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MICROBIOLOGY

A systematic review of the presence of bovine coronavirus on environmental surfaces

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ABSTRACT: The main transmission route of bovine coronavirus (BCoV) is direct contact with secretions and feces, and potentially also via contaminated surfaces. Following PRISMA guidelines, a systematic review was conducted to evaluate the presence of BCoV on environmental surfaces. A literature search, conducted between March and April 2023, utilized databases including Web of Science, PubMed, Scopus, Science Direct, and Springer. The review adhered to the PEO structure: Population - environmental surfaces; Exposure - contact with BCoV; Outcome - identification of presence or infectivity. Primary data were recorded using an extraction form organizing methods for detecting BCoV presence, assessing BCoV infectivity, types of surfaces evaluated, and study type. Search terms "Bovine coronavirus" and "BCoV" yielded 2703 articles. After removing 964 duplicates and excluding 1546 articles not mentioning BCoV in titles, 193 studies underwent abstract reading. Following exclusion criteria not addressing BCoV presence in the environment, three articles were selected for comprehensive review. These articles identified BCoV presence on various types of surfaces, with detection possible up to 81 hours after contamination, depending on surface type. Despite limited studies on BCoV presence on surfaces, findings suggested potential transmission via contaminated surfaces due to the virus's ability to remain infectious for up to 24 hours on fomites. This review underscores the need for further research on BCoV persistence in farm environments, an area currently lacking focused studies.
Key words: BCoV, cattle, infectivity, transmission routes, environment contamination.

Revisão sistemática da presença de coronavírus bovino em superfícies ambientais

RESUMO: A principal via de transmissão do coronavírus bovino (BCoV) é o contato direto com secreções e fezes, podendo ocorrer também por meio de superfícies contaminadas. Uma revisão sistemática, seguindo as recomendações PRISMA, foi realizada para avaliar a presença de BCoV em superfícies ambientais. A busca na literatura foi realizada em março e abril de 2023 e as bases de dados utilizadas foram Web of Science, PubMed, Scopus, Science Direct e Springer. A revisão sistemática seguiu a estrutura PEO: População – superfícies ambientais; Exposição – contato com BCoV; Resultado – identificação da presença ou infectividade de BCoV. As informações primárias foram registradas por meio de um formulário de extração, organizando métodos para detectar a presença de BCoV, avaliar a infectividade de BCoV, tipos de superfícies avaliadas e tipo de estudo. Os termos de pesquisa "coronavírus bovino" e "BCoV" resultaram em 2703 artigos. Após remover 964 duplicatas e excluir 1546 artigos que não mencionavam o BCoV nos títulos, 193 estudos foram submetidos à leitura de resumos. Seguindo os critérios de exclusão, que não abordavam a presença do BCoV no ambiente, três artigos foram selecionados para revisão abrangente. Nestes artigos foi identificada a presença de BCoV em vários tipos de superfícies, sendo possível a detecção até 81 horas após a contaminação, dependendo do tipo de superfície. Apesar do número limitado de estudos que investigam a presença de BCoV em superfícies, os resultados sugerem o potencial de transmissão de BCoV por meio de superfícies contaminadas, devido à capacidade do vírus permanecer infeccioso por até 24 horas em fômites. Esta revisão destaca a necessidade de mais pesquisas sobre a persistência de BCoV em locais de criação de bovinos, uma área atualmente carente de estudos específicos.
Palavras-chave: BCoV, bovinos, infectividade, rotas de transmissão, contaminação do ambiente.

INTRODUCTION

Coronaviruses (CoVs) constitute a significant group of viruses with a remarkable ability to infect diverse species, including humans (MASTERS, 2006). These viruses, spherical in shape with diameters ranging from 60 to 220 nm, consist of structural proteins such as the hemagglutinin esterase (HE) associated with the envelope, spike glycoprotein (S), matrix glycoprotein (M), envelope protein (E), and nucleocapsid phosphoprotein (N). Additionally, several open reading frames (ORFs) encode non-structural proteins responsible for the replication of single-stranded RNA viruses. Notably, CoVs possess the largest genome among all known RNA viruses (LAI & CAVANAGH, 1997; VIJGEN et al., 2005).

