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Bovine coronavirus and SARS-CoV-2 seroprevalence in livestock: marked host-species differences and insights from the first large-scale neutralization survey

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Bovine coronavirus (BCoV) and SARS-CoV-2, both belonging to the *Betacoronavirus* genus, are major pathogens affecting cattle and humans, respectively. BCoV causes respiratory and enteric diseases in cattle, leading to significant economic losses, while the detection of SARS-CoV-2 in various animal species raises concerns about interspecies transmission. This study assessed the seroprevalence of both viruses in cattle and buffaloes from southern Italy, considering species and regional origin as potential risk factors. Among 945 animals analyzed (491 cattle, 454 buffaloes), 435 (46%) tested positive for BCoV and 27 (2.8%) for SARS-CoV-2. Significant differences were observed between species and regions (BCoV: $p < 0.0001$; SARS-CoV-2: $p = 0.0029$). Among BCoV-positive samples, 67.1% of cattle but no buffaloes showed neutralizing antibodies ($p = 0.0006$). Twelve SARS-CoV-2-positive cattle were also BCoV-positive. However, the observed SARS-CoV-2 ELISA reactivity cannot be conclusively interpreted as confirmed SARS-CoV-2 exposure and does not allow discrimination between true exposure and non-specific serological reactivity. Longitudinal and molecular studies are needed to further clarify infection dynamics and possible antibody cross-reactivity among *Betacoronaviruses*.

Coronaviruses (CoVs) are enveloped, positive-sense, single-stranded, non-segmented RNA viruses[1]belonging to the family of *Coronaviridae*, subfamily *Orthocoronavirinae*. They are responsible for respiratory, gastrointestinal, neurological, and hepatic disorders in humans as well as in a wide range of domestic and wild animal species[2].

The family *Coronaviridae* is divided into four genera: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*. Among these, the genus *Betacoronavirus* includes two viruses of major concern for either public health or livestock production, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and bovine coronavirus (BCoV), respectively, which share several pathogenic traits, suggesting analogous mechanisms of transmission[3].

BCoV exhibits both respiratory and enteric tropism. Respiratory infections affect cattle of all ages, typically causing mild or subclinical disease characterized by fever, coughing, and rhinitis; however, animals younger than six months may develop a severe form known as shipping fever pneumonia, especially following transportation or stress[4]. The enteric form of infection, in contrast, is associated with neonatal calf diarrhea, accounting for 10–30% of cases of diarrhea in newborn calves[5], and with winter dysentery in adult cattle[4].

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Due to the quick fecal–oral and respiratory transmission, as well as the presence of carrier animals in infected herds, the virus shows a global distribution and causes significant economic losses in livestock due to mortality in calves, reduced growth performances and milk yield for beef and dairy cattle[6,7].

BCoV displays a remarkably broad host range compared to other coronaviruses. Over the past decade, numerous BCoV-like coronaviruses have been identified as causes of gastrointestinal and/or respiratory diseases in several ruminant species, highlighting their capacity for cross-species transmission and their ability to overcome interspecies barriers[6,8]. Such interspecies transmission events can generate recombinant strains capable of evading immune responses and spreading to new hosts, including humans[9]. Indeed, due to multiple recombination events and interspecies exchanges, members of the species *Betacoronavirus 1* (now *Betacoronavirus gravedinis*) appear to share a common ancestral origin, as indicated by their close antigenic and genetic relatedness[9–11]. Viruses genetically and antigenically related to BCoV have been detected in dogs with respiratory disease, in humans, and in several wild ruminant species, suggesting frequent interspecies transmission events. Experimental studies have shown that BCoV can replicate in dogs, mainly causing subclinical infections, and can infect avian species such as young turkeys, although not chicks, supporting the hypothesis that dogs, wild birds, and wild ruminants may act as potential reservoirs or intermediate hosts for BCoV-like coronaviruses[6]. Furthermore, BCoV appears to evolve continuously under immune pressure, accumulating mutations in key antigenic epitopes that may reduce vaccine efficacy[12].

Comparable patterns have been observed for SARS-CoV-2, which since its emergence in 2019 has been detected in numerous domestic and wild animal species, reflecting a high spillover capacity and a remarkably broad host range. This virus is believed to have originated from a spillover event from bats to humans, followed by multiple spillback episodes to companion and wild animals, primarily through anthropozoonotic transmission[13,14]. Repeated interspecies transmission poses a significant risk for accelerated viral evolution and the emergence of novel strains, as demonstrated by the bidirectional transmission between humans and farmed minks, where viral adaptation in minks led to subsequent zoonotic transmission back to humans[13]. It has been proven that the amino acid sequences of ACE-2 receptor is highly conserved in vertebrates, thus making several animal species susceptible to SARS-CoV-2 infection, including farmed animals, such as cows, buffaloes, goats, sheep, and swine, along with other wild species, could serve as potential intermediate hosts for SARS-CoV-2[15–17].

In Italy, data related to the prevalence of BCoV and SARS-CoV-2 in ruminants are scarce. Therefore, the aim of this study was to conduct a serosurvey on BCoV and SARS-CoV-2 in cattle and buffalo samples from the Campania and Calabria regions, to reveal the presence of possible virus contact and possible cross-reactions between these two betacoronaviruses[11] through virus serum neutralization assay (VSN), that is species-independent and is based on the principle of in vitro interaction between specific neutralizing antibodies and the investigated virus[16,18]. Finally, the role of species and area of origin was also assessed as possible risk factors.

Methods

Ethics information

The present study complies with all relevant ethical regulations and adheres to the ARRIVE guidelines. Sample collection was carried out by Local Veterinary Competent Authorities during official routine activities within the compulsory brucellosis eradication campaign in livestock, in accordance with national and European regulations (Commission Delegated Regulation (EU) 2020/689 of 17 December 2019 supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council as regards rules for surveillance, eradication programs, and disease-free status for certain listed and emerging diseases). Moreover, the Istituto Zooprofilattico Sperimentale del Mezzogiorno (IZSM) operates as an official laboratory of the Italian Ministry of Health, as designated by Article 9, paragraph 1, letter b of the Italian Legislative Decree No. 27/2021, which implements Regulation (EU) 2017/625 on official controls. Therefore, in compliance with national and regional regulations and internal policy, formal ethical approval was not required and was consequently waived.

