1   **Title:** Evidence of reduced viremia, pathogenicity and vector competence in a re-

2   emerging European strain of bluetongue virus serotype 8 in sheep

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4   **Running title:** Bluetongue virus serotype 8 infection in sheep

5

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16

17   **Summary:** The re-emerging Bluetongue virus serotype 8 still presents a threat to

18   naïve ruminants in Europe despite reduced virulence

19

20   **Keywords:** Bluetongue virus, infection kinetics, European serotypes, virus isolation

21   RT-qPCR, Arboviruses

22

23   **Summary**

24   The outbreak of bluetongue virus (BTV) serotype 8 (BTV-8) during 2006-2009 in

25   Europe was the most costly epidemic of the virus in recorded history. In 2015, a

26   BTV-8 strain re-emerged in France which has continued to circulate since then. To

27   examine anecdotal reports of reduced pathogenicity and transmission efficiency, we

28   investigated the infection kinetics of a 2007 UK BTV-8 strain alongside the re-

29   emerging BTV-8 strain isolated from France in 2017. Two groups of eight BTV-naïve

30   British mule sheep were inoculated with 5.75 $\log_{10}TCID_{50}$ ml^{-1} of either BTV-8 strain.

31   BTV RNA was detected by 2 dpi in both groups with peak viremia occurring between

32   5-9 dpi. A significantly greater amount of BTV RNA was detected in sheep infected

33   with the 2007 strain (6.0-8.8 $\log_{10}$ genome copies mL^{-1}) than the re-emerging BTV-8

34   strain (2.9-7.9 $\log_{10}$ genome copies mL^{-1}). All infected sheep developed BTV-specific

35   antibodies by 9 dpi. BTV was isolated from 2 dpi to 12 dpi for 2007 BTV-8-

36   inoculated sheep and from 5 to 10 dpi for sheep inoculated with the remerging BTV-

37   8. In *Culicoides sonorensis* feeding on the sheep over the period 7-12 dpi, vector

38   competence was significantly higher for the 2007 strain than the re-emerging strain.

39   Both the proportion of animals showing moderate (as opposed to mild or no) clinical

40   disease (6/8 vs 1/8) and the overall clinical scores (median 5.25 vs 3) were

41   significantly higher in sheep infected with the 2007 strain, compared to those infected

42   with the re-emerging strain. However, one sheep infected with the re-emerging strain

43   was euthanized at 16 dpi having developed severe lameness. This highlights the

44   potential of the re-emerging BTV-8 to still cause illness in naïve ruminants with

45   concurrent costs to the livestock industry.

46   **Introduction**

47   Bluetongue (BT) is an infectious hemorrhagic disease of ruminants caused by

48   bluetongue virus (BTV) which is transmitted via *Culicoides* biting midges (Carpenter

49   et al., 2013). BTV is the type species of the genus *Orbivirus* within the family

50   *Reoviridae* and is a serologically and genetically diverse virus. The BTV genome is

51   comprised of 10 double-stranded RNA segments encoding several structural and non-

52   structural proteins. BTV segment-2 encodes the most variable BTV protein (VP2)

53   which contains the majority of epitopes that interact with neutralising antibodies

54   (Maclachlan et al., 2014). Since 1998, a number of BTV serotypes have caused both

55   sporadic and widespread incursions into the EU (Belbis et al., 2017).

56   In August 2006, a BTV serotype 8 (BTV-8) strain of sub-Saharan origin

57   (Maan et al., 2008) was detected within animal holdings in The Netherlands, the first

58   time that the virus had been identified in northern Europe. This BTV-8 strain

59   successfully re-emerged in 2007 and subsequently spread throughout most northern

60   European countries, causing widespread clinical disease and major economic damage

61   to the farming sector (Wilson and Mellor, 2009). However, in conjunction with

62   animal movement restrictions, the implementation of vaccination campaigns by EU

63   member states was effective in reducing and eventually preventing further

64   transmission of BTV-8 and only sporadic outbreaks of disease occurred in the EU by

65   2009.

66   In August 2015, BTV-8 re-emerged in France and subsequently spread

67   throughout the entire country (Sailleau et al., 2017). How BTV-8 persisted in areas

68   that were thought to have been free of virus transmission remains unknown, although

69   it has been recently proposed that low-level circulation of BTV-8 occurred in France

70   prior to the detection in 2015 (Courtejoie et al., 2018). Importantly however,

3

71   anecdotal observation suggested that changes had occurred in the epidemiology of the

72   re-emerging strain. Whereas the 2007-2009 BTV-8 strain caused widespread clinical

73   signs in cattle and sheep (Elbers et al., 2009, Zanella et al., 2013), the re-emerging

74   BTV-8 strain has thus far only caused mild clinical illness (Sailleau et al., 2017). In

75   addition, the rate of spread of the virus in France appeared slower than the original

76   BTV-8 strain. At the time of the BTV-8 re-emergence in 2015, it has been estimated

77   that the ruminant herd immunity in France was 18% (Bournez et al., 2018).

78   Genetically, the re-emerging BTV-8 strain (GenBank accession numbers:

79   KP56990- KP56999) differs from a 2007 UK BTV-8 strain (accession numbers:

80   KP820957, KP821077, KP821199, KP821319, KP821439, KP7821559, KP821681,

81   KP821801, KP821921 and KP822042) in just 11 amino acids occurring in segments 1

82   (3), 2 (1), 3 (1), 4 (1), 8 (1), 9 (3) and 10 (1). The high degree of amino acid similarity

83   between the 2007 and 2015 strains underpins the hypothesis that this is not a new

84   introduction of BTV-8 (Sailleau et al., 2017).

