraction

Each rectal swab was suspended in 1–2 mL of sterile phosphate-buffered saline (PBS) to produce a uniform sample while maintaining swab integrity. Viral RNA was manually isolated using the NucleoSpin® RNA Virus kit (Macherey-Nagel, Germany) in accordance with the manufacturer's protocol.

Identification and detection of pathogens by RT-qPCR

For rotavirus Group A detection, an initial denaturation step was required due to the double-stranded nature of its RNA genome. Rotavirus RNA was amplified by RT-qPCR using an in-house assay based on the PCL COVID-19 Speedy RT-PCR master mixture, originally developed for SARS-CoV-2 detection. This procedure was carried out using the following primers (Xie et al., 2015), which targeted the VP6 gene of group A rotavirus (forward primer: TCATTTCAATTGATGAGGCCACC; reverse primer: ATTCAATTCTAAGCGTGAGTCCTAC), along with a HEX-labeled probe (AATATGACACCAGCGGTAGCGGC). Thermal cycling conditions started with an initial denaturation step at 95°C for 3 minutes, followed by reverse transcription at 50°C for 15 minutes, a second denaturation at 95°C for 2 minutes, and 40 amplification cycles consisting of 95°C for 15 seconds and 60°C for 30 seconds. Samples were considered positive when a sigmoidal amplification curve was observed in the HEX fluorescence channel with a cycle

threshold (Ct) value of 40 or less (≤ 40), following the manufacturer's guidelines. For BCoV detection, the same master mix was used in combination with primers and probe described by Cho et al. (2010). The forward primer was CTAGTAACCAGGCTGATGTCAATACC, and the reverse primer was GGCGGAAACCTAGTCGGAATA, and a FAM/MGB-labeled probe CGGCTGACATTCTCGATC was used. The thermal profile for reverse transcription and amplification included 50°C for 5 minutes, followed by 95°C for 20 seconds, then 40 cycles of 95°C for 5 seconds and 60°C for 30 seconds. Samples were considered positive for BCoV when a sigmoidal amplification curve appeared in the FAM/MGB fluorescence channel with a Ct value of ≤ 40, following the manufacturer's instructions.

Statistical analysis

Statistical analyses were performed using RStudio software (version 2023). Descriptive statistics were used to summarize the distribution of bovine rotavirus A (RVA) and bovine coronavirus (BCoV) positivity according to the studied factors, including sex, age, geographic region, farming system, dam's vaccination status, and calving rank. Associations between categorical variables and virus positivity were assessed using Pearson's chi-square (χ^2) test. When the expected cell counts were less than five, Fisher's exact test was used instead. A p-value < 0.05 was considered statistically significant.

RESULTS

Among the 889 calves examined, 130 (14.6%) presented with diarrhea. RT-qPCR analysis of these samples indicated that 34% (44/130) tested positive for RVA, while 62% (80/130) tested positive for BCoV. Notably, 24% (31/130) of the diarrheic calves were co-infected with both viruses (Graph 1). Multiple factors were assessed for their potential association with RVA and BCoV detection, such as calf sex, region, age, dam vaccination status, calving rank, and the production system.

Variation by sex

The present results indicated that calf sex had no significant effect on RVA or BCoV positivity rates ($p > 0.05$; Graph 2).

Regional variation

PCR testing revealed that the Rabat region had the highest RVA positivity rate (58%), whereas the Fez region showed the highest BCoV positivity rate (81%, Figure 2). Pearson's chi-square test showed that BCoV positivity differed significantly among geographic regions ($\chi^2 = 11.148$, $p < 0.05$), whereas no significant regional differences were observed for RVA positivity.

Variation by calf's age

The current findings indicate that the 8-14-day age group had the highest RVA infection rate, at 42%. The infection remained at high levels until approximately 30 days of age. However, BCoV prevalence was highest in the 21-29-day age group, reaching 75% (Graph 3). The current results indicated that these differences in both RVA and BCoV by calf age were not statistically significant ($p > 0.05$).

