Experimental infection of European red deer (*Cervus elaphus*) with bluetongue virus serotypes 1 and 8

Jorge Ramón López-Olvera[1]*, Caterina Falconi[2,5], Paloma Férnandez-Pacheco[3], Jovita Fernández-Pinero[3], Miguel Ángel Sánchez[3], Agustín Palma[3], Irene Herruzo[3], Joaquín Vicente[2], Miguel Ángel Jiménez-Clavero[3], Marisa Arias[3], José Manuel Sánchez-Vizcaíno[4], Christian Gortázar[2]

1 Servei d'Ecopatologia de Fauna Salvatge (SEFaS), Universitat Autònoma de Barcelona (UAB), Bellaterra, Barcelona, Spain

2 Instituto de Investigación en Recursos Cinegéticos (IREC; CSIC-UCLM-JCCM), Ciudad Real, Spain

3 Centro de Investigación en Sanidad Animal (CISA-INIA), Valdeolmos, Madrid, Spain

4 Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense de Madrid (UCM), Madrid, Spain

5 Via E. De Magistris 9, 09123, Cagliari, Italy

* Corresponding author:
Servei d'Ecopatologia de Fauna Salvatge (SEFaS), Universitat Autònoma de Barcelona (UAB), Bellaterra, E-08193, Barcelona, Spain

E-mail address: Jordi.Lopez.Olvera@uab.es

Telephone: +34935868190 Fax: +34935812006

Keywords: Bluetongue; red deer; BTV RNA; antibodies; wildlife; reservoir

Abstract

Bluetongue (BT) is a climate change-related emerging infectious disease in Europe. Outbreaks of serotypes 1, 2, 4, 6, 8, 9, 11, and 16 are challenging Central and Western Europe since 1998. Measures to control or eradicate bluetongue virus (BTV) from Europe have been implemented, including movement restrictions and vaccination of domestic BTV-susceptible ruminants. However, these measures are difficult to apply in wild free-ranging hosts of the virus, like red deer (*Cervus elaphus*), which could play a role in the still unclear epidemiology of BT in Europe. We show for the first time that BTV RNA can be detected in European red deer blood for long periods, comparable to those of domestic ruminants, after experimental infection with BTV-1 and BTV-8. BTV RNA was detected in experimentally-infected red deer blood up to the end of the study (98-112 dpi). BTV-specific antibodies were found in serum both by enzyme-linked immunosorbent assay (ELISA) and virus neutralization (VNT) from 8-12 dpi to the end of the study, peaking at 17-28 dpi. Our results indicate that red deer can be infected with BTV and maintain BTV RNA for long periods, remaining essentially asymptomatic. Thus, unvaccinated red deer populations have the potential to be a BT reservoir in Europe, and could threaten the success of the European BTV control strategy. Therefore, wild and farmed red deer should be taken into account for BTV surveillance, and movement restrictions and vaccination schemes applied to domestic animals should be adapted to include farmed or translocated red deer.

1. Introduction

Formerly considered an exotic viral disease of wild and domestic ruminants, several outbreaks of bluetongue (BT) virus serotypes 1, 2, 4, 6, 8, 9, 11, and 16 have challenged Europe since 1998. BT is an emerging infectious disease related to climate change (Purse et al., 2005; Breard et al., 2007; Enserink, 2008; Rodríguez-Sánchez et al., 2008; Eschbaumer et al., 2009; European Commission, 2009).

Measures to control bluetongue virus (BTV) in Europe include movement restrictions and vaccination (European Commission, 2009), and surveillance systems for BT are being established (Hadorn et al., 2009). As of May 2009, the largest restriction zones in Europe correspond to BTV serotypes 1 (BTV-1), which is expanding northwards since its first introduction in Southern Spain in 2007, and 8 (BTV-8), spreading throughout Europe since it appeared in The Netherlands in 2006 (Purse et al., 2005; Rodríguez-Sánchez et al., 2008). The target of the vaccination campaign is to achieve at least 80% coverage of domestic ruminants using killed vaccines, although doubts have arisen about its effectiveness (Enserink, 2008; Rodríguez-Sánchez et al., 2008).

Red deer (*Cervus elaphus*) population density in Europe ranges from 2 to 30 individuals per square kilometre (up to 70 for food supplemented populations) (Acevedo et al., 2008; Lovari et al., 2009), which could account for a significant percentage of the BTV-infection susceptible ruminant population in certain regions.

