

Characterization of an outbreak of equine coronavirus infection in adult horses in Switzerland

N. Fouché¹, F. Remy-Wohlfender¹, D. Blau², J. Franzen³, C. Gurtner³, T. Seuberlich⁴, L. Unger^{1,§}, V. Gerber^{1,§}

¹Swiss Institute of Equine Medicine, Vetsuisse Faculty, University of Bern; ² National Horse Center, Bern;

³Institute of Animal Pathology, Vetsuisse Faculty, University of Bern;

⁴ Division of Neurological Sciences, Vetsuisse Faculty, University of Bern

Beschreibung eines Equinen Coronavirus-Ausbruchs bei erwachsenen Pferden in der Schweiz

Ausbrüche von Infektionen mit dem Equinen Coronavirus (ECoV) wurden in verschiedenen Teilen der Welt, einschliesslich Europa, beschrieben. Das Ziel dieses Berichts war es, klinische Anzeichen, diagnostische Aufarbeitung und die Ergebnisse des ersten dokumentierten Ausbruchs von ECoV in der Schweiz zu beschreiben, dies um das Bewusstsein für die Krankheit und ihre verschiedenen klinischen Präsentationen zu schärfen. Der Ausbruch ereignete sich auf einem Reitstall mit 26 Pferden. Sieben Pferde entwickelten eine klinische Erkrankung, die von leichten Krankheitsanzeichen wie Fieber und Anorexie bis zu einer schweren, akuten Kolitis reichte. Ein Pferd verstarb an einer schweren Endotoxämie mit Kreislaufchock infolge einer schweren akuten nekrotisierenden Enteritis und Kolitis. Von den 26 Pferden wurden fünf positiv auf ECoV getestet, darunter zwei Ponys ohne klinische Anzeichen. Die geringe Anzahl positiver Fälle sollte jedoch mit Vorsicht interpretiert werden, da die Tests nur einmal, nach mehr als einen Monat nach Auftreten der klinischen Symptome im ersten Verdachtsfall, durchgeführt wurden. Dieser Bericht hebt die Bedeutung diagnostischer Tests und der frühzeitigen Umsetzung von Biosicherheitsmassnahmen in einem Betrieb mit einem ECoV-Ausbruch hervor. Ausserdem soll das Bewusstsein für mögliche Krankheitsüberträger mit unspezifischen und milden klinischen Anzeichen wie Fieber und Anorexie geschärft werden.

Schlüsselwörter: Kolitis, Durchfall, Equiden, Infektionskrankheit, Virusinfektion

Summary

Outbreaks of equine coronavirus (ECoV) infections have been described in different parts of the world including Europe. The aim of this report was to describe clinical signs, diagnostic work-up and outcome of the first documented outbreak of ECoV in Switzerland in order to raise the awareness for the disease and its various clinical presentations. The outbreak occurred on a farm with 26 horses. Of these, seven horses developed clinical disease ranging from mild signs such as fever and anorexia to severe signs of acute colitis. One horse died due to severe endotoxemia and circulatory shock secondary to severe acute necrotizing enteritis and colitis. Out of the 26 horses, five horses tested positive for ECoV, including two ponies without any clinical signs of infection. The low number of positive cases should nevertheless be interpreted with caution as testing was only performed on one occasion, over a month after the onset of clinical signs in the first suspected case. This report highlights the importance of diagnostic testing and early implementation of biosecurity measures on a farm with an ECoV outbreak. It should furthermore raise the awareness for unspecific and mild clinical signs such as fever and anorexia in affected animals that are potentially able to spread the disease.

Keywords: colitis, diarrhoea, equid, infectious disease, viral infection

<https://doi.org/10.17236/sat00372>

Eingereicht: 20.12.2021
Angenommen: 14.07.2022

§These authors contributed equally to this article

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Introduction

Equine coronavirus (ECoV) is a *Betacoronavirus* causing gastrointestinal disease in equids. ECoV was initially documented in foals⁴ and has later been associated with enteric disease in adult horses.^{20,24} Individual horses might be affected but disease outbreaks have also been reported in Japan, the USA and Europe.^{3,12,16,19–21,24} The first detection of ECoV in Europe (France) was documented in 2014,¹⁶ with further reports from the UK and Ireland in the following years.^{3,19} ECoV infections have previously been diagnosed in Switzerland but these were so far limited to single cases.⁶

The description and characterization of such outbreaks allows the collection of clinical data in order to refine the knowledge about the clinical presentation of ECoV infections in horses.²³ It also helps raising the awareness of the potential spread of the disease in a population and the importance of early implementation of quarantine measures. It is therefore of great value to document and describe clinical outbreaks of ECoV infections in different geographic areas.