Sample collection

From January 2023 to March 2024, a total of 945 serum samples were obtained by 491 cattle and 454 buffaloes from different provinces of Calabria and Campania regions, southern Italy. In particular, among 491 cattle, 311 belonged to herds located in Calabria region and 180 in the Campania region, and, among 454 buffaloes, 16 came from Calabria and 438 from the Campania region. All sampled animals were over 1 year of age, as they were tested within the official brucellosis eradication program, in accordance with national and European regulations, and none of them was reported to have received a vaccination against BCoV. The sampling strategy was defined according to the species predominantly farmed in the two regions involved. Specifically, the Calabria region is characterized by a predominance of cattle farms, while the Campania region, particularly the provinces of Salerno and Caserta, hosts the highest concentration of water buffalo herds. The sampled animals originated from 34 farms in total (25 cattle farms and 9 buffalo farms).

Samples were conferred to the Istituto Zooprofilattico Sperimentale del Mezzogiorno laboratories under refrigeration conditions and, after centrifugation at 1300 × g for 10 min (Centrifuge 5810, Eppendorf, Hamburg, Germany), the aliquots were frozen at − 20 °C until use[19].

Enzyme-linked immunosorbent assay and virus serum neutralization

All the serum samples underwent serological examinations by enzyme-linked immunosorbent assay (ELISA) in order to detect the presence of IgG against SARS-CoV-2 nucleocapsid (N) and IgG against BCoV using ID Screen SARS-CoV-2 Double Antigen Multi-Species ELISA Kit (ID VET, Montpellier, France) and SVANOVIR BCV-Ab Antibody Test (SVANOVA, INDICAL BIOSCIENCE GmbH, Leipzig, Germany), respectively.

SARS-CoV-2 ELISA was performed following manufacturer's instructions as previously described by Fusco et al.[16]. The microplates were read using iMark Microplate Absorbance reader (Bio-rad Laboratories, Hercules,

California, USA) at 450 nm. The test was considered valid if the positive control optical density (OD) value was > 0.350 and the ratio of the mean values of positive and negative control was > 3. Thus, following the datasheet guidelines, samples showing S/P% < 50% were considered negative, S/P% between 50 and 60% were considered doubtful, while samples with S/P% > 60% were considered positive.

The presence of antibodies against bovine coronavirus (BCoV) was assessed using the SVANOVIR BCV-Ab Antibody Test (SVANOVA), following the manufacturer's instructions and the protocol reported by Ferrara et al.[20]. Briefly, 100 µl of Dilution Buffer were dispensed in microtiter plates with odd columns coated with viral antigen and even columns with control antigen. Next, 4 µl of serum of each animal were added, in the presence of negative and positive controls, in both odd and even columns. After an incubation of 1 h at 37 °C, the wells were discharged and washed three times using 300 µl of Wash Solution. Next, 100 µl of horseradish peroxidase conjugated anti-bovine IgG monoclonal antibodies were added and the plate was incubated for 1 h at 37 °C, discharged, and washed three times, as aforementioned. Then, after the addition of 100 µl of Substrate Solution, followed by an incubation of 10 min at room temperature (18–25 °C), 50 µl of Stop Solution were added to stop the reaction. The microplate was read using iMark Microplate Absorbance reader (Bio-rad Laboratories) at 450 nm. The OD values in wells coated with BCV viral antigen were corrected by subtracting the OD values of the corresponding wells containing the control antigen. Therefore, all corrected OD values for the samples, as well as the negative control, were related to the corrected OD value of the positive control as follows: PP = OD corr (Sample or negative control)/OD corr (positive control) × 100.

The test was considered valid if the corrected OD value of the positive control was > 0.5 and the Percent Positivity Values (PP) of the negative control was < 10. Thus, following the datasheet guidelines, samples showing PP < 10 were considered as negative, while samples with PP ≥ 10 were considered as positive.

ELISA-positive sera were further tested by virus serum neutralization (VSN) assays for SARS-CoV-2 and BCoV under BSL-3 conditions, according to the procedures described by Fusco et al.[16] and Fiorito et al.[21], using a standardized micro-method already described by Cardillo et al.[22]. Samples were considered as positive when a titer ≥ 1:20 was observed and as negative with a titer < 1:20.

Statistical analysis

Prevalence was calculated at the 95% confidence level by dividing the number of positive animals by the total number of animals tested. According to the manufacturer's indications regarding the diagnostic performance of the kits, the true prevalence (TP) was calculated using the Sensitivity (Se) and Specificity (Sp) parameters reported in the validation protocols. In particular, the SVANOVIR BCV-Ab protocol reported 84.6% Se and 100% Sp (https://web.archive.org/web/20210125131917/https://www.svanova.com/content/dam/internet/ah/svanova/dk_EN/documents/bovine/BCV_infosheet_V03.pdf) while for ID SCREEN SARS-COV-2 DOUBLE ANTIGEN MULTI SPECIES, two sets of Se and Sp were considered, 100% Se and 97.8% Sp as reported in the validation protocol[23], obtaining TP1, and 38.7% Se and 100% Sp as reported by Fernández-Bastit et al.[24], obtaining TP2.

Descriptive statistics were used to summarize the distribution of animals positive for BCoV and SARS-CoV-2. Subsequently, chi-square (X^2) statistics were applied in a univariate analysis at the animal level to assess potential risk factors for antibody positivity. The variables considered (species and region of origin) were treated as categorical variables, as already reported by Ferrara et al.[20]. A p-value ≤ 0.05 was considered statistically significant. All statistical analyses were performed using MedCalc Statistical Software (MedCalc Software Ltd., Ostend, Belgium; version xx; available at https://www.medcalc.org/en/index.php; accessed 7 October 2025).