85   In this study we directly compare the infection of sheep with the original and

86   re-emerging European BTV-8 strains by assessing viremia, clinical signs, antibody

87   production and transmission to *Culicoides* biting midges. By using British mule

88   sheep, representative of the UK flock from a region not originally exposed to BTV-8

89   infection, the aim is to understand if the changes in epidemiology and pathogenicity

90   observed in France will also occur in regions beyond those affected by outbreaks in

91   2006-2009. The findings from this study will inform response in both the UK and

92   other countries previously unaffected by BTV-8 and allow for evidence-based

93   surveillance and control measures to be implemented.

94   **Materials and methods**

95   **Ethical statement for animal studies**

96   All animal experiments were carried out in accordance with the UK Animal

97   Scientific Procedure Act (ASPA) 1986 which transposes European Directive

98   2010/63/EU into UK national law. The animal studies were approved by the UK

99   Home Office in granting Project licence 70/7819 under the Animal Scientific

100   Procedure Act and all protocols underwent appropriate local ethical review

101   procedures by the Animal Welfare and Ethical Review Board (AWERB) of The

102   Pirbright Institute.

103

104   **Preparation of inocula**

105   A 2017 French BTV-8 strain (FRA2017) which had been isolated on *Culicoides*

106   *sonorensis* (KC) cells through a single passage was provided by the French BTV NRL

107   (Animal Health Laboratory, ANSES, Maisons-Alfort). BTV-8 isolate UKG2007/03

108   (UKG2007), isolated through a single passage from the 2007 UK index case was

109   obtained from the Orbivirus Reference Collection, (Pirbright Institute, UK). Both the

110   UKG2007 and FRA2017 strains were re-passaged once on KC cells. Following

111   harvest, viral inocula were titrated on KC cells using a 96-well titration assay in

112   combination with immunofluorescence microscopy. Briefly, 10-fold serial dilutions of

113   the virus were added to KC cells immediately following addition of the cells to 96

114   well tissue culture plates. After 5 days incubation at 26°C, the cells were fixed using

115   4% paraformaldehyde for 45 min and following multiple washes with PBS were

116   permeabilized with 0.2% Triton X-100 for 20 min following. Viral proteins were

117   labelled using an anti-BTV polyclonal guinea-pig sera (ORAB279) and anti-guinea-

118   pig-Alexa488 and immunofluorescence was visualized on a Nikon Eclipse TE300

5

119   microscope to calculate viral titers according to Spearman-Kärber. Subsequently, the

120   inocula were adjusted using Schneiders media to yield a final titer of 5.75 $\log_{10}$

121    $TCID_{50}$/mL.

122

123   **Study design**

124   Eighteen adult female British mule sheep, representing the most common British

125   mixed breed of > 7 years of age were chosen for use in this study. All sheep were

126   previously tested as BTV-antibody negative by C-ELISA. The sheep were randomly

127   assigned (see supplementary information) into two groups of 9 individuals. The two

128   groups were housed in separate rooms of the Pirbright Institute's high containment

129   animal facility. Eight sheep per group were inoculated with 1 mL subcutaneously in

130   the left neck and 0.5 mL intradermally distributed over 5 inoculation sites into the

131   inner left thigh of either UKG2007 or FRA2017. One sheep in each respective group

132   was kept uninfected as a contact transmission control (sheep 2 and sheep 18). Blood

133   samples (EDTA and whole blood) were taken from the jugular vein at -1, 2, 3, 5, 6, 7,

134   8, 9, 10, 12, 14, 16, 19, and 20/21 dpi. Sheep body temperatures were recorded daily

135   and clinical scoring was performed throughout as described previously (Darpel et al.,

136   2007).

137

138   **Vector competence**

139   *Culicoides sonorensis* from a colony maintained at The Pirbright Institute (PIRB-s-3

140   strain) were fed on two sheep from each group at 6 dpi (UKG2007) or 7 dpi

141   (FRA2017) to coincide with determined peak viremia as described previously (Baylis

142   et al., 2008). Engorged individuals were incubated at 25°C for 8 days and surviving

143   individuals were homogenized in GMEM using a tissue lyser (Qiagen) and made up

144   to a final volume of 1 mL per sample (Veronesi et al., 2013). *Culicoides* samples were

145   analyzed in pools of eight individuals for BTV genome detection by RT-qPCR as

146   described below. Pools that reported a cycle threshold (C_T value) less than that of the

147   original blood meal in midges harvested on the day of feeding were considered to

148   contain one or more individuals competent for BTV transmission.

149

150   **Molecular analyses**

151   **Preparation of plasmid for quantitation in real-time RT-PCR assay**

152   Plasmid pGEM-3Zf(+) carrying a 97 bp sequence from BTV segment 10 (derived

153   from the assay described by (Hofmann et al., 2008)) was transformed into JM109

154   competent cells (Promega), purified and then quantified using a nanodrop to create

155   working stocks of 10^6 BTV genome copies/µL.

156

157   **RNA extraction and RT-qPCR analysis**

158   BTV RNA was extracted from 100 µL of EDTA blood and eluted into 80 µl buffer

159   using the KingFisher Flex automated extraction platform and the MagVet Universal

160   nucleic acid extraction kit (ThermoFisher Scientific, Paisley, UK). Ten microliters of

161   sample RNA was analyzed as per the assay described by (Hofmann et al., 2008) with

162   modifications (Flannery et al., 2018) using the Express One-Step qRT-PCR kit

163   (ThermoFisher) on an Applied Biosystems 7500 Fast instrument (ThermoFisher). A

164   log-dilution series of the plasmid (1×10^0 to 1×10^6 copies per µL) was included in

165   triplicate on each RT-qPCR run. BTV RNA copies were determined by comparing

166   sample C_T values to the standard curves and expressed as BTV genome copies/mL.