The purpose of this report is to document the first outbreak of ECoV infection in Switzerland, focussing on the characterization and description of the clinical signs and diagnostic test results and compare the findings to previously published reports.

History

The outbreak occurred on a farm with 26 horses of various age-groups and breeds. All the horses were stabled in individual box stalls in three different barns with access to daily pasture turnout in groups. Some of the horses were regularly exercised in an indoor riding arena located nearby.

The suspected index case (case 1) was presented to a private practitioner with acute onset of fever and coughing at the end of December 2020. Haematology revealed leucocytosis, neutrophilia and lymphopenia. The horse was treated with non-steroidal anti-inflammatory drugs, analgesics, corticosteroids and antibiotics and was euthanized 10 days after the onset of clinical signs due to persistent tachycardia, tachypnea and fever. This horse was not tested for ECoV.

The cases 2–5 were presented to the same private practitioner with fever and anorexia. One of these horses also showed signs of colic that were attributed to a caecal impaction. Haematology showed varying degrees of leucopenia with neutropenia and/or lymphopenia (cases 2–4), while blood work was not performed in one of the affected horses (case 5). Clinical signs persisted for 2–6 days and treatment included non-steroidal anti-in-

flammatory drugs, administration of laxatives via naso-gastric tube and gastro-protective drugs. Cases 2–5 all recovered. They were tested for ECoV (RT-PCR test of fecal samples) at the farm 17–25 days after the onset of initial clinical signs (see section “Diagnostic test results in the home yard”) and one horse (case 5) tested positive at that time point (18 days after the onset of clinical signs) (Table 1).

Clinical findings and diagnostic test results in the first hospitalized case (case 6)

The 6th case, an 8-year-old Freiburger gelding was referred to the equine hospital mid of January 2021 for further evaluation of fever of 2 days duration. The horse had been treated by the private practitioner with non-steroidal anti-inflammatory drugs and analgesics and was referred due to a worsening of clinical signs. It had become increasingly lethargic, fever increased and the horse had developed loose faeces. Upon presentation to the hospital, the horse had diarrhea and anorexia. The horse became more lethargic and showed signs of endotoxemia and circulatory shock shortly after arrival to the equine hospital: It had a heart rate of 80 beats per minute, a respiratory rate of 60 per minute and a rectal temperature of 38,6°C. Mucous membranes were dry, dark red with a toxic line and a capillary refill time of 6 seconds. Peristalsis was normal on auscultation although the horse had diarrhoea. The abdominal and thoracic ultrasound examinations were unremarkable. Significant complete blood count (CBC) findings included an increased haematocrit (0,55 L/L; reference interval (RI) 0,34–0,45), thrombocytopenia (53 giga/L; RI 104–244), leucopenia (0,63 giga/L; RI 5,3–10,3), neutropenia (0,08 giga/L; RI 2,5–6,0) and lymphopenia (0,42 giga/L; 1,5–4,0). A serum biochemistry profile disclosed hyponatraemia (130 mmol/L; RI 135–143), hypochloremia (80 mmol/L; RI 95–105), hyperphosphatemia (1,72 mmol/L; RI 0,54–1,26), hypermagnesemia (1,15 mmol/L; RI 0,6–0,95), hyperglycemia (5,55 mmol/L; RI 2,91–5,15 mmol/L) and azotaemia (urea 9,9 mmol/L; RI 3,2–6,5 and creatinine 176 µmol/L; RI 86–173). Furthermore, the horse had hyperbilirubinemia (61,4 µmol/L; RI 7,8–42,4), increased alkaline phosphatase (475 U/L; RI 0–227), aspartate transaminase (629 U/L; RI 200–602), gamma-glutamyltransferase (γ-GT) (146 U/L; RI 11–26), sorbitol dehydrogenase (116 U/L; RI 3–16) and serum amyloid A (3450 µg/mL; RI <20) activities. A belly tap was performed and submitted for analysis. The abdominal fluid was transparent and of a dark yellow color with a total protein of 20 g/L and a lactate of 6 mmol/L. Initial treatment consisted of intravenous fluid therapy at double maintenance rate (lactated Ringer’s solution, Ringer-Lactat „Bichsel“ ohne/sans Glucose, Grosse Apotheke Dr. G. Bichsel AG, Interlaken, Switzerland and Equibiserol, Laboratorium Dr. G. Bichsel AG, Weissenaustrasse 73, 3800 Interlaken, Switzerland, 4 mL/kg/hour), Polymyxin B (Polymyxin-B,