Results

A total of 945 animals, including 491 cattle and 454 buffaloes, originating from two Italian regions, Campania (180 cattle and 438 buffaloes) and Calabria (311 cattle and 16 buffaloes), were analyzed for the presence of BCoV and SARS-CoV-2 antibodies. A remarkably high BCoV seroprevalence was detected, with 435 animals testing positive, corresponding to an overall apparent prevalence (AP) of 46% (95% CI 42.8–49.1%). In contrast, SARS-CoV-2 antibodies were identified in 27 animals, resulting in an AP of 2.8% (95% CI 1.7–3.8%). Based on the diagnostic performance reported in the validation protocols of the ELISA kits, the true prevalence (TP) was calculated considering both sensitivity and specificity. Specifically, for the BCoV ELISA kit, a sensitivity of 84.6% and a specificity of 100% were reported, resulting in an overall TP of 54.3%. In contrast, for the Double Antigen ELISA kit targeting anti-N antibodies of SARS-CoV-2, a sensitivity of 100% and a specificity of 97.8% were reported for bovine samples[23], yielding a TP1 of 0.6%. Nevertheless, a recent article of Fernández-Bastit et al.[24] reported lower sensitivity of SARS-CoV-2 ELISA kit which detect anti-N compared to anti-RBD antibodies. Therefore, to minimize the impact of the sensitivity of the assay on the prevalence, sensitivity and specificity parameters reported by these authors were also considered. Specifically, 38.7% Se and 100% Sp were used, thus obtaining 7.23% TP2.

When potential risk factors were examined, species and region of origin emerged as major determinants of exposure. Cattle exhibited a strikingly higher BCoV seroprevalence (429/491; 87.3% AP; 100% TP) compared to buffaloes (6/454; 1.3% AP; 1.5% TP) ($p < 0.0001$), highlighting a strong host-species barrier in BCoV circulation (Table 1).

Likewise, a marked geographical difference was observed, with significantly higher seroprevalence in Calabria (117/155; 81% AP; 95.7% TP) than in the Campania Region (170/618; 27.5% AP; 32.5% TP) ($p < 0.0001$), suggesting that local epidemiological dynamics and management practices may influence viral spread Cattle showed a higher seroprevalence (22/491; 4.4% AP; 2.24% TP1; 11.36% TP2) than buffaloes (5/454; 1.1% AP; 0% TP1; 2.84% TP2) ($p = 0.0029$), while animals from Calabria Region again exhibited a higher prevalence (10/155; 6.4% AP; 4.29% TP1; 16.5% TP2) compared with those from Campania (10/618; 1.6% AP; 0% TP1; 4.13% TP2) ($p = 0.0017$). When evaluating the prevalence at farm level, among the 34 establishments, 23 (67.6%) tested

	BCoV ELISA				BCoV VSN				SARS-CoV-2 ELISA				SARS-CoV-2 VSN			
	Total	Pos	%	95% C.I.	Total	Pos	%	95% C.I.	Total	Pos	%	95% C.I.	Total	Pos	%	95% C.I.
Species																
Cattle	491	429	87.3	84.3–90.2	368	247	67.1	62.7–71.9	491	22	4,4	2.6–6.2	22	0	0	n.a.
Buffaloes	454	6	1.3	0.26–2.3	6	0	0	n. a.	454	5	1.1	0.1–2	5	0	0	n.a.
X^2	702.36				11.82				8.85				n.a.			
p value	< 0.0001*				0.0006*				0.0029*				n.a.			
Region/Province																
Campania	**618**	**170**	**27.5**	**23.9–31**	**164**	**110**	**67**	**59.8–74.1**	**618**	**10**	**1.6**	**0.6–2.5**	**10**	**0**	**0**	**n.a.**
Caserta	**30**	**11**	**36.6**	**19.4–53.8**	**9**	**5**	**55.5**	**23–88**	**30**	**1**	**3.3**	**0–6.8**	**1**	**0**	**0**	**n.a.**
Cattle	20	10	50	28–71.9	8	5	62.5	29–96	20	1	5	0–14.5	1	0	0	n.a.
Buffaloes	10	1	10	0–28.6	1	0	0	n.a.	10	0	0	n.a.	0	0	0	n.a.
Salerno	**588**	**159**	**27**	**23.4–30.5**	**155**	**105**	**67.7**	**60.3–75**	**588**	**9**	**1.5**	**0.5–2.5**	**9**	**0**	**0**	**n.a.**
Cattle	160	154	96.2	93.1–99.2	150	105	70	62.6–77.3	160	4	2.5	0.1–4.9	4	0	0	n.a.
Buffaloes	428	5	1.1	0.1–2	5	0	0	n.a.	428	5	1.1	0.1–2	5	0	0	n.a.
Calabria	**327**	**265**	**81**	**76.7–85.2**	**210**	**137**	**65.2**	**58.7–71.6**	**327**	**17**	**5.1**	**2.7–7.4**	**17**	**0**	**0**	**n.a.**
Cosenza	**155**	**117**	**75.4**	**68.6–82.1**	**92**	**58**	**63**	**53.1–72.8**	**155**	**10**	**6.4**	**2.6–10.2**	**10**	**0**	**0**	**n.a.**
Cattle	139	117	84.1	78–90.1	92	58	63	53.1–72.8	139	10	7.2	2.9–11.5	10	0	0	n.a.
Buffaloes	16	0	0	n.a.	0	0	0	n.a.	16	0	0	n.a.	16	0	0	n.a.
Catanzaro	**42**	**25**	**59.5**	**44.7–74.3**	**14**	**0**	**0**	**n.a.**	**42**	**0**	**0**	**n.a.**	**0**	**0**	**0**	**n.a.**
Cattle	42	25	59.5	44.7–74.3	14	0	0	n.a.	42	0	0	n.a.	0	0	0	n.a.
Crotone	**34**	**30**	**88.2**	**77.3–99**	**22**	**0**	**0**	**n.a.**	**34**	**0**	**0**	**n.a.**	**0**	**0**	**0**	**n.a.**
Cattle	34	30	88.2	77.3–99	22	0	0	n.a.	34	0	0	n.a.	0	0	0	n.a.
Vibo Valentia	**96**	**93**	**96.8**	**93.2–100**	**82**	**79**	**96.3**	**92.22–100**	**96**	**7**	**7.2**	**2.1–12.3**	**7**	**0**	**0**	**n.a.**
Cattle	96	93	96.8	93.2–100	82	79	96.3	92.22–100	96	7	7.2	2.1–12.3	7	0	0	n.a.
X^2	246.41				0.13				9.86							
p value	< 0.0001*				0.7104				0.0017*							
Total	945	435	46	42.8–49.1	374	247	66	61.2–70.8	945	27	2.8	1.7–3.8	27	0	0	n.a.