Variation by dams' vaccination status

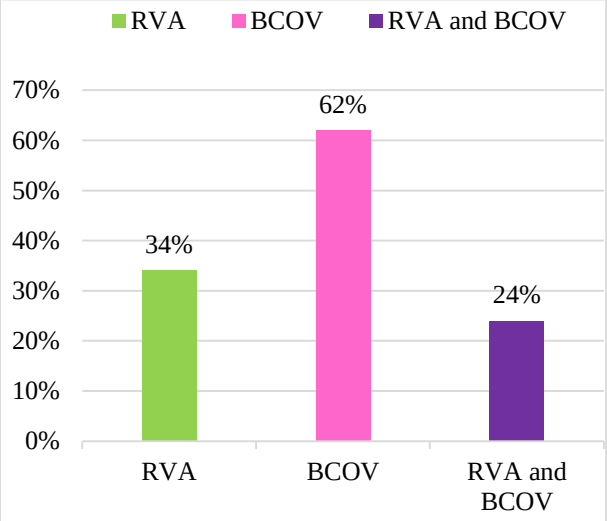
Graph 4 illustrates the positivity rates for RVA and BCoV by maternal vaccination status. Regarding RVA infection, calves born to vaccinated dams had a higher positivity rate (40%) than those born to unvaccinated dams (20%; $p < 0.05$). For BCoV infection, the positivity rate was marginally higher in calves born to vaccinated dams (64%) than in their unvaccinated counterparts (60%), although this difference was not statistically significant ($p > 0.05$).

Variation by calving rank

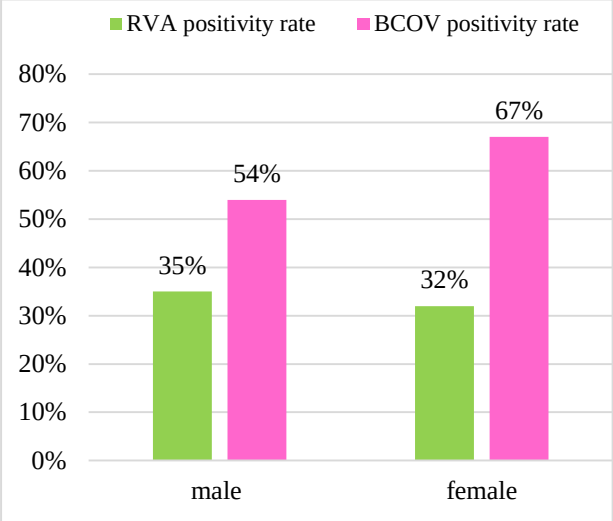
No significant difference was observed in RVA positivity between calves born to heifers and multiparous dams ($p > 0.05$). However, calves from heifers exhibited a significantly higher BCoV positivity rate compared with those from multiparous cows (Graph 5).

Variation by farming system

RT-qPCR analysis indicated that calves from industrial farms exhibited a significantly higher infection rate than those from small- or medium-sized farms ($p < 0.05$; Graph 6).



Graph 1. Positivity rates of rotavirus group A (RVA) and bovine coronavirus (BCoV) in diarrheic calves in Morocco from November 2022 to March 2023



Graph 2. Variation of rotavirus group A (RVA) and Bovine Coronavirus (BCoV) positivity rates by calf's sex in Morocco from November 2022 to March 2023