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The *Coronaviridae* family, subdivided into the subfamily *Orthocoronavirinae*, is further categorized into four genera of significant importance due to their role in causing diseases in humans and various animal species: *Alphacoronavirus*, *Betacoronavirus*, *Deltacoronavirus*, and *Gammacoronavirus* (ICTV, 2023; WOO et al., 2023). The *Betacoronavirus* genus encompasses bovine coronavirus (BCoV), along with other prevalent viruses such as equine coronavirus (ECoV), porcine hemagglutinating encephalomyelitis virus (PHEV), human coronavirus OC43 (HCoV-OC43), and severe acute respiratory syndrome-related coronavirus (SARS-CoV1 and SARS-CoV2) (MIRANDA et al., 2021). The close genetic relationship among different viruses of *Betacoronavirus* genus elucidates their potential for zoonotic infections (HODNIK et al., 2020).

BCoV, endemic in the cattle population, exhibits dual tropism for enteric and respiratory cells and is associated with neonatal diarrhea in calves, winter dysentery in adults, and respiratory infections in cattle across various age groups (SAIF, 2010). Clinical infections due to BCoV can lead to diminished growth performance in confined cattle, a decrease in milk production, and elevated morbidity rates in calves (SAIF, 2010; LORENZETTI et al., 2013; RIBEIRO et al., 2016; BOK et al., 2018; BRUNAUER et al., 2021).

BCoV infection can occur through direct contact, either via the fecal-oral route or through aerosol inhalation (SAIF, 2010). Transmission between herds can occur directly via the transfer of live cattle (OMA et al., 2016). Nevertheless, the potential for indirect transmission of BCoV should not be dismissed, as it could occur through contamination by inert or inanimate surfaces (OMA et al., 2018).

Similar to other CoVs (VAN DOREMALEN et al., 2020; RIDDELL et al., 2020; LIU et al., 2021), it is hypothesized that BCoV may retain its infectivity on abiotic surfaces and fomites for extended periods due to its genetic similarity with previously studied CoVs (VIJGEN et al., 2005; BIDOKHTI et al., 2012).

Comprehensive knowledge of all transmission routes is crucial for the prevention of infectious diseases. Given that the survival of BCoV on surfaces could directly impact clinical practices, such as hygiene guidelines and disinfection strategies, this study systematically reviewed the presence of BCoV on various surfaces.

METHODOLOGY

A systematic literature review was conducted between March and April 2023, adhering to the revised Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines as recommended by PAGE et al. (2021). Study selection, evaluation, and data extraction were independently carried out by two reviewers (CRCF and SHW), with any discrepancies resolved through consensus. The study protocol was not entered into any registry.

Search strategy

The initial search aimed to answer the question: “Is BCoV present on environmental surfaces?” A comprehensive database search was performed using the mandatory search term “bovine coronavirus” or “BCoV” and the terms “survival,” “surface,” “environment,” “stability,” “contamination,” “infectivity,” “environment surface,” or “inanimate surfaces.” However, no specific BCoV studies could be identified using these combinations. For this reason, a broader protocol search was conducted using only “Bovine coronavirus” or “BCoV”. The systematic review followed the PEO structure described by MOOLA et al. (2015), focusing on Population: environmental surfaces; Exposure: contact with BCoV; Outcome: identification of BCoV presence or infectivity. Databases searched included Web of Science, PubMed, Scopus, ScienceDirect, and Springer.

Inclusion and exclusion criteria

Inclusion criteria encompassed original research articles addressing BCoV and its presence on both living organisms and inanimate surfaces, without language restrictions. Exclusion criteria included duplicate articles and those not directly relevant to BCoV based on their titles. Studies exclusively assessing animals were also excluded.

Data extraction

Data from selected articles were extracted using a pre-established form, including detection methods for BCoV presence, BCoV infectivity identification methods, evaluated surface types, study type (experimental or observational), and the assessment of BCoV as a model for other CoVs.