167

168   **Serological analyses**

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169   Whole blood samples were centrifuged at $3000 \times g$ for 5 min and serum was decanted

170   and stored at +4°C until analysis. BTV antibodies were detected using the ID Screen®

171   Bluetongue Competition kit (ID Vet, Grabels, France) in accordance with the

172   manufacturer's instructions. The serum neutralisation test (SNT) against BTV-8 was

173   performed as described previously (Batten et al., 2012).

174   **Virus isolation**

175   EDTA blood cells were washed 3× with PBS and sonicated as described in the World

176   Organisation for Animal Health (OIE) manual (Savini, 2014). KC cells were

177   inoculated with 100 µl of washed blood and incubated at 26°C in Schneiders media

178   (1% Amphotericin B, 1% Penicillin/Streptomycin and 10% FBS). Media was

179   replenished 24 hours post infection and cells were incubated for a further 6 days.

180   Following the 7-day incubation, cells were harvested, centrifuged at $3000 \times g$ for 5

181   min and the supernatant tested for BTV using RT-qPCR as described above. Virus

182   was considered to have been isolated if the BTV C_T value of the harvested material

183   was 3-C_T values less than that of the original EDTA-blood inoculum.

184

185   **Statistical analysis.**

186   Body temperatures and clinical scores were analyzed by comparing the maximum

187   values and the dpi at which they occurred for each strain using Wilcoxon rank-sum

188   (also known as Mann-Whitney U) tests. The proportion of sheep showing moderate

189   (as opposed to mild or no) clinical disease in each group was compared using a Fisher

190   exact test. Viremia for sheep infected with each strain was analyzed by comparing the

191   maximum titer, the dpi at which it occurred and the area under the curve (a measure

192   of total virus production; computed using the trapezium rule) using Wilcoxon rank-

193   sum tests. Non-parametric tests were preferred because of the small group sizes and

194   potential non-normality of the data.

195   The vector competence for each BTV-8 strain was calculated from the number

196   of positive pools and the number of pools tested using a binomial likelihood and

197   noting that the probability of a positive pool is equal to $1-(1-b)^n$, where b is the vector

198   competence (i.e. the probability of an individual midge becoming infected) and n is

199   the number of midges in the pool. A second model was also considered in which

200   vector competence was independent of strain, but depended on the viral titer ($\log_{10}$

201   genome copies/mL) in the sheep on which each pool of midges fed, so that

202    $b=1/(1+\exp(-a_0-a_1\times\text{titer}))$.

203

9

204 **Results**

205 **Clinical observations**

206 Typical clinical signs of BTV infection (fever, depression, facial edema, reddening of

207 the mucosal membrane and coronary bands) were seen more frequently and were

208 more pronounced in the UKG2007-inoculated sheep compared to the FRA2017-

209 inoculated sheep (figure 1). Overall, 6/8 UKG2007-inoculated sheep were classed as

210 moderately ill, while two sheep were classed as mildly ill. By contrast, only one sheep

211 was classed as moderately affected in the FRA2017-inoculated sheep, with the

212 remaining 7/8 being classed as mildly affected. The proportion of sheep showing

213 moderate (as opposed to mild) clinical disease was significantly ($p = 0.04$) higher in

214 sheep infected with UKG2007 compared with those infected with FRA2017. In

215 addition, the maximum clinical score was significantly ($p = 0.014$) higher in sheep

216 infected with UKG2007 (median score 5.25, range 2-6) compared with those infected

217 with FRA2017 (median score 3, range 1.5-3.5), but the time at which the maximum

218 score occurred did not differ significantly ($p = 1.0$) between the two groups of BTV-8

219 inoculated sheep.

220 Five of eight UKG2007-inoculated sheep showed pyrexia (>40.5°C), in

221 contrast with 2/8 FRA2017-inoculated sheep (figure 1). However, the difference

222 between the groups was not statistically significant for either maximum temperature

223 ($p = 0.49$) or the dpi at which this occurred ($p = 0.76$).

224 Two sheep were euthanized on welfare grounds during the study, one in each

225 group. The sheep from the UKG2007-inoculated group was euthanized at 9 dpi for

226 reaching the humane endpoint (at the end of the moderate clinical spectrum) of the

227 moderate study protocol. In this sheep, typical hemorrhagic lesions on mucosal

228 membranes, coronary band, lymph nodes, pulmonary artery as well as the epithelium

10

229   of the reticulum and omasum were observed during necropsy. The sheep from the

230   FRA2017-inoculated group developed acute lameness on all four feet late in infection

231   and was euthanized on humane grounds at 17 dpi.

232   The two transmission control sheep remained clinically unaffected other than

233   exhibiting a mild nasal discharge over a two-day period during the experiment.

234

235   **Molecular analyses**

236   Figure 2 shows the BTV concentrations in EDTA blood over the study period. In 5

237   UKG2007-inoculated sheep and in 3 FRA2017-inoculated sheep BTV RNA was

238   detected by RT-qPCR at 2 dpi. BTV RNA was detected in all other inoculated sheep

239   at 5 dpi with the exception of sheep 17 (that had been inoculated with FRA2017).