Grosse Apotheke Dr. G. Bichsel, Interlaken, Switzerland, 3 mio units i.v.), metamizol (Vetalgin[®], MSD Animal Health GmbH, Luzern, Switzerland, 40 mg/kg i.v.), penicillin (Penicillin Natrium Streuli[®] ad us. vet., Streuli Pharma AG, Uznach, Switzerland, 30000 IU/kg i.v.) and gentamicin (Pargenta[®] ad us. vet., Dr. E. Graeb AG, Bern, Switzerland, 6,5 mg/kg i.v.). Approximately 2 hours after arrival at the hospital, the horse collapsed and died of endotoxic circulatory shock. Fecal samples had been submitted prior to death for detection of endoparasites (quantitative flotation: negativ), clostridia (culture: negativ), salmonella (culture: negativ) and equine coronavirus (RT-qPCR: positiv). Necropsy revealed a severe, diffuse, acute, necrotizing enterocolitis with microthrombi affecting blood vessels in the lamina propria and tunica submucosa. Immunohistochemistry was performed on small intestine, large intestine, adrenal gland, heart, lung, liver and the kidney of the horse as previously published⁸ using a slightly modified protocol,¹⁰ Labelling was identified in affected parts of the large colon but not in the other organs, confirming ECoV as the causative agent of the disease.

Clinical findings and diagnostic test results in the second hospitalized case (case 7)

A 23-year-old Swiss Warmblood gelding from the same farm was referred one week after case 6 for evaluation of lethargy, anorexia and mild diarrhoea of acute onset. The horse had been treated with analgesics by the private practitioner and was referred for further diagnostics and surveillance. A CBC and blood biochemistry profile had been performed by the private practitioner the same day and revealed leucopenia with lymphopenia and neutropenia, thrombocytopenia, hyperbilirubinemia and an elevated γ -GT. Upon presentation to the hospital, the horse was bright and alert. The gelding had a heart rate of 48 beats per minute, a respiratory rate of 16 per minute and a rectal temperature of 37,5°C. Mucous membranes were moist and pink with a capillary

refill time of 2 seconds. Peristalsis was hypermotile on auscultation and the horse had mild diarrhoea. The abdominal ultrasound examination the following day showed thickened colon walls (up to 6 mm) and increased fluid accumulation in the large intestine. Supportive treatment consisted of intravenous fluid therapy at maintenance rate for two days (lactated Ringer's solution, Ringer-Lactat „Bichsel“ ohne/sans Glucose, Grosse Apotheke Dr. G. Bichsel AG, Interlaken, Switzerland and Equibiserol, Laboratorium Dr. G. Bichsel AG, Weissenaustrasse 73, 3800 Interlaken, Switzerland, 2 mL/kg/hour) and di-tri-octahedral smectite for 5 days (Bio-Sponge[®] Platinum Performance, Buellton, California, USA, 97,5 g twice daily). Fecal samples were submitted for detection of endoparasites (quantitative flotation: negativ), clostridia (culture: negativ), salmonella (culture of five consecutive samples: negativ) and equine coronavirus (RT-qPCR: positiv). The horse responded well to the treatment, did not show any diarrhea after 3 days of treatment and was discharged after 11 days of hospitalisation. Serum samples of the horse were submitted for the detection of antibodies against ECoV using the validated S1 protein-based ELISA as previously reported.²⁷ The horse had developed clinical signs on the 19th of January and serial serum samples were submitted from days 2, 4, 6 and 8 after the onset of clinical signs. All four samples were positive with optical density sample to positive control ratios (S/P value) well above the cut-off of 0,130. The S/P value increased from day 2 (0,462) to day 4 (1,622) and remained at that high level on the days 6 and 8 (1,676 and 1617) confirming seroconversion at day 4.