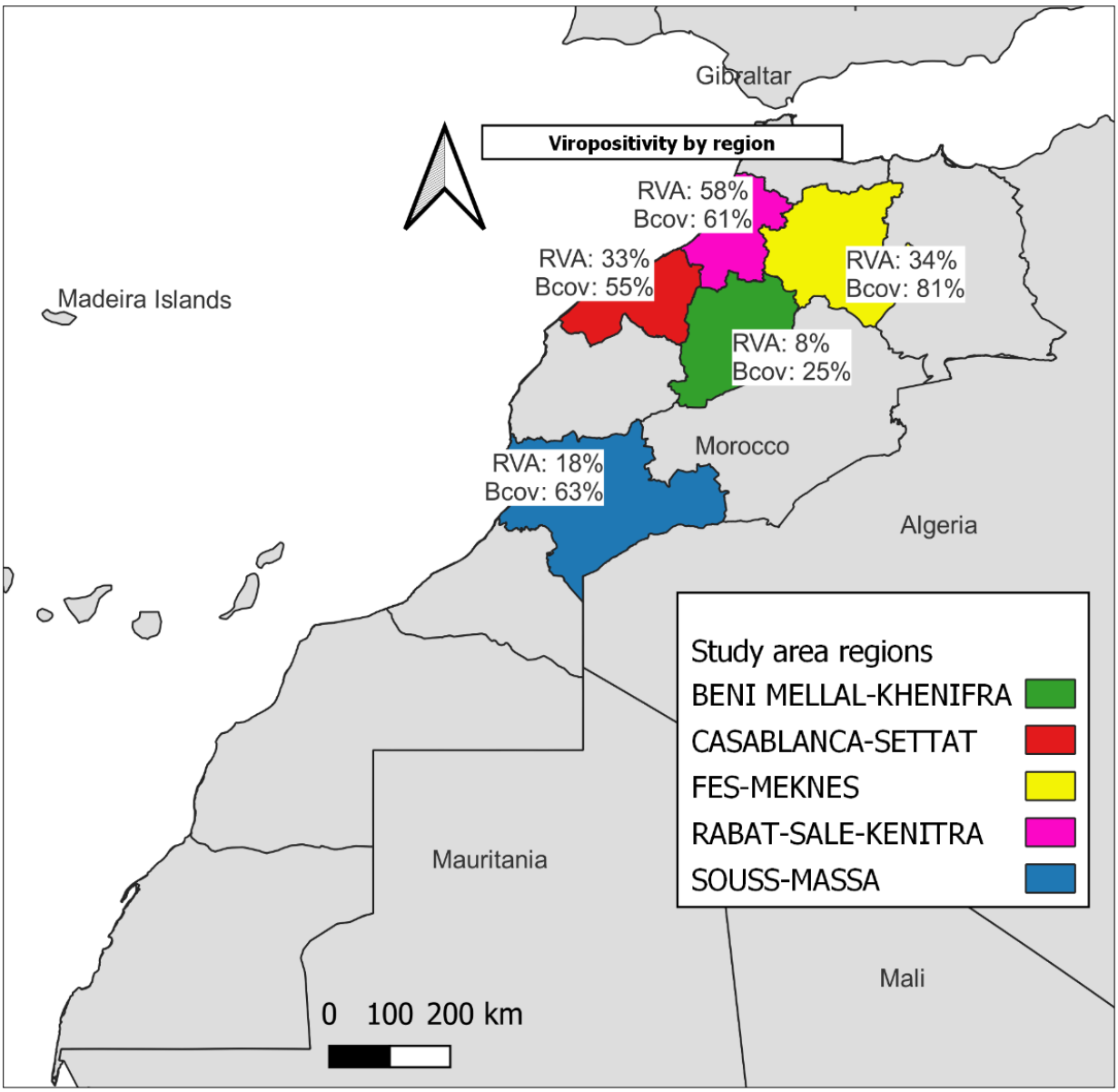
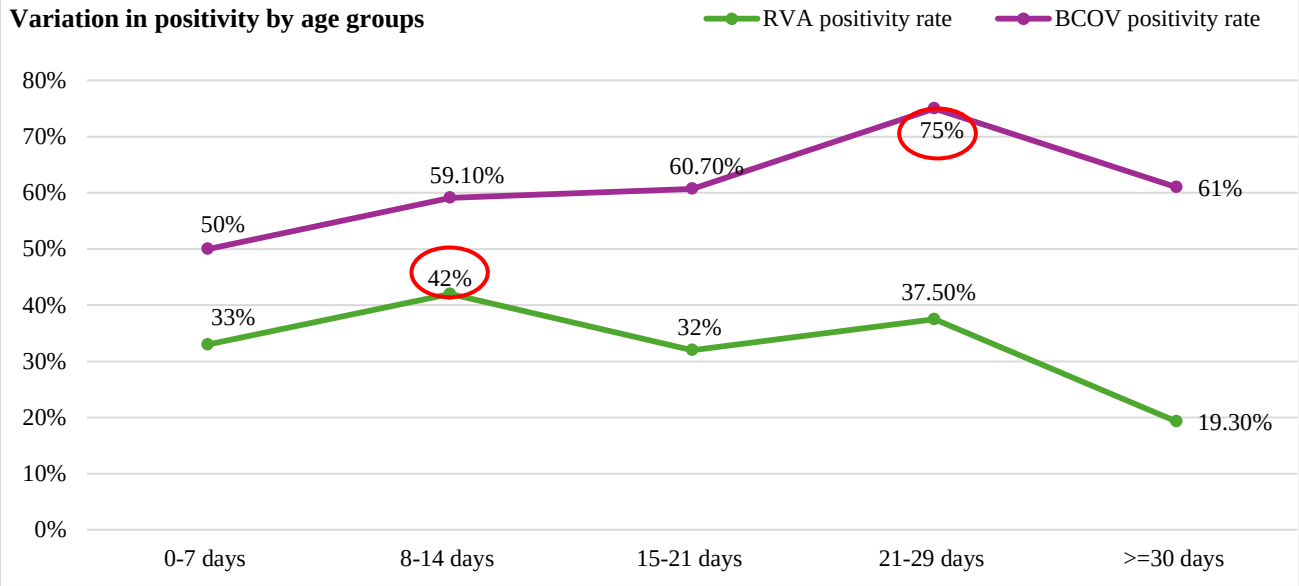
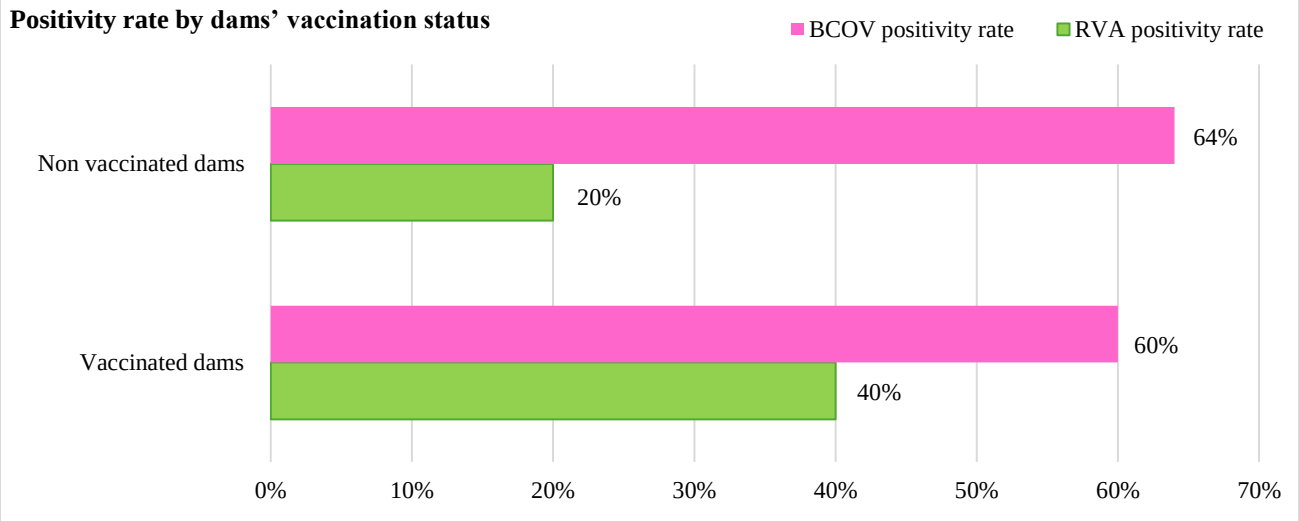