Wild ruminants are included in the European Council Directive 2000/75/EC of 20 November 2000, laying down specific provisions for the control and eradication of bluetongue, but vaccination and movement restrictions can only be applied in farmed or managed ruminants, being almost impossible in wild free-ranging hosts of the virus.

High prevalence of serum antibodies against BTV has been reported in several species of wild ungulates, including red deer (Linden et al. 2008; Ruiz-Fons et al. 2008; García et al. 2009), suggesting widespread contact of wild ruminants with BTV. Moreover, BTV RNA has been recently detected in farmed red deer in Spain (serotypes 1 and 4) (Rodríguez-Sánchez et al. 2010) but BTV infection seems not to result in a significant mortality in red deer (Linden et al. 2008), although sporadic fatal disease with BTV isolation has been reported in mouflon (*Ovis aries*) (Fernández-Pacheco et al. 2008). BT is considered endemic in wild ruminants in parts of Africa and North America (Stallknecht et al. 1996; Gerdes, 2004), but up to now little is known about the role deer could play in the epidemiology of BT in Europe. The aim of this study is to determine the dynamics of BTV serotypes 1 and 8 infection in red deer, thus assessing the potential of this species as a wild reservoir for BT.

2. Materials and methods

Eleven seven month-old red deer females were transported into the insect-proof biosecurity level 3 (BSL3) facilities of the Centro de Investigación en Sanidad Animal (CISA) in Valdeolmos (Madrid) on January 19th 2009. The deer were kept in three different boxes (four in boxes A and B and three in box C). According to the routine BSL3 procedures, each box was isolated from the others, sampling material was exclusively used in the same box and the operators changed clothes before and after working in a box, having a shower to exit from each box. One week after arriving to the CISA, four of the deer (deer 1 to deer 3 in box A and deer 4 in box C) were inoculated intravenously (iv) with 2,5x10^{6} $TCID_{50}$ of BTV-1 strain Algeria/2006. This strain was received from the Institue for Animal Health in Pirbright, and underwent five cell

93 culture passages prior to inoculation. Other four deer (deer 5 to deer 7 in box B and deer
94 8 in box C) were inoculated iv with $2{,}5x10^6$ $TCID_{50}$ BTV-8 isolate 202326
95 (Belgium/2006). This strain was received from the Istituto Zooprofilattico Sperimentale
96 Della Lombardia e dell'Emilia Romagna (IZSLER), and was inoculated after four
97 culture passages. Finally, the three remaining deer (deer 9 in box A, deer 10 in box B,
98 and deer 11 in box C) received iv an equivalent volume of cell culture medium, acting
99 as controls. The deer were monitored daily from 0 days post-infection (dpi) to 12 dpi
100 and on 14, 17, 21, 24, 28, 31, 38, 50, 60, 66, 71, and 78 dpi. Monitoring included
101 exploration for clinical signs of bluetongue (rectal temperature, facial oedema,
102 erythema, coronitis, stomatitis, conjunctivitis), as well as collection of blood samples
103 with anticoagulant for real time RT-PCR analysis and without anticoagulant for serum.
104 Skin biopsies were taken at 14 dpi from the eight deer at boxes A and B (three BTV-1
105 inoculated, three BTV-8 inoculated and two controls). The deer were euthanized on 98
106 (four deer at box B), 105 (four deer at box A), and 112 dpi (three deer at box C).
107 Viral BTV RNA in blood was assessed using a modification of an already described
108 semi-quantitative real-time RT-PCR (Toussaint et al., 2007). Serum antibodies were
109 analyzed by commercial ELISA (Pourquier Bluetongue competitive ELISA, Institut
110 Pourquier, Montpellier, France), and by standard virus neutralization test (VNT) using
111 the inoculation virus as antigen, similarly to the methodology previously reported
112 (Hamers et al., 2009). Virus isolation was attempted in Vero and BHK cells only at
113 peak genome detection (12 dpi) and at late stagesof the infection (78 dpi) one BTV-1
114 (deer 4) and one BTV-8 (deer 7) inoculated deer.
115 This study was approved by the INIA Ethics Committees on Animal
116 Experimentation and Biosafety. Handling procedures and sampling frequency were
117 designed to reduce stress and health risks for subjects, according to European (86/609)

and Spanish laws (R. D. 223/1988, R. D. 1021/2005), and current guidelines for ethical use of animals in research (ASAB, 2006).