Diagnostic test results and biosecurity measures implemented in the home yard

In total, 25 horses were tested for ECoV by RT-qPCR at different time points. All results are displayed in Table 1. The hospitalized horses were tested first (case 6 on the 14th of January and case 7 on the 20th of January),

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Tabelle 3: Clinical and diagnostic results from the horses involved in the ECoV outbreak in Switzerland

Case	Clinical signs	Onset of clinical signs	Duration of clinical signs	Date of fecal PCR testing for ECoV	Result of fecal ECoV PCR	Outcome
1	fever respiratory signs (cough)	21.12.2020	10 days	not performed	–	euthanized
2	fever anorexia	04.01.2021	5 days	29.01.2021	negative	survived
3	fever mild anorexia	06.01.2021	2 days	29.01.2021	negative	survived
4	fever anorexia colic (caecal impaction)	11.01.2021	6 days	29.01.2021	negative	survived
5	fever anorexia	12.01.2021	3 days	29.01.2021	positive	survived
6	fever anorexia diarrhea endotoxic shock	12.01.2021	2 days	14.01.2021	positive	died
7	anorexia diarrhea	19.01.2021	3 days	20.01.2021	positive	survived
8	none	–	–	29.01.2021	positive	survived
9	none	–	–	29.01.2021	positive	survived
10–26	none	–	–	29.01.2021	negative	survived

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both while showing clinical signs. Afterwards, twenty-three horses at the farm were tested for ECoV at the end of January. Case 5 had previously shown mild clinical signs and still tested positive on the 29th of January, 18 days after the onset and 15 days after the cessation of clinical signs. Two ponies (cases 8 and 9) tested positive, but never showed any clinical signs associated with ECoV infection. The cases 2, 3 and 4 had previously shown mild clinical signs but were tested negative on the 29th of January. One horse was never tested for ECoV infection (suspected case 1).

Biosecurity measures on the farm were implemented at the end of January by the attending private practitioner in accordance with Equinella guidelines.⁶ In the present outbreak, biosecurity measures included testing of all horses on the premise and isolation of the whole barn to restrict contact with other horses for a total of 4 weeks after the last manifestation of clinical signs. Rectal temperatures were taken twice daily and the animals were closely monitored for the development of clinical signs such as anorexia, colic or diarrhoea. Horses developing clinical signs were isolated from other horses and general hygiene measures such as dedicated and single-use clothing or boots, environmental and hand disinfection were implemented. Due to financial constraints, horses were only tested once.

Discussion

This report highlights the spectrum of clinical presentations and potential outcomes associated with an outbreak of ECoV infection in Switzerland. Whole genome sequencing of the virus involved in this outbreak revealed that it was genetically closely related to ECoVs reported from the USA and Japan¹⁰, thus allowing comparison of the clinical data collected from the horses in this outbreak with data from previously published outbreaks.

The goal of this report is to raise awareness for this emerging disease among equine practitioners in Switzerland and to discuss the difficulties of establishing causality between a pathogen and a disease. The presence of clinical signs compatible with ECoV infection, the exclusion of other infectious agents and the detection of ECoV in faeces is considered adequate to diagnose the disease.¹ Nevertheless, horses can shed ECoV in their faeces without apparent clinical signs and positive RT-PCR therefore does not confirm that the virus is responsible for the disease. Establishing causality conclusively can be achieved by a post-mortem examination using immunohistochemistry or with the help of serology showing seroconversion, often not available to the private practitioner. In this report, case 6 showed

clinical signs and haematological findings (leukopenia with neutropenia and/or lymphopenia) compatible with ECoV infection. ECoV was furthermore detected via RT-PCR and by immunohistochemistry. Other pathogens were ruled out, although testing for salmonella was only performed on one sample in case 6 as the horse died before further samples could be collected. Case 7 was diagnosed on the basis of the AAEP guidelines¹ and demonstration of seroconversion, whereas in the other cases the diagnosis was based solely on clinical signs and positive faecal RT-PCR for ECoV. The most uncertain case is case 1 in which respiratory signs were predominant and haematological findings were not typical for ECoV infections. Because this was the first potential case in the outbreak, we decided to report it.