240   Peak viremia occurred earlier for UKG2007-inoculated sheep (between 5 and 6 dpi)

241   than for FRA2017-inoculated sheep (5-9 dpi), but this difference was not statistically

242   significant ($p = 0.11$). At peak viremia, BTV concentrations in blood ($\log_{10}$ genome

243   copies/mL) were significantly ($p = 0.021$) higher for UKG2007-inoculated sheep

244   (median 7.0; range: 5.5-8.3) compared with FRA2017-inoculated sheep (median 5.4;

245   range: 1.6-7.3). Furthermore, the area under the curve (a measure of total virus

246   production) was significantly ($p = 0.028$) greater for UKG2007-inoculated sheep

247   (median 7.5; range: 6.0-8.8) compared with FRA2017-inoculated sheep (median 5.9;

248   range: 2.9-7.9).

249   At 21 dpi in the remaining animals, 3 sheep inoculated with the FRA2017

250   strain had cleared the infection. BTV remained detectable in all 7 sheep and in 3

251   sheep inoculated with UKG2007 and FRA2017 strains, respectively. At the end of the

252   experiment (21 dpi), mean BTV concentrations were 5.26 (range: 4.08-5.70) and 3.87

253   (range: 2.63 and 4.97) $\log_{10}$ genome copies/mL for UKG2007 and FRA2017-

254   inoculated sheep, respectively. BTV RNA was not detected in the transmission

255   controls or in sheep 17 throughout the experiment.

256

257   **Vector competence**

258   A total of 272 individual *C. sonorensis* fed on sheep 6 and sheep 9 that were infected

259   with the UKG2007 strain survived incubation and this equated to 34 pools of midges

260   for testing. A total of 280 individuals fed on sheep 11 and sheep 12 that were infected

261   with FRA2017 were tested in 36 pools. Three midge pools from the UKG2007-

262   inoculated sheep had lower C_T values than the mean value obtained in *C. sonorensis*

263   tested on the day of feeding (C_T 30.9). *C. sonorensis* tested on the day of feeding on

264   FRA2017-infected sheep had mean C_T value of 34.7. BTV RNA was not detected in

265   any midge pools from the FRA2017-inoculated sheep.

266   Based on these results for pooled midges, the estimated vector competence

267   (i.e. probability of an individual midge becoming infected) was 1.2% (95%

268   confidence interval (CI): 0.3% to 3.0%) for UKG2007 and 0% (95% CI: 0% to 0.7%)

269   for FRA2017, which are significantly different. Viral titers ($\log_{10}$ genome copies/mL)

270   in the sheep infected with UKG2007 were higher on the day of *Culicoides* feeding

271   than for those infected with FRA2017 (8.3 and 8.0 compared with 7.3 and 6.6,

272   respectively). An increase in competence was associated with an increase in titer, but

273   this was not statistically significant (estimate for coefficient, a_1: 2.6; 95% CI: -0.1 to

274   3.6).

275

276   **Serological analyses**

277   Figure 3 shows the BTV antibody response using C-ELISA. In the sheep inoculated

278   with UKG2007, five of the eight seroconverted by 7 dpi, and by 8 dpi all inoculated

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279   sheep had seroconverted (defined as <40% inhibition by the manufacturer). In the

280   group inoculated with FRA2017, none of the sheep seroconverted at 7 dpi yet all had

281   seroconverted by 9 dpi. Based on the ELISA results, SNTs were performed on serum

282   samples obtained at 7, 9, 12, 14 and 21 dpi. Neutralizing antibodies were first detected

283   at 7 and 9 dpi for UKG2007 and FRA2017-inoculated sheep, respectively. By 14 dpi,

284   all sheep had neutralizing antibodies with titers (log_{10}) between 1.18 and 2.68, rising

285   to between 1.60 and 2.81 at 21 dpi. The mean antibody titers at 21 dpi were 2.01 and

286   1.54 for the UKG2007 and FRA2017-inoculated sheep, respectively. The

287   transmission control sheep in both groups did not seroconvert.

288

289   **Virus isolation**

290   Virus isolation from EDTA blood was performed for all inoculated sheep at 2, 5, 7,

291   10, 12, 14, 16 and 21 dpi. For UKG2007-inoculated sheep, virus was isolated at 2 dpi

292   (3/8 sheep), 5 dpi (7/8 sheep), 7-10 dpi (8/8 sheep) until 12 dpi (1 sheep). For

293   FRA2017-inoculated sheep, virus was isolated at 5-7 dpi (6/8 sheep) until 10 dpi (4/8

294   sheep).

13

295   **Discussion**

296   Constant and largely unpredictable outbreaks of BTV present a significant global

297   challenge in terms of proportionate response. This is exacerbated by our inability to

298   accurately define the pathogenicity of emerging and re-emerging BTV strains, which

299   is the main determining factor in vaccine production by industry and subsequent

300   uptake by farmers. The aim of this study was to compare infection kinetics and

301   clinical severity of a re-emerging BTV-8 strain in sheep to understand the impact of

302   this strain in areas not affected by BTV-8 during 2006-2009. The study confirms

303   anecdotal evidence from the field that viremia and clinical signs are reduced in the re-

304   emerging strain. However, this strain may significantly impact upon naïve sheep

305   populations as evidenced by one sheep developing acute lameness during the

306   convalescence phase of infection.