Table 1. Bovine coronavirus and SARS-CoV-2 ELISA and virus serum neutralization results and statistical analysis. 95% C.I., 95% Confidence Intervals; n.a., not applicable; VSN, virus serum neutralization. *Results were considered as statistically significant with a p value < 0.05.

positive for BCoV, with 84% of cattle farms (21/25) and 22.2% of buffalo farms (2/9), showing a statistically significant difference (Chi-squared: 11.2006; $p = 0.0008$), while 32.3% overall farm prevalence was revealed for SARS-CoV-2 (11/34), which 36% (9/25) were bovine farms and 22.2% were buffalo farms (2/9), with no statistically significant difference (Chi-squared: 0.5571; $p = 0.4554$). Moreover, among the 11 SARS-CoV-2 positive farms, 9 showed a co-infection with BCoV (9 bovine and 1 bubaline farms).

All ELISA-positive samples were subsequently subjected to a virus serum neutralization (VSN) assay to confirm antibody specificity and rule out potential cross-reactivity. Among the 435 BCoV-positive sera, 61 were excluded due to cytotoxicity, and 247 of the remaining 374 samples (66%) showed neutralizing antibodies. Notably, neutralizing antibodies were detected exclusively in cattle (247/368; 67.1%), whereas none of the buffalo sera (0/6) tested positive, revealing a statistically significant interspecies difference ($p = 0.0006$). No regional differences were observed in neutralization titers, suggesting that species-specific immune responses, rather than geographical origin, primarily drive BCoV susceptibility. Interestingly, when performing VSN among the 27 SARS-CoV-2 ELISA-positive samples, none of them showed SARS-CoV-2 specific neutralizing antibodies, both in cattle and buffaloes. In this context, among the 27 positive sera, 12 tested positive also for BCoV, which were all cattle, and 4 of them also showed detectable BCoV-neutralizing antibodies. Therefore, the serological reactivity cannot be certainly attributed to prior SARS-CoV-2 exposure, as cross-reactions with other betacoronaviruses, including BCoV, could have occurred, leading to the detection of non-specific antibodies.

Discussion

The present seroepidemiological survey aimed to evaluate two relevant betacoronaviruses, BCoV and SARS-CoV-2, among livestock populations to assess the spread of these two viruses, the impact of the species and the geographical area of origin as possible risk factors, as well as the real status of the animals through the detection of neutralizing antibodies by VSN. VSN is an optimal tool to quantify the antibody response, thus shed light on infection status and immunity[6,25], and to rule out eventual cross-reactions[11].

Previous studies have demonstrated high rates of infection for BCoV in bovine populations. Across different geographic area, BCoV seroprevalence in cattle shows remarkable variability, ranging from 11.0 to 97.8% at the animal level and 72.2 to 100% at the herd level, reflecting the virus widespread distribution and its ability to persist under diverse environmental and management conditions[20,26–31]. In Italy, data related to the prevalence

of BCoV are scarce. Our findings demonstrated an overall prevalence of 46% in Southern Italy, being in line with data already reported among other European countries[3]. In particular, a study conducted by Ferrara and colleagues (2023)[20]in the Campania Region showed an overall prevalence of 30.8%, with a positivity of 49.2% among cattle and 5.3% among buffaloes. In this context, our findings corroborate this sharp contrast between cattle (87.3%) and buffaloes (1.3%) in exposure, showing a statistically significant difference ($p < 0.0001$), also corroborated by the difference in herd prevalence ($p = 0.0008$). This remarkable difference in seroprevalence between these two species strongly suggests that species-specific factors, such as receptor distribution, innate immune responses[20,32,33], may play a fundamental role in coronavirus susceptibility. Indeed, Kiser et al.[32] by the evaluation of whole blood samples of cattle, identified at least three *loci* which could be associated to predisposition to BCoV infection. Moreover, previous studies have reported the presence of a bovine-like coronavirus in buffaloes, named bubaline coronavirus (BuCoV), which showed a very high degree of sequence identity (98 to 99%) with BCoV, but marked differences in accessory proteins, in particular between the S and E protein regions[33–35]. Therefore, the homology with BCoV but the presence of different biological properties[33,36] may be the basis of the observed differences in seroprevalence and susceptibility between cattle and buffaloes, thus resulting not only from host-related factors, as BCoV preferentially infects bovine respiratory and intestinal epithelia, whereas water buffaloes may exhibit lower receptor affinity or reduced viral replication efficiency[36], but also from virus-specific adaptations.

Although vaccines against bovine coronavirus (BCoV) are available, including an intranasal vaccine authorized for use in the European Union and in Italy, none of the animals included in this study had been vaccinated against this virus. Furthermore, the possibility that seropositivity could result from maternally derived antibodies transferred via colostrum can be reasonably excluded. Indeed, all sampled animals were older than one year, a period far exceeding the known persistence of passively acquired antibodies in calves. Passive immunity to BCoV, primarily mediated by colostral IgG1[37], typically wanes within the first months of life, with protective titres declining substantially between 2 and 4 months of age, showing the lowest antibody titer level at the 4th month[38].