Risk of bias assessment and quality analysis

A quality analysis was conducted to evaluate the risk of bias in the selected studies. Two evaluators (SHW and GSS) independently rated the studies and with any disagreements resolved through discussion and consensus. Ratings included low risk, some concern, and high risk. The revised Cochrane risk-of-bias tool for randomized trials (RoB 2) was utilized, assessing five domains: 1. Randomization process; 2. Deviations from intended interventions; 3. Missing data; 4. Measurement of the outcome; 5. Selection of the reported result (STERNE et al., 2019).

RESULTS

In the systematic literature search, 2703 articles were identified for analysis (Figure 1). After removing 964 (35.7%) duplicate articles, 1739 remained for further scrutiny. Based on the exclusion criteria by title, 1546 articles were subsequently excluded. The primary reasons for these exclusions were: studies on mathematical and physical sciences (n = 131); studies focusing on the molecular aspects of BCoV (n = 103); studies involving species other than cattle (n = 107); treatment and vaccination studies (n = 38); and studies on other pathogens and viruses (n = 30). Of the remaining 193 studies subjected to abstract reading, 190 were excluded as they did not address the presence of BCoV in the environment (Figure 1). Consequently, three studies were selected for further investigation: MULLIS et al. (2012), OMA et al. (2018), and WATANABE et al. (2022). In the overall risk-of-bias judgement, all the three included studies were judged as low risk of bias.

MULLIS et al. (2012) utilized BCoV as a model to investigate the infectivity and potential transmission of CoV, with a particular focus on severe acute respiratory syndrome (SARS) cases, due to the inclusion of SARS-CoVs in a list of viruses with potential for foodborne transmission (FAO & WHO, 2008). To achieve their objectives, the authors investigated the presence and infectivity of the BCoV 88 strain on the surfaces of romaine lettuce leaves. To mimic mild and heavy fecal contamination, 0.1% and 10% bovine fecal suspensions were created, corresponding to 1 ppm and 100 ppm, respectively. Additionally, minimum essential medium (MEM) was used to dilute the BCoV. Each lettuce piece

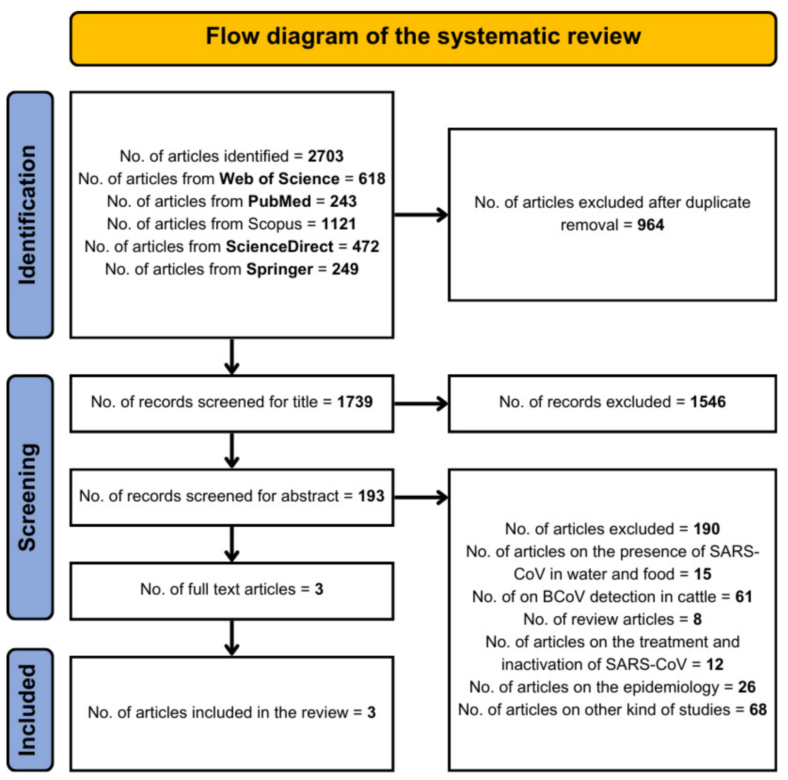

Figure 1 - Flowchart of the research and number of articles identified in the systematic review of BCoV presence on environmental surfaces.