307   British mule sheep were used in this study as representative of the UK flock

308   and seem slightly more resistant to the UKG2007 strain than pure-bred Dorset Poll

309   sheep when infected with the UK BTV-8 strain (Moulin et al., 2012). Indeed, one

310   sheep inoculated with the 2007 strain had almost cleared the infection by the end of

311   the experiment (21 dpi). This variability in the severity of BTV infection has been

312   attributed to numerous host factors, such as the breed, health and age of the ruminant

313   (Maclachlan et al., 2009, Caporale et al., 2014). It has been previously reported that in

314   field settings, environmental factors, such as exposure to sunlight, elevated

315   temperatures, stress and bacterial or viral co-infections can exacerbate BT clinical

316   signs in sheep (Kyriakis et al., 2015). Therefore, the clinical presentation of infection

317   with the BTV-8 strains in the field is likely to be more severe than what we have

318   reported in this study where animals were housed within the high-sanitary and

319   controlled-husbandry conditions of the containment facilities. This highlights that

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320   infection with the re-emerging BTV-8 strain may still have a considerable economic

321   impact in the field.

322   Interestingly, one of the sheep inoculated with the re-emerging BTV-8 strain

323   did not develop detectable viremia throughout the entire study, yet seroconverted and

324   developed a neutralizing antibody response. This suggests that the sheep did receive

325   the inoculum. Historically studies have previously reported that ruminants

326   experimentally-inoculated with BTV did not develop detectable viremia (in the

327   presence or absence of seroconversion), although viremia detection was based on

328   virus isolation rather than the more sensitive use of real-time PCR (Parsonson et al.,

329   1987, Flanagan et al., 1982, Baylis et al., 2008). Other studies have further noted

330   seroconversion of individual BTV-inoculated sheep or cattle either in the presence of

331   very low detectable RNA levels by RT-qPCR (van der Sluijs et al., 2013) or in the

332   absence of detectable viremia, however, the latter was associated with either a low-

333   titer (Baylis et al., 2008, Di Gialleonardo et al., 2011) or a high-passage inoculum

334   (Janowicz et al., 2015). Interestingly, a recent study also highlighted that a highly-

335   passaged BTV-8 strain infected and was detectable in the skin and draining lymph

336   nodes of inoculated sheep however did not result in detectable RNA levels in the

337   systemic blood (Melzi et al., 2016). Overall all these studies, including the data

338   presented here, demonstrate that manifestation of clinical disease and viremia varies

339   in individual animals even when infected by the same route and with the same dosage

340   of virus, highlighting differences in individual susceptibility to BTV infection.

341   Alternatively, it is possible that this sheep had residual immunological memory from

342   previous vaccination which could provide some protection from infection even in the

343   absence of detectable antibodies. BTV antibodies can be detected in sheep up to 2.5

344   years following vaccination (Batten et al., 2013) and it has been estimated that they

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345   can persist for 5-6 years following infection or vaccination (Bournez et al., 2018). The

346   sheep used in our study were at an advanced age (>7 years old), as reported by the

347   farmer and by assessing the general condition of the sheep.

348   Significantly greater RNA copy numbers were detected in sheep inoculated

349   with the 2007 strain than the re-emerging strain. In addition, BTV was isolated more

350   frequently from sheep inoculated with the 2007 strain. By the end of the experiment, 3

351   sheep inoculated with the re-emerging strain had also cleared infection (BTV RNA

352   was not detected). This reduced viremia was reflected in the quantity of BTV RNA

353   imbibed by *C. sonorensis* implying that monitoring through the venous route was

354   representative of the quantity of viral RNA in the skin. Furthermore, *C. sonorensis*

355   demonstrated a higher vector competence (1.2%) towards the 2007 strain than the re-

356   emerging strain. The lower vector competence observed using FRA2017 is possibly

357   related to lower viral titers in the blood of sheep infected with this strain, though the

358   small number of sheep used for the vector infection studies preclude drawing robust

359   conclusions about the relationship between competence and viremia. Overall, these

360   results suggest that the re-emerging strain elicits a lower viremia, for a shorter

361   duration in sheep and is less efficient at infecting *Culicoide*s biting midges. These

362   findings are consistent with the relatively slow spread of the re-emerged BTV-8 strain

363   which has remained largely within the French borders since circulation was detected

364   in August 2015.

365   The infection kinetics of the two BTV-8 strains were similar to each other and

366   were in-line with those reported for other BTV serotypes in sheep (Schulz et al., 2018,

367   van der Sluijs et al., 2013). However, the re-emerging strain was found to be less

368   virulent than the 2007 strain. It has been postulated that the re-emerging strain has

369   been circulating asymptomatically in wildlife since the end of the 2006-2009 BTV-8

370   epizootic (Sailleau et al., 2017). The reason behind the attenuation of BTV strains

371   over a period of time has been attributed to selective pressures placed on circulating

372   BTV serotypes during replication within their mammalian hosts and vectors (Caporale

373   et al., 2014). The respective lineages of the two BTV-8 strains used in this study differ

374   by a few amino acids, thus, the impact of future genomic mutations in the re-emerging

375   BTV-8 strain is uncertain. Nonetheless, detailed genomic comparison between

376   different BTV strains used in experimental infection studies may increase our overall

377   understanding of the molecular determinants of BTV virulence.

378   We have performed this experimental infection study in sheep; therefore, the

379   impact of the re-emerged BTV-8 strain in cattle is yet to be determined

380   experimentally. Previous experimental infection studies of other European BTV-8

381   strains in cattle did not result in overt disease or caused mild clinical signs which were

382   in contrast with the clinical illness reported in sheep (Darpel et al., 2007, Martinelle et

383   al., 2011, Di Gialleonardo et al., 2011). An experimental comparison of BTV-8 strain-

384   specific virulence (similar to our study here) is likely to be more challenging in cattle.