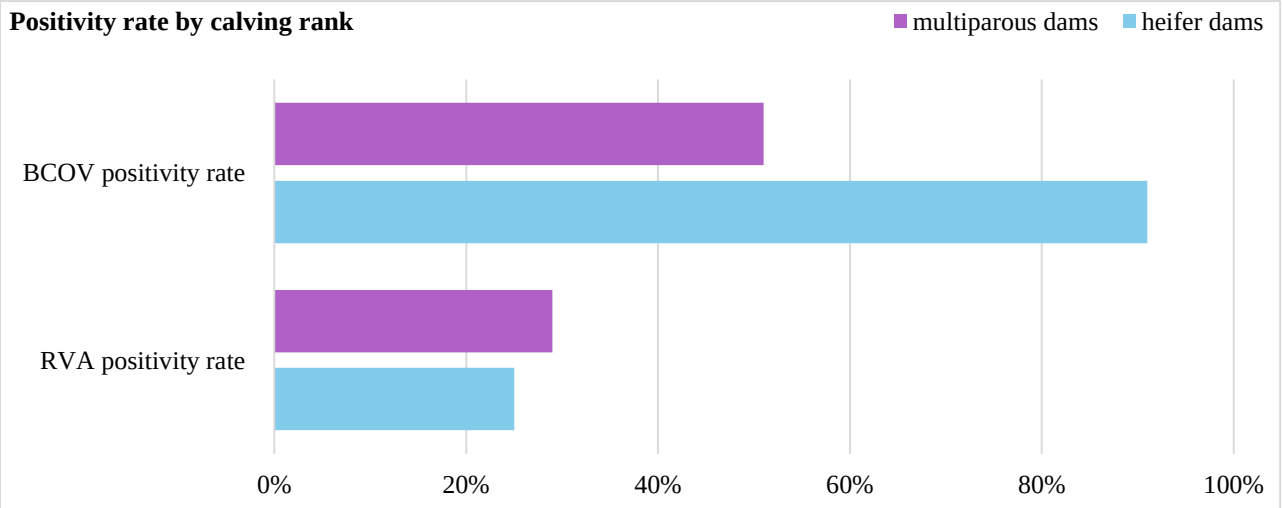
Figure 2. Variation of Rotavirus group A (RVA) and Bovine Coronavirus (BCoV) positivity rates according to regions in Morocco from November 2022 to March 2023