was sprayed with 100 mL of the virus preparations, left to dry for 2 hours in a biosafety cabinet, and subsequently frozen at 4 °C until elution and virus presence analysis via qRT-PCR. Viral infectivity tests were conducted by culturing with a plaque assay on 3- to 5-day-old HRT-18 cell monolayers. These monolayers were rinsed and incubated with MEM without fetal bovine serum (FBS) for 3 hours at 37 °C in a 5% CO_2 atmosphere. The experiment revealed the presence of viral RNA on the lettuce surface at all time points (up to 30 days); although, RNA levels significantly fluctuated based on the suspension used. BCoV retained its infectivity for a minimum of 14 days. The study concluded that vegetables contaminated with the virus could potentially serve as vehicles for the zoonotic transmission of coronaviruses to humans.

The study by OMA et al. (2018) determined the duration of viral RNA carriage and the infectivity of viral particles on fomites and human nasal mucosa after exposure to BCoV. This study marked the first description of natural fomites contamination by infectious BCoV. The experiment was conducted during an outbreak of BCoV infection in 10 calves aged 6 – 12 weeks. Human nasal mucosa samples from 16 persons as well as from 44 sources of fomites (such as clothes, boots, watches, and stethoscopes) were collected before and after exposure to the infected calves at different times to assess the presence of BCoV. Subsequently, virus infectivity was tested on five samples that showed the highest levels of BCoV RNA, using cell culture integrated with RT-qPCR. After 30 minutes of exposure, the virus was detected in 46% (n = 80) of human nasal mucosa swabs. After two, four, and six hours, 15%, 5%, and 0% of swabs tested positive, respectively. Among the fomite samples, 97% (n = 44) carried a high viral RNA load (per log10 copy numbers) 24 hours after exposure. Although, no virus with infectivity was detected in the nasal mucosa, it was detected in 2 out of 3 fomite samples. Therefore, the authors concluded that fomites (such as clothes, boots, watches, and stethoscopes) exposed to the virus represent a high-risk source for cattle, as infective virus particles were detected even after 24 hours of exposure.

WATANABE et al. (2022) conducted a study to investigate the infectivity potential of SARS-CoV-2 on various porous and non-porous environmental surfaces and the CS5 strain of BCoV propagated in HRT-18G cell culture in Dulbecco's modified Eagle's medium was used as the model. The culture underwent a five-day incubation at 37 °C in a 5% CO_2 environment, then purified and diluted tenfold. Following incubation, the cells were examined for a virus-induced cytopathic effect (CPE). Virus titers were determined using the Reed–Muench method. Subsequently, the virus was introduced to 15 different porous and non-porous surfaces to assess the recovery time and infectivity. BCoV was detectable after 4 hours on brass, 7 hours on soft polyvinyl chloride, 22 hours on ceramics, 25 hours on melamine resin, 29 hours on stainless steel, 35 hours on glass, 48 hours on polystyrene, 62 hours on polypropylene, 65 hours on polyethylene, and 81 hours on acrylic resin. In contrast, the recovery time for SARS-CoV-2 on non-porous substrates was 3 hours on soft polyvinyl chloride and 11 hours on ceramic tiles. On porous substrates, such as disposable masks, it took 19 hours. Surface roughness did not directly influence the survival capacity of BCoV. The levels of infectious BCoV recovery after surface fixation varied between substrates with similar physical characteristics. The study demonstrated the value of BCoV as a model virus for testing the recovery of infectious SARS-CoV-2 from surfaces.