385   Considering that morbidity rates in field conditions are likely to be higher than

386   what we have reported here and coupled with animal movement restrictions, it is

387   probable that future incursions of the re-emerged BTV-8 will have an economic

388   impact on the livestock industry. Therefore, appropriate surveillance and vaccination

389   strategies should be considered in advance of the likely incursion of this re-emerged

390   BTV-8 strain.

391

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400   **Conflict of Interest Statement**

401   The authors have no conflicts of interest to declare.

402

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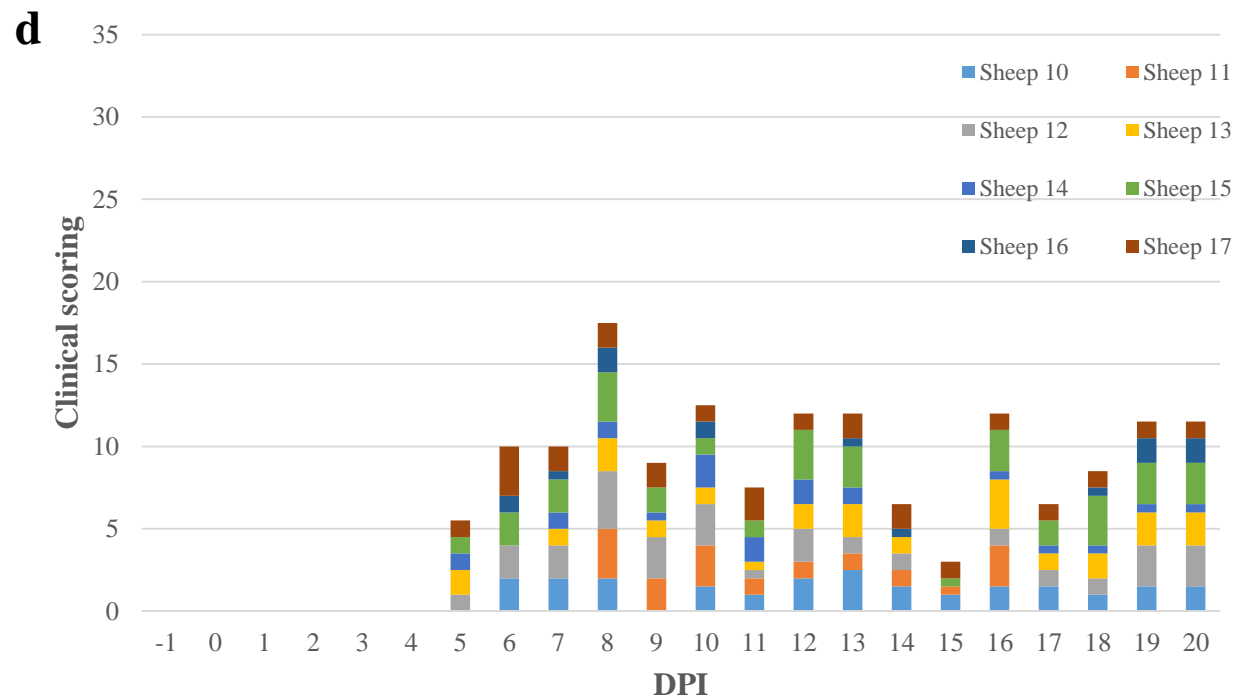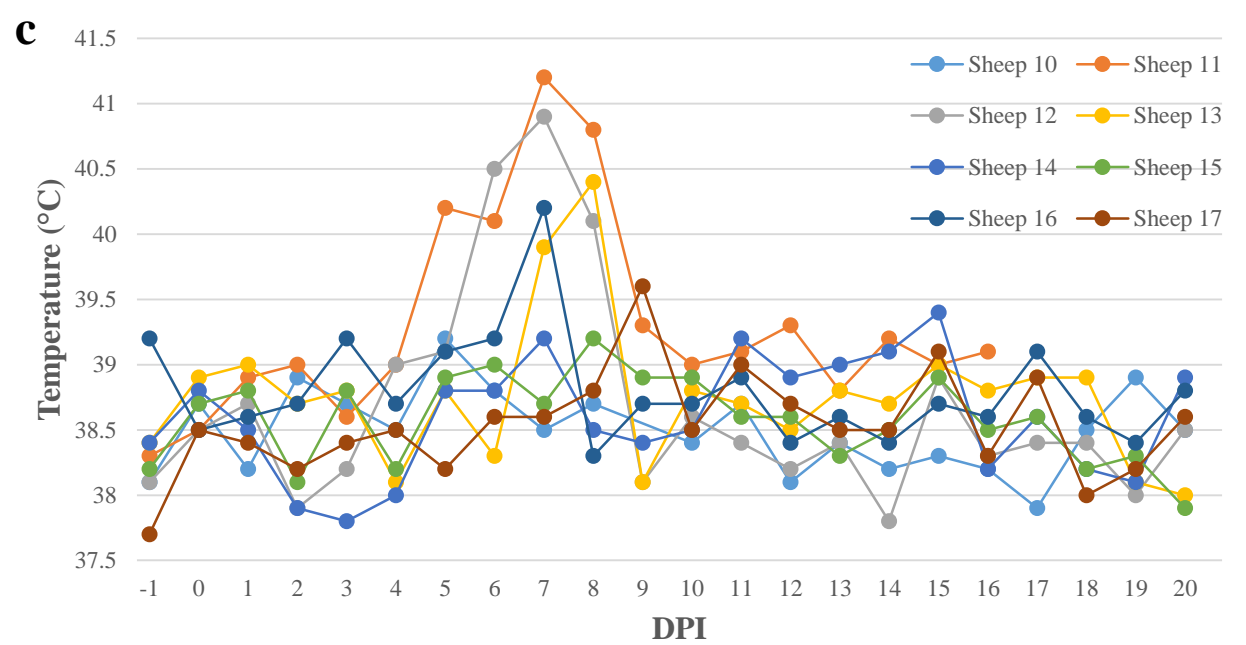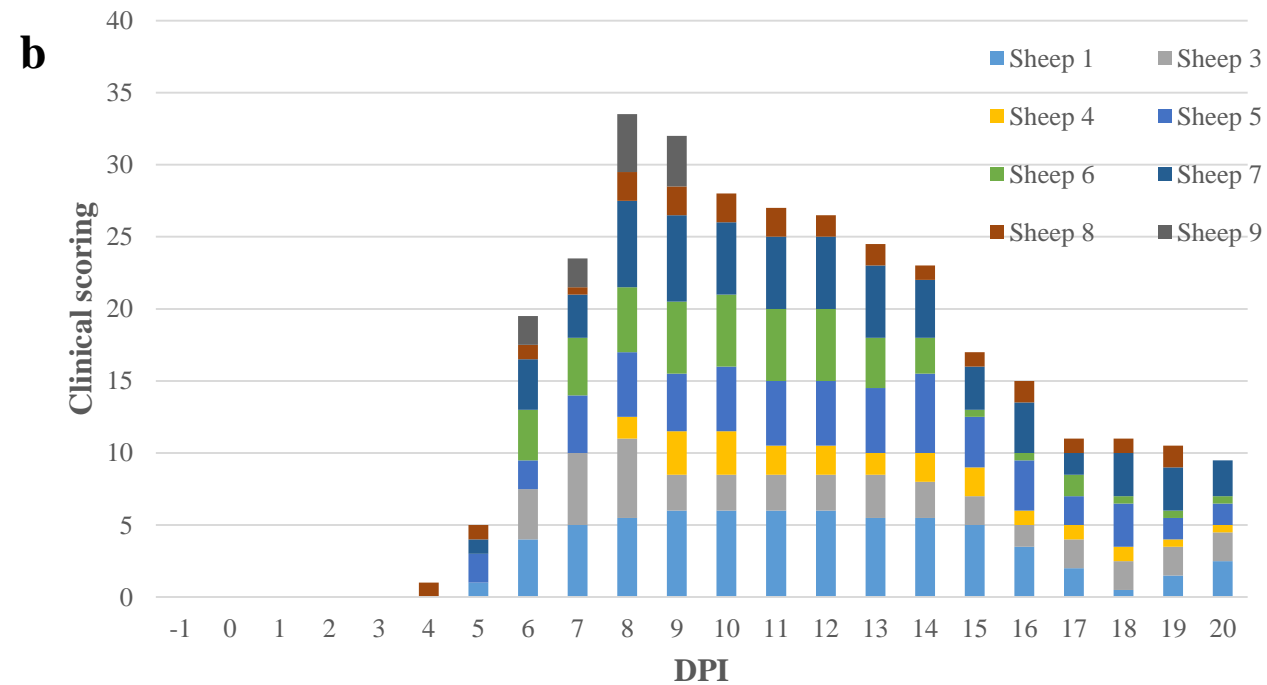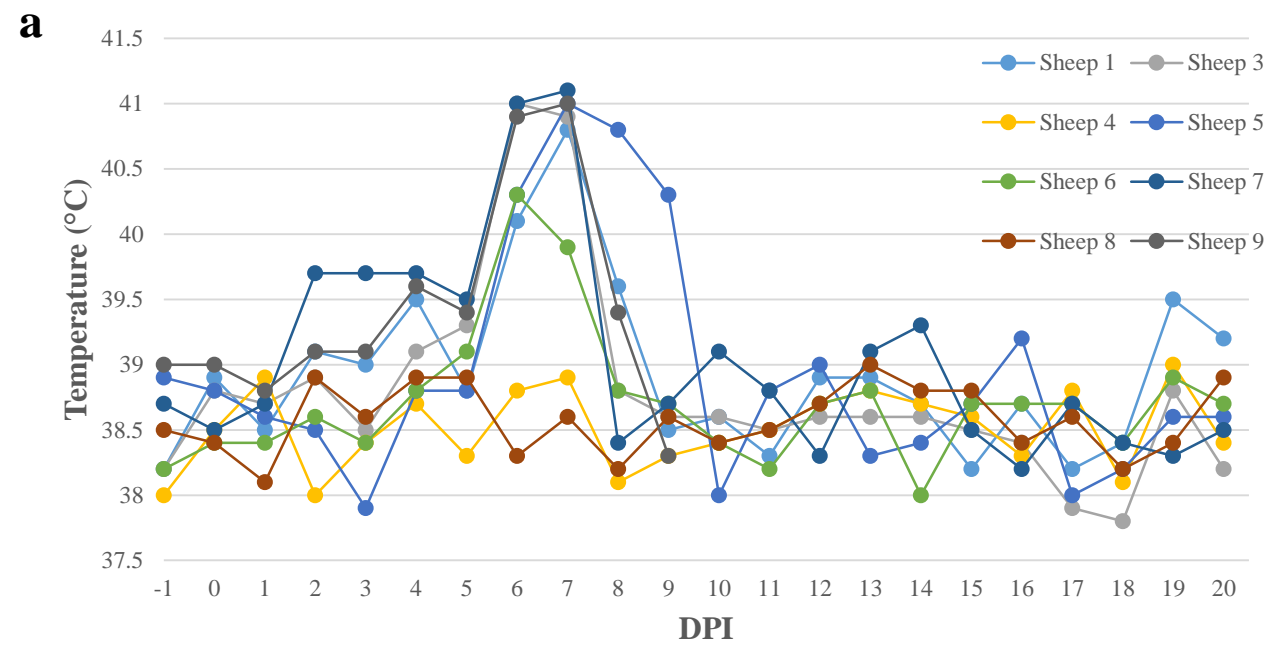
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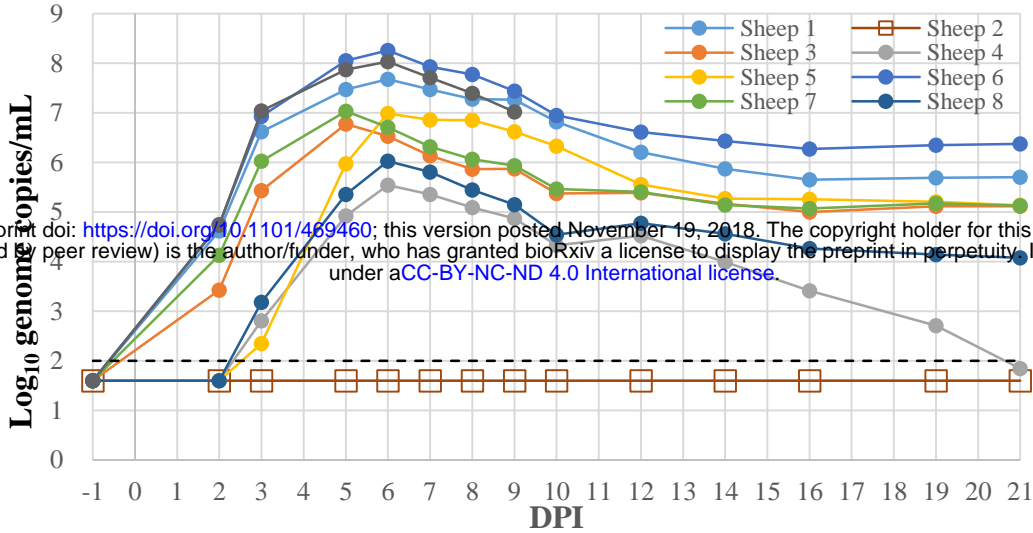502   *Emerg. Dis.,* 60, 231-237.