A similar pattern was detected for SARS-CoV-2 antibodies. This marked-host difference was also revealed for SARS-CoV-2, with a statistically significant difference between cattle (4.4%) and buffaloes (1.1%) ($p = 0.0029$), which may undergo same influences of BcoV, although no difference was revealed at farm level ($p = 0.4554$).

Since its first emergence, SARS-CoV-2 has rapidly shown high tendence of spillover and spillback events[14]. Indeed, as of 25 September 2025, Food and Agriculture Organization of the United Nations (FAO) reported the detection of SARS-CoV-2 in 68 naturally infected animal species across 49 countries and territories, including domestic and wild ruminants[39]. The first serological evidence of SARS-CoV-2 natural infection in domestic ruminants was revealed in the Campania Region[21] in lactating cows, where it was observed that out of 24 cows, 15 tested positive by ELISA and 13 also showed neutralizing antibodies. Later, in the same area, it was evaluated the natural exposure of small ruminants to SARS-CoV-2 by ELISA among 612 animals, showing a prevalence of 3.48% and 4.83% in sheep and goats, respectively, although only one sheep showed detectable neutralizing antibodies[16].

Although domestic ruminants are considered poorly susceptible to SARS-CoV-2[40], showing an overall AP of 2.8% in the present study, and they do not show the ability of intraspecies transmission[16,21,41,42], the detection of positive animals is not negligible. Furthermore, the identification of antibodies anti-SARS-CoV-2 in livestock reinforces the concept that coronavirus transmission is not strictly confined to humans or wildlife but may extend to domesticated species. While current evidence does not suggest active transmission cycles in cattle or buffaloes, continuous monitoring is essential to detect possible adaptation events that could influence the emergence of novel variants at the human–animal interface[16,21,42].

Nevertheless, limitations of ELISA kits based on the detection of anti–SARS-CoV-2 nucleocapsid (N) antibodies have been reported. In particular, a discrepancy has been noted between the 100% sensitivity and 97.8% specificity for bovine samples declared in the kit validation protocol[23] and the diagnostic performance observed for N-based assays when compared with ELISAs targeting the receptor-binding domain (RBD)[24]. Indeed, Fernández-Bastit et al. (2025)[24], through a comparative evaluation of three SARS-CoV-2 ELISA assays, demonstrated that the kit targeting antibodies against the N protein, while exhibiting high diagnostic specificity (100%), showed a markedly reduced sensitivity (38.7%) when benchmarked against the pseudovirus neutralization test (pVNT). In contrast, the two RBD-based ELISA kits displayed substantially superior overall performance. One kit achieved both high sensitivity (96.9%) and specificity (97.1%), whereas the second showed intermediate diagnostic accuracy, with a sensitivity of 51.7% and a specificity of 65.5%. Accordingly, ELISA assays targeting the RBD, which is directly involved in virus–receptor interactions, appear to provide more reliable estimates that better reflect neutralizing antibody activity. Conversely, N-based ELISAs, including the ID Screen kit, may underestimate seropositivity, particularly in cross-species surveillance contexts[24].

It should be noted, however, that these performance data were generated in dogs and cats. Although these findings may reasonably be extrapolated to cattle, species-specific evidence for cattle remains limited. To minimize potential bias due to interspecies differences, the present study estimated true prevalence using both the manufacturer-reported performance characteristics of the kit[23] and the parameters reported by Fernández-Bastit et al.[24]. This approach resulted in estimated an overall true prevalence value of 0.6% and 7.23%, respectively.

Our findings also revealed that, beyond species differences, the region of origin represents a significant determinant influencing the prevalence of both BCoV and SARS-CoV-2 infection ($p < 0.0001$). While Ferrara et al.[20] reported no significant differences among provinces within the Campania region, suggesting that location did not constitute a risk factor for BCoV infection, our study identified a markedly higher seroprevalence between regions. This discrepancy likely reflects the influence of local epidemiological drivers, including herd management systems, animal movement patterns, climatic conditions, and potential interactions with wildlife reservoirs. In particular, open-grazing practices, poor biosecurity measures, and contact with wild ruminants

may facilitate viral maintenance and circulation in specific areas[8,30], as well as co-living of different domestic ruminants, as the case of cattle and buffaloes mixed breeding, which facilitate the contagion and spillover events[20].

Finally, a very high percentage of BCoV ELISA positive samples showed detectable neutralizing antibodies (67.1%). Nevertheless, the exclusive detection of neutralizing antibodies in cattle may indicate that functional humoral immunity develops more efficiently in this species, while buffaloes may experience not only a limited exposure but also a distinct immune response pattern[20,32,33]. On the other hand, it can be hypothesized that cross-reactions may have occurred with other betacoronaviruses, that could have implied the positivity by ELISA but negative results by VSN, in particular, among buffaloes, where the BCoV-like BuCoV may have been responsible for the six ELISA positive animals. Conversely, among the 27 SARS-CoV-2 ELISA positive sera none of them showed neutralizing antibodies. It is widely reported that ELISA screening positive results should be carefully considered and should be confirmed by pVNT, VSN, and plaque reduction neutralization test[24,43,44]. Nevertheless, a recent study by Ramasamy and colleagues (2025) showed the presence of cross-reactions in pre-pandemic bovine sera using pVNT that did not correspond to positivity by other serological tests, including VNT, therefore showing it is not the most performing assay for SARS-CoV-2 diagnosis and confirmation[45]. In this context, the use of the VSN shall be considered a methodologically essential step for confirming the ELISA screening results, which enables the detection of functional neutralizing antibodies directed against both the S and N proteins, thereby providing a higher level of evidence. This approach strengthens the interpretive robustness of the dataset and minimizes the risk of overestimating seropositivity.