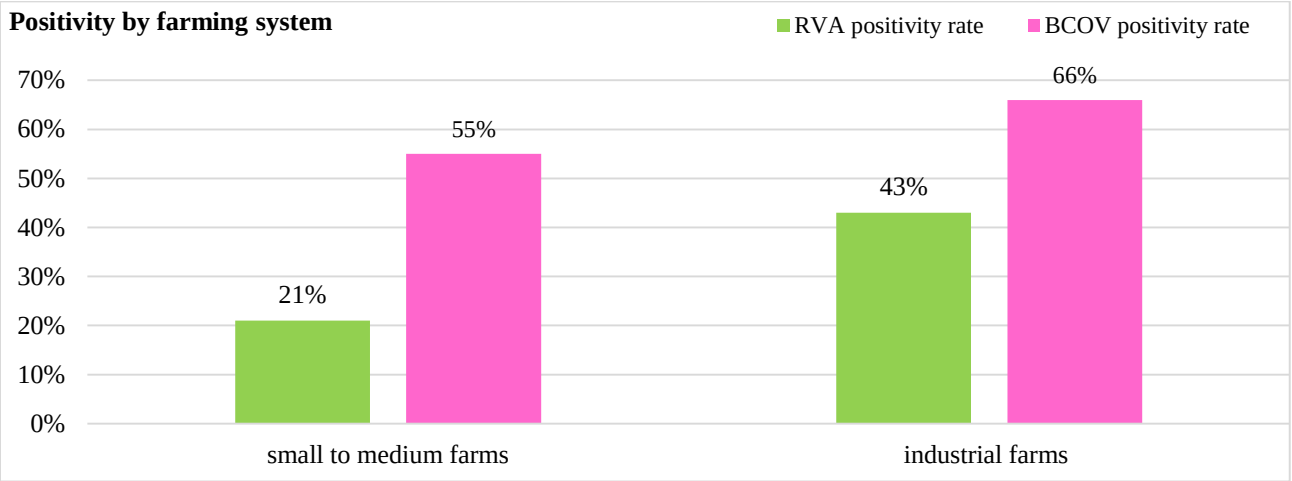
Graph 3. Variation of rotavirus group A (RVA) and Bovine Coronavirus (BCoV) positivity rates by calf's age in Morocco from November 2022 to March 2023



Graph 4. Variation of rotavirus group A and Bovine Coronavirus positivity rates according to dams' vaccination status in Morocco between November 2022 and March 2023



Graph 5. Variation of rotavirus group A and Bovine Coronavirus positivity rates according to the calving rank in Morocco between November 2022 and March 2023



Graph 6. Variation in rotavirus group A and bovine coronavirus positivity rates according to farming system in Morocco between November 2022 and March 2023

DISCUSSION

Neonatal calf diarrhea is a complex syndrome that impacts cattle farms, affecting both productivity and animal welfare. Neonatal calf diarrhea is recognized as the leading cause of illness and death in newborn calves on dairy farms worldwide (Urie et al., 2018; Caffarena et al., 2021). The health of calves in their early days is crucial for the success of any dairy operation. High rates of diarrhea-related illnesses result in substantial economic losses attributable to increased mortality rates and treatment costs (Rocha et al., 2019). Investigations into neonatal calf diarrhea in Morocco predominantly date back to studies conducted during the 1980s and 1990s (Fassi-Fihri et al., 1988). Only one recent study, employing a rapid immunochromatographic test, was conducted in 2017 (Zouagui et al., 2017). Consequently, the existing epidemiological data on this syndrome in Moroccan livestock remained outdated, despite the ongoing incidence of neonatal diarrhea in the country, underscoring the importance of conducting the present study.

Diarrhea prevalence in the study area

The current study identified a prevalence of neonatal diarrhea at 14.6% among Moroccan calves, exceeding the 10% rate reported by Zouagui et al. (2017). This prevalence was considerably lower than the high rates recorded in the 1980s and 1990s, which were 75% and 74.1%, respectively (Fassi-Fihri et al., 1988; El-Haous, 1990). The prevalence of neonatal calf diarrhea differed considerably across countries. For instance, rates of 30%, 37.5%, and 33.9% have been reported in Kuwait (Majeed and Alazemi, 2023), Korea (Chae et al., 2021), and Ethiopia (Umer Seid et al., 2020), respectively. In Canada, morbidity rates of neonatal calf diarrhea ranged from 20% to 40% (Bongers et al., 2022). In Morocco, the incidence of neonatal calf diarrhea has declined markedly from over 75% in the 1980s and 1990s to approximately 10% in 2017. This decline could be due to improvements in calf health, advances in husbandry practices, increased farmer awareness of hygiene protocols, the implementation of vaccination programs, and greater colostrum management through early ingestion, freezing methods, and immunoglobulin measurement to ensure adequate immunity in newborn calves against the pathogens responsible for neonatal diarrhea (Zouagui et al., 2017). Despite these control measures, neonatal diarrhea is still a persistent and possibly increasing problem in Moroccan cattle farming. The persistent prevalence of neonatal diarrhea in calves (14%) highlighted the need for updated epidemiological monitoring and the use of advanced diagnostic tools.

