

1 **Serological screening suggests single SARS-CoV-2 spillover events to cattle**

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13 **Abstract**

14 Widespread human SARS-CoV-2 infections pose a constant risk for virus transmission to
15 animals. Here, we serologically investigated 1000 cattle samples collected in late 2021 in
16 Germany. Eleven sera tested antibody-positive, indicating that cattle may be occasionally
17 infected by contact to SARS-CoV-2-positive keepers, but there is no indication of further
18 spreading.

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20 **Keywords:** SARS-CoV-2, COVID-19, animal, reservoir, cattle, ruminants, livestock, serology,
21 epidemiology

22 **Text**

23 Since its first detection at the end of 2019, the betacoronavirus SARS-CoV-2 is keeping
24 the world in suspense. This novel virus, which induces coronavirus disease 2019 (COVID-19) in
25 humans, very rapidly spread around the world, thereby causing a massive global pandemic that
26 resulted in more than five millions of deaths in less than two years of virus circulation (1). Since
27 the beginning of the pandemic, the role of livestock and wildlife species at the human-animal
28 interface was discussed. A special focus was placed on the identification of susceptible species
29 and potential intermediate or reservoir hosts. Under experimental conditions, various animal
30 species could be infected with SARS-CoV-2, among them non-human primates, felines, canines,
31 mustelids, white-tailed deer and several *Cricetidae* species, while e.g. poultry or swine are not
32 susceptible (2). For domestic ruminants such as cattle, sheep or goat a very low susceptibility
33 was demonstrated following experimental inoculation, as only a small proportion of animals
34 could be infected without animal to animal transmission (3-5). Furthermore, 26 cattle exposed in
35 the field to SARS-CoV-2 via contact to their infected keepers tested negative by RT-PCR (6).
36 However, given the very short time frame of only one to two days at which cattle test RT-PCR
37 positive after experimental infection (3,7), serological screenings could be more beneficial to
38 identify previously infected animals, in order to estimate the rate of spill-over infections in the
39 field.

40 Here, 1000 available samples of cattle kept in 83 holdings located in four German federal
41 states (Bavaria, Lower Saxony, Saxony-Anhalt and Thuringia) were analyzed. The sampling
42 dates were autumn 2021 and early winter 2021/22 when a massive wave of infections in the
43 human population driven by the Delta variant of concern (VOC) occurred. Two to 20 randomly
44 selected serum or plasma samples were analyzed per holding. Farm 31 was sampled twice, in

45 between the animal owner was quarantined. Whether this quarantine was due to contact to an
46 infected person or whether the owner himself tested SARS-CoV-2 positive is not known to the
47 authors. All bovine samples were tested by an RBD-based multispecies ELISA performed as
48 described previously (8). During the initial test validation and during an experimental SARS-
49 CoV-2 infection study in cattle, it could be shown that the ELISA does not cross-react with the
50 bovine coronavirus (BCoV) (3,8). Here, additional 100 cattle control samples randomly collected
51 across Germany in 2016 were investigated and all of them tested negative.

52 Of the animals sampled in 2021, 11 cattle from nine farms tested positive by the RBD-
53 ELISA, among them one animal kept in farm 31 and sampled after the quarantine of the owner
54 (Figure 1). All but one (farm 8) positive ELISA results could be confirmed by an indirect
55 immunofluorescence assay (iIFA) using Vero cells infected with the SARS-CoV-2 strain
56 2019_nCoV Muc-IMB-1 (multiplicity of infection of 0.1) as antigen matrix (3). The titers ranged
57 between 1/8 and 1/512, where the highest titer was measured in the seropositive animal from
58 farm 31 (Table 1). To further confirm the reactivity towards SARS-CoV-2, the 11 samples that
59 reacted positive in the RBD-ELISA were additionally tested by a surrogate virus neutralization
60 test (cPass SARS-CoV-2 Surrogate Virus Neutralization Test (sVNT) Kit, GenScript, the
61 Netherlands). This test allows for the detection of neutralizing antibodies by mimicking the
62 interaction between SARS-CoV-2 and the host cell's membrane receptor protein ACE2. It was
63 reported to be highly specific but only moderately sensitive for animal samples, since it does not
64 detect low antibody titers (9). Four cattle samples scored also positive by the sVNT (farms 11,
65 31, 47 and 74; Table 1).

66 In conclusion, our findings of a low number of individual seropositive cattle in several
67 farms demonstrate that cattle might be occasionally infected by contact to infected humans and

68 seroconvert. However, in keeping with experimental infection studies (3), intraspecies
69 transmission seems likewise not to occur in the field. Nevertheless, cattle farms should be
70 included in future monitoring programs, especially as another coronavirus, i.e. BCoV, is highly
71 prevalent in the cattle population and a BCoV infection did not prevent a SARS-CoV-2 infection
72 in a previous study (3). Furthermore, we do not know the susceptibility of animal hosts for the
73 new VOC Omicron.

74 Resulting double infections of individual animals could potentially lead to recombination
75 between both viruses, a phenomenon well-described for other coronaviruses (10). Although, the
76 emergence is highly unlikely due to the low susceptibility of cattle for SARS-CoV-2, a
77 conceivable chimera between SARS-CoV-2 and BCoV could represent an additional threat.
78 Hence, also ruminants should be included in outbreak investigations and regular screenings
79 should be performed to exclude any spread of new variants in the livestock population.