The complete absence of detectable neutralizing antibodies may open two different scenarios. In the first instance, the rapid decay of neutralizing antibodies that are described for livestock, which, as soon as one month following intranasal experimental infection, show very low neutralizing antibody titres or even non-existent titres[16,40]. Therefore, the poor and short time of SARS-CoV-2 shedding after exposure, together with the absence of a robust and long-term persistence of neutralizing antibodies that characterize livestock, may be the reason underlying our findings[16,39]. Another hypothesis, which may be more consistent, could be the presence of cross-reactions with other betacoronaviruses, including BCoV. Indeed, among the 27 SARS-CoV-2 positive animals, 12 also tested positive to BCoV, and four of them showed BCoV specific neutralizing antibodies. In this context, the comparative sequence analysis between SARS-CoV-2 and other betacoronavirus, in particular coronavirus belonging to the subgenus *Embecovirus*, which include BCoV species, has revealed a low degree of amino-acid identity (38%) with HCoV-OC43[46], which is closely related to BCoV[47]. From an immunological perspective, this level of sequence conservation is not of extent to support extensive serological cross-reactivity, although limited cross-reaction cannot be excluded. However, recently, it has been evidenced that the BCoV spike protein, when expressed in a vaccine vector, elicits humoral and cellular immune responses in mice that cross-react with various SARS-CoV-2 spike variants, although the antibodies fail to neutralize it[11]. Altogether, the possible presence of cross-reactivity cannot be completely ruled out. Therefore, the interpretation of the ELISA-positive samples is challenging. Indeed, the available data do not allow discrimination between true SARS-CoV-2 exposure and non-specific serological reactivity arising from non-specific binding or cross-reactivity with other coronaviruses. For this reason, the observed N-reactive serological signals should be interpreted with caution and cannot be related as conclusive evidence of SARS-CoV-2 exposure and/or circulation in cattle or buffalo populations but, more plausibly, as a result of detection of antibodies elicited by other viruses with high structural homology. Thus, further studies should be addressed to evaluate possible cross-reactions between SARS-CoV-2 N-based ELISAs and other betacoronaviruses.

A limitation of our study is the lack of pre-pandemic sera from the same animal species and locations, which would have allowed a more definitive assessment of baseline cross-reactivity. Nevertheless, several studies conducted on human and animal samples provide a useful approximation of such baseline. Cross-reactivity with SARS-CoV-2 antigens has been reported following exposure to heterologous betacoronaviruses in pre- and post-pandemic sera and colostrum, which can cross-react with SARS-CoV-2 S and N proteins[45,48,49], but they failed to neutralize SARS-CoV-2 infection in vitro, thereby showing that BCoV-neutralizing animal sera do not neutralize by VNT[48], as well as Ramasamy et al. showed a low extent of cross-reactions in pre-pandemic sera using pVNT but they did not correspond to positivity by other serological tests, including VSN[45]. Finally, other authors observed cross-reactions in the pre-pandemic colostrum, even though they concluded that antibodies to BCoV are unlikely to neutralize SARS-CoV-2[49]. Altogether, bibliographic data can represent the baseline for the pre-pandemic situation which, associated with the use of VSN, may be the basis for reliable results, even in the absence of pre-pandemic sera.

Conclusions

The present study shed light on the complex ecology of betacoronaviruses within livestock populations, emphasizing how species-specific susceptibility and regional factors may shape viral circulation. Although serological reactivity to both BCoV and SARS-CoV-2–related antigens was observed in cattle and buffaloes, the present study does not provide definitive support for previous exposure to or circulation of SARS-CoV-2 in the investigated livestock species. In this context, our results underline the necessity of confirmatory assays, such as virus serum neutralization (VSN). These findings reinforce the importance of adopting a One Health perspective when investigating coronavirus dynamics across domestic and wild species, while also highlighting the challenges posed by the high homology among betacoronaviruses, particularly in serological investigations. By documenting serological evidence of both BCoV and SARS-CoV-2 in cattle and buffaloes, this study reinforces the importance of a One Health perspective in understanding coronavirus dynamics across domestic and wild species. Furthermore, given the high homology of betacoronaviruses that could result in cross-reactions, mostly in serological investigations, our findings highlight the importance of conducting confirmation tests such as VSN that could help to increase the credibility of positive results, and to assess the real distribution and host

range of coronaviruses. Altogether, these considerations highlight the need for further longitudinal surveillance, coupled with molecular detection and genomic sequencing, to elucidate the infection dynamics, persistence, and potential interspecies transmission of these viruses. Such studies could also clarify the role of co-infections or antibody cross-reactivity in shaping coronavirus evolution and host range[20].

Data availability

All data referred to the present study are included within the manuscript.