3. Results

BTV RNA was detected in all deer inoculated with BTV-1 (deer 1 to 4) and three out of the four deer inoculated with BTV-8 (deer 5 to 7) from one dpi to the end of the study, with a peak around twelve dpi for both serotypes, and a slow decline thereafter. Figure 1 shows dynamics of BTV RNA detected in blood of the infected red deer, as assessed by real-time RT-PCR. Detection of BTV RNA was low and transient for BTV-8-infected deer 8 (box C), disappearing after 14 dpi. No virus RNA was detected in none of the control deer until 38 dpi. However, on 38 dpi BTV RNA was detected in control deer 11 (box C), followed by seroconversion between 38 and 50 dpi. The virus infecting this deer was characterized as BTV-1 both by VNT and BTV-1/BTV-8 multiplex real-time RT-PCR (Fernández-Pinero et al., in prep.). From all the four samples where BTV isolation was tried, only BTV-1 was recovered at 12 dpi from deer 4 (inoculated with BTV-1) in Vero cells. Only mild transient unspecific clinical signs, which could be compatible with BT, were observed in the infected deer, and no statistically significant difference, peak or trend in rectal temperature was evidenced.

Serum antibodies against BTV were detected in all inoculated deer both by VNT and ELISA tests. ELISA revealed BTV specific antibodies by 10 dpi in the BTV-1 group, and between 9 and 12 dpi in the BTV-8 group, antibodies being present throughout the whole study period for both serotypes. In deer inoculated with BTV-1, the neutralizing antibody response was first detected at 8-11 dpi, with peak titres of 1/1280 around 17-21 dpi. In deer inoculated with BTV-8 neutralizing antibodies

appeared at 8 dpi, with peak titres of 1/640 to 1/1280 between 17 and 28 dpi. Controls 9 and 10 showed no specific antibodies throughout the whole experiment. However, control deer 11, the one where BTV RNA was first detected at 38 dpi, seroconverted by 45 dpi, showing antibodies against BTV-1 until the end of the study (Figure 2).

BTV RNA was detected in the skin biopsies obtained at 14 dpi in five out of the six BTV-inoculated deer (three inoculated with BTV-1 and two with BTV-8) analyzed. The result was doubtful for the remaining BTV-8-inoculated deer, and negative for the two control deer sampled.

4. Discussion

Our results confirm that Iberian red deer get asymptomatically infected with BTV serotypes 1 and 8, BTV RNA being reliably detected for long periods, comparable in intensity and duration to that of domestic ungulates (Luedke, 1969; MacLachlan et al. 1990; Puentes et al., 2008). RT-PCR has been validated as a detection technique for BTV, at least as sensitive as viral titration on Vero cells (Hamers et al. 2009). BTV viraemia with mild or no clinical signs following experimental infection has been reported in North American elk (*Cervus elaphus canadensis* and *Cervus elaphus nelsoni*) (Murray and Trainer, 1990; Ellis et al., 1993). BTV was isolated as long as 105 dpi from the blood of experimentally infected elk after 95 days of negative results followed by cortisone injection (Murray and Trainer 1970). However, duration and intensity of both the virus dynamics and the immune response remains to be fully described. To the best of our knowledge, this is the first report to address this issue in European red deer. The kinetics of the antibody response of our experimentally-infected red deer during the study period was similar to those of experimentally infected

168 domestic ruminants and North American elk (Murray and Trainer, 1970), showing the
169 validity of ELISA and serum VNT to monitor contact with BTV in red deer. The
170 clinical picture observed among BTV-1 and BTV-8 infected deer was similar to the
171 mild subclinical effects of BTV observed in cattle (MacLachlan et al., 1990) and elk
172 (Murray and Trainer, 1970; Ellis et al., 1993), rather than to the more severe clinical
173 pictures often described in sheep (MacLachlan et al., 2008) and white-tailed deer
174 (Vosdingh et al., 1968).