Clinical signs of ECoV-infected horses in this report ranged from subclinical to severe signs of endotoxemia and shock associated with enteritis and a fatal outcome. Nevertheless, most of the horses showed self-limiting fever and anorexia as the major clinical signs, followed by gastro-intestinal disorders such as colic and diarrhoea. Respiratory signs were only observed in one case and ECoV infection was not conclusively confirmed, as necropsy was not performed in this patient. The range of disease severity and the predominance of mild and non-specific clinical signs in these horses agrees with previous descriptions of natural^{2,3,16,20,21,24} and experimental infections with ECoV.¹⁸ The lack of enteric signs such as diarrhoea in the majority of cases might be explained by the fact that enteritis of the small intestine is the main characteristic of the infection in most patients²³, usually not causing diarrhoea in adult horses, whereas colitis seems to develop only in a subset of adult patients.^{2,15} Clinical and clinicopathological findings of the most severely affected animal in this report are similar to other reported cases of colitis^{5,26} and are consistent with¹⁵ but not specific for ECoV-associated colitis. Recent research showed that disease features were similar in horses suffering from enteric salmonellosis and equine coronavirus infection.¹⁴ Therefore the importance of ruling out salmonella as the pathogen of origin cannot be emphasized enough, especially as salmonellosis is a reportable zoonotic disease in Switzerland. In this outbreak, one horse was euthanised and one died spontaneously, consistent with the previously reported range of mortality rates of 3–27%^{2,7,24}. It is to date unclear which host, viral and environmental factors determine the outcome of ECoV infections in horses.²³ Age, breed, co-morbidities, viral strain and load may all influence the course of disease transmission and the development of clinical signs, but these observations need further investigation.^{7,9,12,13,23} Furthermore, a seasonal disease pattern has been described with more positive

tested cases during the winter months^{3,25}, again similar to this outbreak.

Diagnostic testing was based on fecal RT-qPCR testing which is commercially available, routinely performed and recommended in suspect cases and in-contact horses.²³ Further diagnostic tests included immunohistochemistry in case 6 in order to confirm ECoV as the causative agent of colitis in this patient. Serological assays were recently developed to detect antibodies against ECoV in horses.^{13,27} Serial serological tests were performed in case 7 and seroconversion was identified, confirming acute infection with ECoV. The rapid serological response in this patient might indicate that the horse had already experienced an ECoV infection earlier in his life and could be an explanation for the mild clinical signs. Further serological testing on the farm could have helped to better characterize the outbreak by retrospectively identifying affected horses that are no longer shedding virus, but samples were unfortunately not available. Furthermore, serological tests could be used in future seroprevalence studies of different geographic regions in Switzerland.

Faecal RT-qPCR testing was only performed on one occasion in this outbreak. Nevertheless, the fact that one horse tested positive for ECoV 18 days after the development of clinical signs might indicate prolonged viral shedding in this patient. This is longer than the reported typical shedding times of 3–9 days, but long-term shedding after natural infections in horses with or without clinical signs has been described.^{9,12,16} This should be taken into account when establishing a biosecurity plan, but it is presently unclear how long-term shedders should be addressed apart from isolating these horses from other susceptible animals. Official biosecurity recommendations for ECoV outbreaks are missing to date and therefore mostly include measures such as isolation of affected horses and monitoring of stable – or herdmates.²³ The recommended duration of isolation was chosen slightly longer⁶ than the previously recommended 2–3 weeks²³ because shedding of virus up to 5 weeks has been described.¹⁶

There are currently no specific treatment recommendations for horses displaying mild clinical signs associated with ECoV infections as these are often self-limiting. Horses showing severe clinical signs associated with colitis should promptly receive supportive treatment and intensive care as previously described.¹¹

Currently no licensed vaccine is available against ECoV. Two recently published studies reported that vaccines against bovine Coronavirus (BCoV) resulted in a measurable serological response in horses^{17,22} that could potentially be protective through cross-reactivity against ECoV due to the close genetic homology of the viruses. Since efficacy data is lacking, these vaccines cannot be recommended for routine use in horses to date. Strict biosecurity policies remain the most important measure to prevent viral spread.

Whenever possible, horses that die in an outbreak should undergo a pathological examination in order to confirm the diagnosis and causation between clinical signs and ECoV infection. The post mortem histopathological examination of case 6 showed similar changes to previously reported cases of fatal ECoV infections.⁸

The main limitation of this report is the fact, that horses in this outbreak were tested only once for ECoV, which might potentially have led to negative results in horses not shedding virus anymore. It would furthermore have been interesting to follow-up on positive cases and determine the duration of faecal shedding in this outbreak.