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References

1. Lai, M. M., Liao, C. L., Lin, Y. J. & Zhang, X. Coronavirus: How a large RNA viral genome is replicated and transcribed. *Infect. Agents Dis.* **3** (2–3), 98–105 (1994).
2. Khamassi Khbou, M., Daaloul Jedidi, M., Bouaicha Zaafouri, F. & Benzarti, M. Coronaviruses in farm animals: epidemiology and public health implications. *Vet. Med. Sci.* **7** (2), 322–347. https://doi.org/10.1002/vms3.359 (2021).
3. Zhu, Q., Li, B. & Sun, D. Advances in bovine coronavirus epidemiology. *Viruses* **14** (5), 1109. https://doi.org/10.3390/v14051109 (2022).
4. Colina, S. E., Serena, M. S., Echeverría, M. G. & Metz, G. E. Clinical and molecular aspects of veterinary coronaviruses. *Virus Res.* **297**, 198382. https://doi.org/10.1016/j.virusres.2021.198382 (2021).
5. Alfieri, A. A., Ribeiro, J., de Carvalho Balbo, L., Lorenzetti, E. & Alfieri, A. F. Dairy calf rearing unit and infectious diseases: diarrhea outbreak by bovine coronavirus as a model for the dispersion of pathogenic microorganisms. *Trop. Anim. Health Prod.* **50** (8), 1937–1940. https://doi.org/10.1007/s11250-018-1592-9 (2018).
6. Saif, L. J. Bovine respiratory coronavirus. *Vet. Clin. N. Am. Food Anim. Pract.* **26**, 349–364. https://doi.org/10.1016/j.cvfa.2010.04.005 (2010).
7. Saif, L. J. & Jung, K. Comparative pathogenesis of bovine and Porcine respiratory coronaviruses in the animal host species and SARS-CoV-2 in humans. *J. Clin. Microbiol.* **58**, e01355–e01320. https://doi.org/10.1128/JCM.01355-20 (2020).
8. Vlasova, A. N. & Saif, L. J. Bovine coronavirus and the associated diseases. *Front. Vet. Sci.* **8**, 643220. https://doi.org/10.3389/fvets.2021.643220 (2021).
9. Goldstein, S. A., Brown, J., Pedersen, B. S., Quinlan, A. R. & Elde, N. C. Extensive recombination-driven coronavirus diversification expands the pool of potential pandemic pathogens. *Genome Biol. Evol.* **14** (12), evac161. https://doi.org/10.1093/gbe/evac161 (2022).
10. Lau, S. K. et al. Molecular epidemiology of human coronavirus OC43 reveals evolution of different genotypes over time and recent emergence of a novel genotype due to natural recombination. *J. Virol.* **85**, 11325–11337. https://doi.org/10.1128/JVI.05512-11 (2011).
11. Cossu, C. et al. Cross-reactive immune response of bovine coronavirus spike glycoprotein to SARS-CoV-2 variants of concern. *Int. J. Mol. Sci.* **25** (21), 11509. https://doi.org/10.3390/ijms252111509 (2024).
12. Russell, T., Lozano, J. M., Challinor, C. & Barry, G. Diversifying selection identified in immune epitopes of bovine coronavirus isolates from Irish cattle. *J. Gen. Virol.* **105** (8), 002019. https://doi.org/10.1099/jgv.0.002019 (2024).
13. Bashor, L. et al. SARS-CoV-2 evolution in animals suggests mechanisms for rapid variant selection. *bioRxiv* 2021.03.05.434135. (2021). https://doi.org/10.1101/2021.03.05.434135
14. Sparrer, M. N. et al. Role of spillover and spillback in SARS-CoV-2 transmission and the importance of one health in Understanding the dynamics of the COVID-19 pandemic. *J. Clin. Microbiol.* 61e01610–61e01622. https://doi.org/10.1128/jcm.01610-22 (2023).
15. Qiu, Y. et al. Predicting the angiotensin converting enzyme 2 (ACE2) utilizing capability as the receptor of SARS-CoV-2. *Microbes Infect.* **22** (4–5), 221–225. https://doi.org/10.1016/j.micinf.2020.03.003 (2020).
16. Fusco, G. et al. First serological evidence of SARS-CoV-2 natural infection in small ruminants: brief report. *Vet. Res. Commun.* **47** (3), 1741–1748. https://doi.org/10.1007/s11259-022-10044-3 (2023).
17. Ma, Y. et al. Susceptibility of bovine to SARS-CoV-2 variants of concern: insights from ACE2, AXL, and NRP1 receptors. *Virol. J.* **20**, 276. https://doi.org/10.1186/s12985-023-02222-9 (2023).
18. Wernike, K. et al. Multi-species ELISA for the detection of antibodies against SARS-CoV-2 in animals. *Transbound. Emerg. Dis.* **68** (4), 1779–1785. https://doi.org/10.1111/tbed.13926 (2021).
19. Yen, L., Henao-Díaz, A., Zimmerman, J. & Giménez-Lirola, L. Considerations on the stability of IgG antibody in clinical specimens. *J. Vet. Diagn. Investig.* **37** (1), 13–26. https://doi.org/10.1177/10406387241296848 (2025).
20. Ferrara, G. et al. Seroprevalence and risk factors for bovine coronavirus infection among dairy cattle and water Buffalo in campania Region, Southern Italy. *Animals* **13** (5), 772. https://doi.org/10.3390/ani13050772 (2023).
21. Fiorito, F. et al. First description of serological evidence for SARS-CoV-2 in lactating cows. *Animals* **12** (11), 1459. https://doi.org/10.3390/ani12111459 (2022).
22. Cardillo, L. et al. SARS-CoV-2 serological and biomolecular analyses among companion animals in campania region (2020–2021). *Microorganisms* **10** (2), 263. https://doi.org/10.3390/microorganisms10020263 (2022).
23. Laidoudi, Y. et al. SARS-CoV-2 antibodies seroprevalence in dogs from France using ELISA and an automated Western blotting assay. *One Health*. **13**, 100293. https://doi.org/10.1016/j.onehlt.2021.100293 (2021).
24. Fernández-Bastit, L. et al. Comparison of three commercial ELISA kits for detection of antibodies against SARS-CoV-2 in serum samples from different animal species. *Viruses* **17** (5), 716. https://doi.org/10.3390/v17050716 (2025).
25. Goto, A. et al. Prediction models for neutralization activity against emerging SARS-CoV-2 variants: A cross-sectional study. *Front. Microbiol.* **14**, 1126527. https://doi.org/10.3389/fmicb.2023.1126527 (2023).
26. Gulliksen, S. M. et al. Respiratory infections in Norwegian dairy calves. *J. Dairy. Sci.* **92** (10), 5139–5146. https://doi.org/10.3168/jds.2009-2224 (2009).
27. Wolff, C., Emanuelson, U., Ohlson, A., Alenius, S. & Fall, N. Bovine respiratory syncytial virus and bovine coronavirus in Swedish organic and conventional dairy herds. *Acta Vet. Scand.* **57** (1), 2. https://doi.org/10.1186/s13028-014-0091-x (2015).
28. Ven, S., Arunvipas, P., Lertwatcharasarakul, P. & Ratanapob, N. Seroprevalence of bovine coronavirus and factors associated with the serological status in dairy cattle in the Western region of Thailand. *Vet. World*. **14** (8), 2041–2047. https://doi.org/10.14202/vetworld.2021.2041-2047 (2021).
29. Socha, W., Larska, M., Rola, J. & Bednarek, D. Occurrence of bovine coronavirus and other major respiratory viruses in cattle in Poland. *J. Vet. Res.* **66** (4), 479–486. https://doi.org/10.2478/jvetres-2022-0059 (2022).
30. Berge, A. C. & Vertenten, G. Bovine coronavirus prevalence and risk factors in calves on dairy farms in Europe. *Animals* **14** (18), 2744. https://doi.org/10.3390/ani14182744 (2024).
31. Perfilyeva, Y. V. et al. Occurrence and epidemiology of bovine coronavirus in cattle in Kazakhstan. *Vet. J.* **313**, 106371. https://doi.org/10.1016/j.tvjl.2025.106371 (2025).