80

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83 study was supported by intramural funding of the German Federal Ministry of Food and
84 Agriculture provided to the Friedrich-Loeffler-Institut.

85 **Ethical Statement**

86 The serum samples represented superfluous material of routine diagnostic submissions
87 taken by the responsible veterinarians in the context of the health monitoring of the respective
88 cattle farm, no permissions were needed to collect these specimens.

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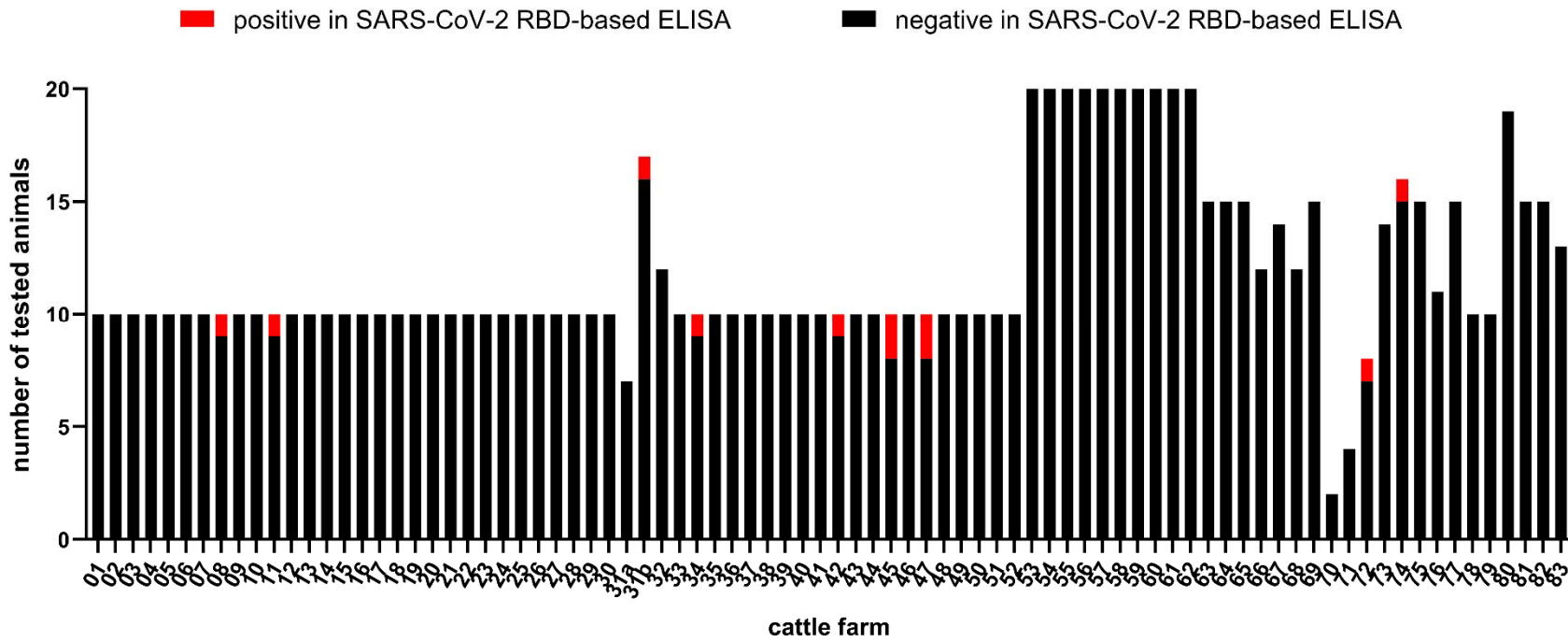
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119 **Table 1.** Detailed information about the results of samples that tested positive by a multispecies
120 SARS-CoV-2 RBD-based ELISA. iIFA = indirect immunofluorescence assay, sVNT = surrogate
121 virus neutralization test (cPass SARS-CoV-2 Surrogate Virus Neutralization Test (sVNT) Kit,
122 GenScript, the Netherlands; cut-off $\geq 30\%$ positive and $< 30\%$ negative)

Cattle farm/animal number	RBD-ELISA (corr. OD - status)	iIFA (titer - status)	sVNT (% inhibition - status)
8/1	0.35 - positive	<1/8 - negative	6.1 - negative
11/1	0.70 - positive	1/32 - positive	36.4 - positive
31/1	1.00 - positive	1/512 - positive	57.8 - positive
34/1	0.50 - positive	1/32 - positive	11.7 - negative
42/1	0.65 - positive	1/16 - positive	5.5 - negative
45/1	0.67 - positive	1/8 - positive	10.6 - negative
45/2	0.33 - positive	1/16 - positive	9.0 - negative
47/1	0.48 - positive	1/8 - positive	37.1 - positive
47/2	0.67 - positive	1/8 - positive	0.6 - negative
72/1	0.52 - positive	1/16 - positive	4.7 - negative
74/1	0.76 - positive	1/32 - positive	54.2 - positive

123 **Figure 1.** Number of cattle per farm tested for antibodies against SARS-CoV-2. Samples that reacted negative in the RBD-based
124 ELISA are depicted in black and positive samples in red. Holding 31 was sampled twice (indicated as 31a and 31b), in between the
125 animal owner was quarantined.

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