Positivity rates

The RT-qPCR analysis revealed an RVA positivity rate of 34%, slightly lower than the 37.2% reported by Zouagui et al. (2017) but higher than the 29.9% reported by Fassi-Fihri et al. (1988). Notably, the RVA prevalence on Moroccan farms has remained relatively unchanged over the past years, despite improvements in breeding practices and vaccination programs. It should be noted that the diagnostic methods differed among studies; Zouagui et al. (2017) employed a rapid immunochromatographic test, whereas Fassi-Fihri et al. (1988) used the Enzyme-Linked Immunosorbent Assay. Comparable findings have been reported in Turkey at 30% (Mamak et al., 2023), whereas lower prevalence has been reported in China at 23.2% (Wang et al., 2023) and Algeria at 20.7% (Ammar et al., 2014). In contrast, BCoV indicated a markedly higher positivity rate in the present study (62%), exceeding the 25% reported by Zouagui et al. (2017). This rate of BCoV was considerably higher than those reported in China (11.8%; Wang et al., 2023), Turkey (8%; Mamak et

al., 2023), Algeria (20.7%; Ammar et al., 2014), and Kuwait (1%; Majeed and Alazemi, 2023). The present results indicated that in Morocco, the RVA positivity rate has slightly changed over time, whereas BCoV demonstrated a marked increase compared with previous Moroccan studies.

Positivity rate by sex

In the present study, the prevalence of RVA and BCoV infections did not notably differ between male and female calves. Similar results have been documented in previous studies conducted by Radhy (2015), Yavru et al. (2016), and Zaitoun et al. (2018). This lack of difference might be attributed to the comparable anatomical, physiological, and hormonal characteristics of calves at an early age, leading to similar susceptibility to infection (Yavru et al., 2016). Conversely, some studies have reported sex-related differences. In Algeria, Ammar et al. (2014) found higher infection rates in males (59.3%) than in females (40.7%) for RVA and BCoV. This finding was attributed to the higher birth weight of males, which increases the risk of dystocia and, consequently, impairs colostrum absorption, predisposing cattle to infection. In Ethiopia, Debelo et al. (2021) observed that male calves were more likely to be infected with RVA, which was consistent with the findings of Ammar et al. (2014). In addition, management practices may influence infection rates, as in some production systems, female calves receive greater health care and husbandry due to their higher economic value, which might explain the lower prevalence of infection observed in females (Chaudhary et al., 2013).

Positivity rate by region

The results of this study showed that the prevalence of RVA did not vary significantly between the regions studied. Similarly, in Turkey, the proportion of RVA-positive calves ranged from 9.7% in Basgedikler to 36.4% in Kumbetli, although these inter-locality differences were not statistically significant ($p > 0.05$) (Erdoğan et al., 2003). Such variations in positivity rates may be influenced by factors including farm hygiene, herd management practices, and regulations on animal movement (Asadi et al., 2015). In contrast, BCoV prevalence exhibited substantial regional variation, consistent with Asadi et al. (2015), who reported differences in BCoV prevalence across geographic locations, with prevalence varying between countries such as Turkey (1.96%; Okur-Gumusova et al., 2007), Costa Rica (9%; Pérez et al., 1998), and Switzerland (7–8%; Uhde et al., 2008), whereas a study in Egypt found no significant association between BCoV prevalence and geographic location (Elgioushy et al., 2025). According to Wang et al. (2023), farm location may influence calves' susceptibility to RVA and BCoV infections, with drier areas associated with higher infection rates, whereas wetter environments, particularly near rivers, were linked to a higher incidence of diarrhea caused by *Cryptosporidium parvum*. In Morocco, environmental conditions were relatively similar across the regions studied, suggesting that the observed differences in viral prevalence are more likely attributable to variations in hygiene practices and herd management rather than geographic or climatic factors.