DISCUSSION

This study observed a scarcity of data regarding the presence of BCoV in the environment, despite the well-established durability of enteric and respiratory viruses in various environmental conditions. Previous research by CASANOVA et al. (2009) highlighted the extended viability of related coronaviruses like mouse hepatitis beta-coronavirus (MHV), which remained viable in water and sewage for 17 days. Similarly, transmissible gastroenteritis virus (TGEV), an alpha-coronavirus found in pigs, exhibited viability for 22 days and persisted on steel surfaces for over 28 days (CASANOVA et al., 2010). Moreover, amidst the recent COVID-19 pandemic, studies have extensively documented the detection of SARS-CoV-2 on surfaces within hospital settings and other environments, further emphasizing the potential for indirect transmission (GUO et al., 2020; RAZZINI et al., 2020; SANTARPIA et al., 2020; VAN DOREMALEN et al., 2020; BIRYUKOV et al., 2020; CHIN et al., 2020; CARRATURO et al., 2020; MORRIS et al., 2021).

Given the available data and the close genetic relationship with human CoVs, it is plausible to consider that indirect transmission may significantly contribute to BCoV infection. However, our understanding of this topic remains limited, and the pathways of BCoV's indirect transmission are yet to be fully elucidated. In the studies encompassed within this systematic review, MULLIS et al. (2012), OMA et al. (2018), and WATANABE et al. (2022) employed RT-qPCR or RT-PCR to detect BCoV RNA on surfaces, offering insights into its presence. These nucleic acid detection assays, considered among the most sensitive currently in use, are frequently employed in the diagnosis of BCoV infections (CHO et al., 2001; KANNO et al., 2018; CASTELLS et al., 2019; SINGH et al., 2019). However, the presence of viral RNA alone does not signify infectivity, necessitating assessments of virus viability (RAZZINI et al., 2020; ZHANG & WANG, 2023). In this scenario, MULLIS et al. (2012) and WATANABE et al. (2022) evaluated BCoV viability on surfaces through dilutions and cell culture techniques, while OMA et al. (2018) suggested the practicality of cell culture for assessing infectivity. Such studies mostly used clonal cell lines derived from HRT-18 cell cultures.

Although cell culture studies are vital for assessing virus viability, they have inherent limitations. These included variations in results based on cell lines used and reduced sensitivity in the presence of certain compounds. Moreover, controlled *in vitro* settings may not fully replicate the complexities of real-world farm environments, where variables such as temperature and humidity significantly influence virus survival. While the reviewed studies did not assess temperature or humidity, existing literature indicates their substantial impact on virus viability (CHAN et al., 2011; GUO et al., 2021).

The choice of surface material also influences virus persistence, with porous materials potentially harboring infectious particles for extended periods. The research conducted by WATANABE et al. (2022) revealed that BCoV can maintain infectivity on porous surfaces, emphasizing the necessity for tailored biosafety measures. Their comparative study on the viability of BCoV on porous and non-porous surfaces detected infectious BCoV on copy paper and polyester fabric for up to 18 hours, suggesting that BCoV could penetrate and become entrapped in the pores of such surfaces without losing its infectivity. Porous materials, such as wood and cement, possess small spaces or pores capable of capturing and retaining BCoV particles, providing an environment conducive to the virus's survival and maintenance of infectiousness over prolonged periods. For instance, in a stable with a cement floor or wooden walls, BCoV particles could adhere to the surface and persist for several days, thereby increasing the risk of transmission to other animals. Additionally, BOILEAU & KAPIL (2010) reported that BCoV could maintain its infectiousness for up to 3 days in the presence of organic matter. Their findings also demonstrated that BCoV exhibited strong binding affinity to clay, clay minerals, and coal *in vitro*, with an adsorption rate of 99%.

The correlation between decreased viral infectivity and moisture absorption by porous surfaces can be attributed to desiccation processes. Desiccation, which involves the removal of moisture from a surface, can result in dehydration and potential damage to viral particles (CHATTERJEE et al., 2021). This phenomenon may also lead to the reduced recovery of virions absorbed into the inner layers of the surface (SHIVKUMAR et al., 2021). As highlighted by CHATTERJEE et al. (2021), porous surfaces possess a greater capacity for moisture absorption, thereby accelerating the desiccation of the coronavirus. In contrast, impermeable surfaces retain more moisture, creating an environment conducive to the prolonged survival of the virus.