503

504   **Figure 1** Daily temperatures of sheep inoculated with UKG2007 (a) and FRA2017 (c), and

505   combined clinical scores of sheep inoculated with UKG2007 (b) and FRA2017 (d).

506

507   **Figure 2** BTV genome concentrations in sheep inoculated with UKG2007 (a) and FRA2017

508   (b) and mean genome concentrations for both groups (c). Sheep 2 and Sheep 18 are the
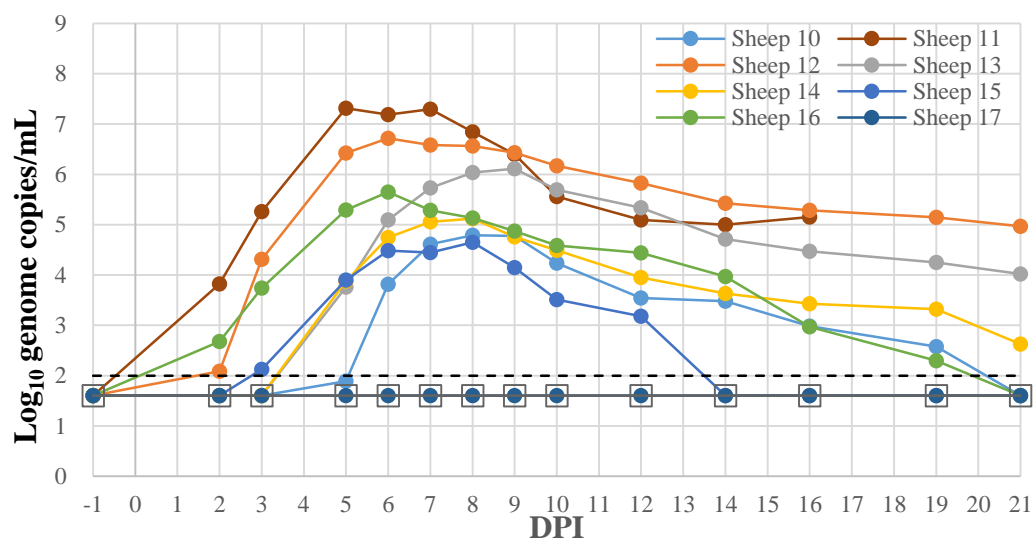
509   control animals.

510

511   **Figure 3** Antibody response of sheep inoculated with UKG2007 (a) and FRA2017 (b) as

512   determined by C-ELISA. The dashed line represents the positive/negative cut off of the

513   ELISA whereby values <40% inhibition are considered positive. Sheep 2 and Sheep 18 are

514   the control animals.

515

a

b

c

a

Sheep 1   Sheep 2   Sheep 3
Sheep 4   Sheep 5   Sheep 6
Sheep 7   Sheep 8   Sheep 9

b

Sheep 10   Sheep 11   Sheep 12
Sheep 13   Sheep 14   Sheep 15
Sheep 16   Sheep 17   Sheep 18