Positivity rate by age

The current findings revealed that calves aged 8-14 days had the highest RVA infection rate (42%), whereas BCoV prevalence peaked in calves aged 21-29 days (75%). These results were consistent with the findings of Ammar et al. (2014), who reported that calves in their second (8-14 days) and fourth (22-30 days) weeks of life are most susceptible to RVA and BCoV, respectively. Similarly, Al-Robaiee and Farwachi (2013) in Iraq and Volkan et al. (2016) in Turkey detected RVA predominantly in calves aged 8-15 days. Broader studies by Zaitoun et al. (2018) and Berber et al. (2021) reported that RVA infections occurred mainly between 3-15 and 4-14 days, respectively, and BCoV infections occurred between 30-37 days and 4-30 days, respectively. In Morocco, Fassi-Fihri et al. (1988) reported a 29.7% incidence of RVA in diarrheic calves, with lower prevalence observed after 20 days of age. In 2017, Zouagui et al. (2017) observed that viral infections, such as RVA and BCoV, were most frequent during the first two weeks of life. The difference between the present results and those of previous Moroccan studies was most apparent during the first week of life, when infection rates were higher than those observed in the present study. This may be explained by the enhanced care provided to calves during the first week of life. After the first week, the decline in passive immunity, combined with limited natural resistance to these pathogens, may increase susceptibility to infection (Okur-Gumusova et al., 2007). Furthermore, the presence of BCoV infection in older calves (22-30 days) could be attributed to group housing practices for calves older than two weeks, rather than the use of individual stalls (Ammar et al., 2014).

Positivity rate by dams' vaccination

The RT-qPCR analysis indicated a notably higher RVA positivity rate in calves born to vaccinated dams (40%) compared to those from unvaccinated dams (20%). For BCoV infection, rates were almost similar between calves from vaccinated and unvaccinated dams at 60% and 64%, respectively. The current results are consistent with Zouagui et al.

(2017), who reported no considerable differences in viral prevalence between vaccinated and unvaccinated farms in Morocco. Similarly, a study conducted in Brazil by Rocha et al. (2017) compared two groups of herds with similar colostrum management practices. Rocha et al. (2017) reported that calves born to dams producing sufficient colostrum (4 liters at the first feeding) had a higher RVA positivity rate when the dams were vaccinated than when unvaccinated (3.92% versus 1.11%). In addition, the clinical appearance of feces was evaluated in both groups, and the proportions of normal and diarrheic samples were nearly identical (27.4% versus 27.3%; Rocha et al., 2017). The higher RVA positivity rate observed in calves born to vaccinated dams indicated that maternal vaccination alone may not be sufficient to prevent viral infection under field conditions (Weaver et al., 2000; Dhama et al., 2009). This finding may reflect the complex interactions across the circulating viral strains, maternal immune response, colostrum management, and herd-level infection pressure. While vaccination is designed to enhance passive immunity, its effectiveness can be influenced by strain diversity and herd management, which may limit the expected reduction in viral circulation (Parreño et al., 2010).

It has been indicated that pre-calving vaccination programs should systematically include pregnant heifers, as they are often overlooked in practice. Excluding heifers from vaccination reduces the passive immunity transferred to their calves, thereby increasing the risk of neonatal diarrhea. Additionally, Viidu and Mõtus highlighted the critical role of feeding calves not only with colostrum but also with hyperimmune transition milk from vaccinated dams for at least the first two weeks of life. This milk, produced in the days after colostrum, retains high levels of antibodies induced by vaccination, providing persistent protection in the gastrointestinal tract. Implementing such feeding practices is crucial to maintain lactogenic immunity during the period when calves are most vulnerable to enteric infections. Bürki et al. (1986) demonstrated notable protective effects of dam vaccination, notably reducing clinical signs and the duration of RVA and coronavirus shedding in calves. Furthermore, Bürki et al. (1986) highlighted the importance of feeding calves' milk from their vaccinated dams for at least 14 days to ensure sustained lactogenic immunity.

These findings indicated that vaccination of pregnant cows in the final third of gestation could substantially reduce the clinical impact of RVA and coronavirus infections in calves. Nevertheless, the effectiveness of vaccination depends on multiple factors, including colostrum quality and hygiene management during the peripartum period. Several studies have reported different causes of vaccination failure. For instance, the absence of reduction in RVA infection despite vaccination might be linked to the virus's high mutation rate. Reassortment and rapid replication enable RVA to acquire new virulence traits that may not be protected against maternal vaccination (Thiry, 2007; Zouagui et al., 2017). Furthermore, management-related issues, such as improper storage, failure to maintain the cold chain, and administering the vaccine close to calving period, can compromise vaccine efficacy and effective immune responses (Quillet et al., 2006). Other factors may interfere with vaccine-induced protection against rotavirus infection, even when vaccination is applied. In Moroccan herds, failures of the maternal immune response following vaccination might be associated with nutrition, parasitism, or immunosuppressive conditions such as bovine viral diarrhea (BVD) or mycotoxicosis (Zouagui et al., 2017). Moreover, Fontaine (2018) reported that a mismatch between vaccine valence and the actual etiological agents responsible for neonatal enteritis was a key factor contributing to vaccination failure in cattle herds. These findings highlighted the importance of identifying circulating viral strains through molecular characterization and sequencing to ensure the relevance of vaccine strains under Moroccan field conditions.