In conclusion, the infections with ECoV in Swiss horses reported here followed the pattern described in previous outbreaks of this emerging equine pathogen. Private practitioners should be aware of the wide range of clinical signs that ECoV can manifest with, in order to promptly test suspect cases and in-contact horses to appropriately implement biosecurity measures and testing strategies on affected premises. Owners of individual ECoV positive horses should be cautioned about the potential spread of the virus within a yard and onto other premises.

Acknowledgments

The authors thank the veterinarians of the ISME-Equine Hospital and the National Horse Center for their help with patient care. The authors would also like to thank the Federal Food Safety and Veterinary Office of Switzerland for supporting this work with funds from Equinella, a surveillance system of non-notifiable equine infectious diseases and clinical signs, in Switzerland. We furthermore thank Dr. Kees van Maanen for performing the serology testing in case 7 and helping with the interpretation of the results.

Funding

Part of this work (diagnostic testing) was funded by the Federal Food Safety and Veterinary Office (FSVO).

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Caractérisation d'un foyer d'infection à coronavirus équin chez des chevaux adultes en Suisse

Des foyers d'infection à coronavirus équin (ECoV) ont été décrits dans différentes parties du monde, y compris en Europe. L'objectif de ce rapport est de décrire les signes cliniques, le diagnostic et les conséquences du premier foyer d'ECoV documenté en Suisse, afin de sensibiliser le public à cette maladie et à ses différents aspects cliniques. L'épidémie s'est produite dans une écurie comptant 26 chevaux. Parmi ceux-ci, sept chevaux ont développé une forme clinique allant de signes légers tels que la fièvre et l'anorexie à des signes sévères de colite aiguë. Un cheval est mort en raison d'une endotoxémie sévère et d'un choc circulatoire secondaire à une entérite nécrosante aiguë sévère et à une colite. Sur les 26 chevaux, cinq ont été testés positifs à l'ECoV, dont deux poneys sans aucun signe clinique d'infection. Le faible nombre de cas positifs doit néanmoins être interprété avec prudence car les tests n'ont été effectués qu'à une seule occasion, plus d'un mois après l'apparition des signes cliniques chez le premier cas suspect. Ce rapport souligne l'importance des tests de diagnostic et de la mise en œuvre rapide de mesures de biosécurité dans une exploitation où un foyer d'ECoV est détecté. Il devrait en outre sensibiliser à la présence de signes cliniques peu spécifiques et bénins tels que la fièvre et l'anorexie chez les animaux atteints qui sont potentiellement capables de propager la maladie.

Mots clés: colite, diarrhée, équidé, maladie infectieuse, infection virale

Descrizione di un focolaio di infezione da coronavirus nei cavalli adulti in Svizzera

Focolai di infezioni da coronavirus (ECoV) sono stati descritti in varie parti del mondo inclusa l'Europa. Lo scopo di questo studio era di descrivere i segni clinici, il percorso diagnostico e l'esito del primo focolaio documentato di ECoV in Svizzera in modo di poter aumentare la consapevolezza sulla malattia e le sue varie presentazioni cliniche. Il focolaio è comparso in un'azienda con 26 cavalli. Fra questi, sette cavalli hanno sviluppato una malattia clinica che comprendeva sintomi lievi come febbre e l'anoressia e sintomi gravi come la colite acuta. Un cavallo è deceduto a causa di una severa endotossiemia e di uno shock circolatorio secondario a una grave enterite necrotizzante acuta e una colite. Tra i 26 cavalli, cinque sono risultati positivi all'ECoV, inclusi due pony senza alcun segno clinico. Tuttavia, il basso numero di casi positivi deve essere interpretato con molta cautela poiché i test sono stati eseguiti una sola volta e a più di un mese dall'insorgere dei primi segni clinici nel primo caso sospetto. Questo rapporto sottolinea l'importanza dei test diagnostici e dell'implementazione tempestiva di misure di biosicurezza negli allevamenti con un focolaio di ECoV. Inoltre, bisognerebbe aumentare la consapevolezza sulla presenza di segni clinici, non specifici e lievi come febbre e anoressia, negli animali affetti, potenzialmente in grado di diffondere la malattia.

Parole chiave: colite, diarrea, equidi, malattia infettiva, infezione virale

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Korrespondenzadresse

Nathalie Fouché
ISME (Institut Suisse de Médecine Equine),
Vetsuisse Fakultät, Universität Bern
Länggassstrasse 124
CH-3012 Bern
Telefon: +41 31 684 22 43
E-Mail: Nathalie.fouche@vetsuisse.unibe.ch