32. Kiser, J. N. & Neibergs, H. L. Identifying loci associated with bovine Corona virus infection and bovine respiratory disease in dairy and feedlot cattle. *Front. Vet. Sci.* **8**, 679074. https://doi.org/10.3389/fvets.2021.679074 (2021).
33. Decaro, N. et al. Biological and genetic analysis of a bovine-like coronavirus isolated from water Buffalo (*Bubalus bubalis*) calves. *Virology* **370** (1), 213–222. https://doi.org/10.1016/j.virol.2007.08.031 (2008).
34. Lau, S. K. et al. First genome sequences of Buffalo coronavirus from water Buffaloes in Bangladesh. *New. Microbes New. Infect.* **11**, 54–56. https://doi.org/10.1016/j.nmni.2016.02.011 (2016).
35. Amer, H. M. Bovine-like coronaviruses in domestic and wild ruminants. *Anim. Health Res. Rev.* **19** (2), 113–124. https://doi.org/10.1017/S1466252318000117 (2018).
36. Decaro, N. et al. Characterisation of Bubaline coronavirus strains associated with gastroenteritis in water Buffalo (*Bubalus bubalis*) calves. *Vet. Microbiol.* **145** (3–4), 245–251. https://doi.org/10.1016/j.vetmic.2010.04.010 (2010).
37. Bok, M. et al. Passive immunity to control bovine coronavirus diarrhea in a dairy herd in Argentina. *Rev. Argent. Microbiol.* **50** (1), 23–30. https://doi.org/10.1016/j.ram.2017.03.007 (2018).
38. Tuncer, P. & Yeşilbağ, K. Serological detection of infection dynamics for respiratory viruses among dairy calves. *Vet. Microbiol.* **180** (3–4), 180–185. https://doi.org/10.1016/j.vetmic.2015.08.025 (2015).
39. Food and Agricultural Organization of the United Nations (FAO). Emerging zoonotic coronaviruses in animals situation update. accessed on October 13, (2025). https://www.fao.org/animal-health/situation-updates/emerging-zoonotic-coronaviruses-in-animals/en?utm_source=chatgpt.com
40. Bosco-Lauth, A. M. et al. Susceptibility of livestock to SARS-CoV-2 infection. *Emerg. Microbes infect.* **10** (1), 2199–2201. https://doi.org/10.1080/22221751.2021.2003724 (2021).
41. Ulrich, L., Wernike, K., Hoffmann, D., Mettenleiter, T. C. & Beer, M. Experimental infection of cattle with SARS-CoV-2. *Emerg. Infect. Dis.* **26** (12), 2979–2981. https://doi.org/10.3201/eid2612.203799 (2020).
42. Hüttl, J. et al. Serological and molecular investigation of SARS-CoV-2 in horses and cattle in Switzerland from 2020 to 2022. *Viruses* **16** (2), 224. https://doi.org/10.3390/v16020224 (2024).
43. Bewley, K. R. et al. Quantification of SARS-CoV-2 neutralizing antibody by wild-type plaque reduction neutralization, microneutralization and pseudotyped virus neutralization assays. *Nat. Protoc.* **16**, 3114–3140. https://doi.org/10.1038/s41596-021-00536-y (2021).
44. EFSA Panel on Animal Health and Welfare (AHAW). SARS-CoV-2 in animals: susceptibility of animal species, risk for animal and public health, monitoring, prevention and control. *EFSA J.* **21** (2), e07822. https://doi.org/10.2903/j.efsa.2023.7822 (2023).
45. Ramasamy, S. et al. Serological assays reveal no evidence of natural SARS-CoV-2 infection in US cattle. *Microorganisms* **13**, 600. https://doi.org/10.3390/microorganisms13030600 (2025).
46. Gupta, A. K. et al. CoronaVR: A computational resource and analysis of epitopes and therapeutics for severe acute respiratory syndrome Coronavirus-2. *Front. Microbiol.* **11**, 1858. https://doi.org/10.3389/fmicb.2020.01858 (2020).
47. Vijgen, L. et al. Complete genomic sequence of human coronavirus OC43: molecular clock analysis suggests a relatively recent zoonotic coronavirus transmission event. *J. Virol.* **79** (3), 1595–1604. https://doi.org/10.1128/JVI.79.3.1595-1604.2005 (2005).
48. Hulst, M. et al. Cross-reactivity of human, wild boar, and farm animal sera from pre- and post-pandemic periods with alpha- and βeta-coronaviruses (CoV), including SARS-CoV-2. *Viruses* **16** (1), 34. https://doi.org/10.3390/v16010034 (2023).
49. Ellis, J. et al. SARS coronavirus 2-reactive antibodies in bovine colostrum. *Can. Vet. J.* **64** (4), 337–343 (2023).

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Author contributions

G.F. and N.D. conceded the study; A.P., G.P., C.d.M., and L.C. drafted the manuscript; A.P., G.P., and S.B. conducted the analysis; A.M., L.B., E.R., and F.F. provided samples, materials, and data from the field; L.C., C.d.M., N.D., and G.F. reviewed and edited the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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