Furthermore, the studies evaluated herein underscore the potential for indirect transmission through fomites, emphasizing the importance of implementing proper cleaning and disinfection protocols as highlighted elsewhere (TAKAHASHI et al., 2020; HASAN et al., 2022). OMA et al. (2018) suggested that fomites, such as clothing, boots, watches, and stethoscopes, pose a risk of infection for cattle when exposed to the virus. These fomites can become contaminated through direct contact with infected animals, subsequently carrying and transmitting BCoV to susceptible hosts. Therefore, regardless of the surface type, the implementation of appropriate cleaning and disinfection procedures is essential to mitigate the risk of BCoV transmission. Coronaviruses, including BCoV, are enveloped viruses characterized by a lipid layer that encases their genetic material and structural proteins (HODNIK et al., 2020), and the exposure to high temperatures can lead to the denaturation of these structures, thereby inactivating the virus and inhibiting its ability to infect host cells (ABOUBAKR et al., 2021). Additionally, the activity of lipases and proteases found in the bovine intestine can contribute to the loss of infectivity. For instance, REN et al. (2020) reported a 10% decrease in BCoV infectivity in bovine fecal suspension compared to the suspension in FBS minimal medium, suggesting that the loss of the spike protein on the virus's surface or the envelope's loss could contribute to this reduction. Consequently, the fragility of the envelope in enveloped viruses may lead to a loss of infectivity, making them susceptible to inactivation by disinfection procedures. These procedures commonly involve the application of ethanol (62 and 71%), hydrogen peroxide (0.5%), or sodium hypochlorite (0.1%) on surfaces for 1 minute (KAMPF et al., 2020).

The reviewed studies primarily extrapolated the presence and infectivity of BCoV in environments from simulated experiments, potentially influenced by various factors. Consequently, the natural spread of BCoV within herds remains largely uncharted territory, despite the morbidity associated with infections (HÄGGLUND et al., 2006). Inter-herd cattle movement represents a notable transmission route, with potential indirect dissemination occurring through individuals traversing between herds, possibly carrying the virus on their clothing or equipment (TOFTAKER et al., 2017; OMA et al., 2018).

The findings of MULLIS et al. (2012), OMA et al. (2018), and WATANABE et al. (2022) hold significant implications for BCoV transmission, suggesting that indirect transmission via contaminated surfaces could be a plausible threat to both animal and human health. Consequently, robust biosafety measures are essential to curtail BCoV propagation, including the consistent use and replacement of personal protective equipment (PPE) by veterinarians and individuals interacting with infected animals or contaminated environments.

In the construction of cattle facilities, prioritizing less porous materials such as metal, tiles, and vitrified cement is advisable due to their ease of cleaning and disinfection. Additionally, ensuring that cattle housing areas remain covered and dry is crucial, as the virus retains viability in damp environments. Covering earthen floors with straw can help mitigate direct contact between cattle and the ground.

The limited data on the transfer and persistence of BCoV in farm environments leaves critical gaps in understanding the dynamics of indirect transmission. Despite extensive research on direct virus detection in cattle, this systematic review reveals that there is a notable scarcity of studies investigating its presence and viability in the environment. Hence, further research is imperative to comprehensively elucidate the interplay among the virus, environment, and management practices, facilitating the development of effective prevention protocols against the indirect spread of BCoV on farms.

CONCLUSION

Despite limited research in the literature, this systematic review highlights the potential for indirect transmission of BCoV via contaminated surfaces. It emphasizes the importance of effective cleaning and disinfection protocols. Understanding the impact of environmental factors on virus viability is crucial for devising strategies to mitigate transmission. Moreover, recognizing the susceptibility of enveloped viruses like BCoV to environmental conditions and disinfection methods is essential for implementing effective control measures. Overall, this review underscores the need for proactive measures to control BCoV spread in agricultural settings.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

CRCF - Conceptualization; Data curation; Formal analysis; Writing - original draft. SHW - Data curation; Formal analysis; Writing - review & editing. GSS - Writing - review & editing. AMDA - Formal analysis; Supervision; Writing - review & editing. RDO - Conceptualization; Formal analysis; Supervision; Writing - review & editing. CSS - Conceptualization; Data curation; Formal analysis; Project administration; Writing - review & editing.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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