Positivity rate by calving rank

Analysis of 39 calves with available breeding-rank data revealed a notably higher positivity rate of BCoV-infected calves born to heifers compared to those born to multiparous dams. This observation is consistent with the findings of Quillet et al. (2006), who reported that the frequency of BCoV infection decreased with increasing calving rank. In contrast, no association between RVA infection and calving rank was detected in either study. Regarding BCoV infection, the higher susceptibility of calves born to heifers may be related to lower antibody levels in first-lactation colostrum compared with those of multiparous cows, as colostral antibody levels typically increase from the third lactation (Guerin, 2009).

Positivity rate by type of farming system

The present findings indicated that calves raised on industrial farms were more frequently infected with RVA and BCoV than those raised on small farms. This finding might be due to higher animal density, which has been reported to increase the risk of diarrheal disease in calves (Erdoğan et al., 2003). High infectious pressure and a large proportion of susceptible animals in close contact can spread the viral infections. Both healthy adult cattle and calves can shed RVA in their feces, thereby contaminating the environment and increasing infectious pressure in crowded herds. Animals in larger herds or groups with close contact are more likely to be exposed to infectious pathogens and experience more stress. Thus, infection pressure, environmental contamination, stress-related immunosuppression, and overcrowding are

key factors that contribute to epidemics in herds where calves and their dams are closely housed (Torres et al., 1985; Wells et al., 1997; Erdoğan et al., 2003).

Positivity rates according to season

In addition to the risk factors examined, the present study was conducted during the optimal season for viral detection, from November to March (Berber et al., 2021). Hassine-Zaafrane (2011), Nour Mohammad Zadeh et al. (2012), and Chen et al. (2022) have reported higher RVA incidence during colder months, as low temperatures and relative humidity prolong viral survival (Brandt et al., 1982). Furthermore, colostral IgG levels in dairy cows are lower in winter compared to other seasons, reducing passive immunity in newborn calves (Gulliksen et al., 2008). Additionally, the prevalence of BCoV was affected by seasonal variations. Consistent with the present findings, Williams et al. (2007) observed a prevalence of 2% during summer and 9% in winter. The increased occurrence of BCoV-associated diarrhea in winter might be due to enhanced viral stability under colder environmental conditions (Radostits et al., 2007). Similarly, Zaitoun et al. (2018) observed that RVA and coronavirus infections in calves peaked during colder months, with lower infection rates recorded during warmer periods.

CONCLUSION

The present study showed that RVA remains a major causative agent of neonatal calf diarrhea, consistent with previous reports. Bovine coronavirus was also frequently detected and showed a higher positivity rate than RVA. The association between both RVA and BCoV and the examined risk factors was evaluated, including calf sex, region, age, dam vaccination status, calving rank, and farming system. The overall diarrhea prevalence (14.6%) confirmed that the syndrome continues to pose a major health and economic challenge to Moroccan cattle farming. The limited effectiveness of vaccination observed in this study indicated that protection against rotavirus and Coronavirus is influenced by multiple other factors, including herd management, colostrum feeding, and biosecurity measures. In addition, the possible mismatch between vaccine valence and circulating viral strains highlights the importance of ongoing molecular surveillance and sequencing of field isolates to ensure vaccine relevance. Altogether, these findings emphasize the need for integrated control strategies combining molecular diagnostics, adapted vaccination, and improved farm management to reduce the burden of neonatal diarrhea in Moroccan calves.

DECLARATIONS

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Authors' contributions

Imane Choukri conducted the fieldwork and laboratory experiments, analyzed the data, and wrote the manuscript. Nadia Touil and Ouafaa Fassi Fihri supervised the laboratory experiment and reviewed the manuscript. Zaid Zouagui supervised the fieldwork and data analysis. Zineb Rhazzar, Hanan El Ouadi, and El Mehdi Belouad participated in the laboratory experiment. Ikhlass El Berbri supervised both field and laboratory activities and contributed to manuscript writing. All authors read and approved the final edition of the manuscript.

Availability of data and materials

The data that support the findings of the present study are available from the corresponding author upon reasonable request.

Ethical considerations

The authors confirm that the manuscript was checked for plagiarism and duplicate submission and has not been submitted to any other journal. All authors reviewed and approved the final version of the manuscript before submission.

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Competing interests

The authors have not declared any conflict of interest.

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