175 Transplacental, oral, and wound contact have been suggested as mechanisms for
176 BTV transmission in absence of the *Culicoides* vector, both in domestic and wild
177 ungulates (Vosdingh et al. 1968; Thomas and Trainer, 1970; Menzies et al., 2008;
178 Backx et al., 2009). Iatrogenic transmission can be discarded for the spontaneously-
179 infected control deer 11 of our study, since material for each deer was prepared
180 individually before entering the enclosure, so oral (the deer bit each other due to
181 hierarchic fights in the small area of the enclosure) or wound transmission would be the
182 most likely explanation. Transmission in absence of the vector is therefore possible in
183 close contact conditions, and although its epidemiological importance is unknown, it
184 could be a concern regarding the overwintering of BTV (Wilson et al., 2008).

185 Which is the role of red deer in the epidemiology of BTV in Europe? Red deer is
186 an abundant wild ruminant in many parts of the northern hemisphere, occurring in BTV
187 affected regions of central and southern Europe (Lovari et al., 2009). Other wild
188 ruminant species are considered maintenance hosts for BTV in Africa and North
189 America, the virus being endemic in their populations (Stallknecht et al., 1996; Gerdes,
190 2004). Antibodies against BTV and BTV RNA have been identified in free-ranging
191 naturally infected red deer in Europe (Linden et al., 2008; Ruiz-Fons et al., 2008; García
192 et al., 2009). Moreover, RNA of BTV-4 was found in red deer blood samples in summer

2007, eight months after the last detection in sympatric domestic ruminants (Rodríguez-Sánchez et al., 2010). Finally, our results indicate that red deer can maintain BTV RNA in blood for long periods, and therefore red deer have the potential to play a role in BT epidemiology. However, the lack of BTV isolation at 78 dpi suggests that only BTV RNA but no viable virus stays detectable long after the peak viraemia. Moreover, it remains unanswered whether the *Culicoides* vector can get infected from red deer. Nevertheless, the detection of BTV in skin samples of our experimentally-infected red deer at 14 dpi seems to point that it could be infectious to midges, at least during the peak of BTV RNA detection.

6. Conclusion

Red deer have the potential to be a reservoir for BT. A natural experiment to test this hypothesis is currently running: livestock has largely been vaccinated (European Commission, 2009), but annual re-vaccination is unlikely to be maintained for long. Vaccine induced immunity against BTV can last for over one year, but it may be lost after a certain time if no annual boost occurs (Hamers et al., 2009). Also, positive results of the vaccination campaigns rely on a minimum coverage of 80% of the susceptible population (Ferrari et al., 2005), and susceptible unvaccinated red deer could affect this minimum required goal. Hence, if repeated BTV outbreaks occur in regions with high wild ruminant densities and no clear link with livestock movements or vaccination failures, the deer reservoir hypothesis will be confirmed. If, in contrast, no BTV circulation takes place and livestock vaccination alone is successful in eradicating bluetongue, the hypothesis will be rejected.

Acknowledgements

We acknowledge the funding from JCCM PAI08-0287-8502, the Government of Scotland, and INIA-MARM CC08-020 (additional support to CISA). Paolo Cordioli, from the Istituto Zooprofilattico Sperimentale Della Lombardia e dell'Emilia Romagna (IZSLER), provided the BTV serotype 8 strain for the inoculum. Caterina Falconi had a grant from the Government of Sardinia. Help from Cristina de la Parra, Sara Espinosa. Teresa García, Ana Robles, Covadonga Pérez, and Concepción Gómez at CISA; and from Mari Paz Martín, Mauricio Durán, Paqui Talavera, Ursula Höfle, Mariana Boadella, Raquel Jaroso, Luca Carrel, and Elisa Maio at IREC is deeply acknowledged. Special thanks to Peter Nettleton, Andrew Voas and Matthew Hartley for scientific and logistic advice, and for their kind help.

References

Acevedo, P., Ruiz-Fons, F., Vicente, J., Reyes-García, A.R., Alzaga, V., Gortázar C., 2008, Estimating red deer abundance in a wide range of management situations in Mediterranean habitats. J. Zool. 276, 37-47.

Association for the study of animal behaviour (ASAB), 2006. Guidelines for the treatment of animals in behavioural research and teaching. Anim. Behav. 71, 245-253.

Backx, A., Heutink, R., van Rooij, E., van Rijn, P., 2009. Transplacental and oral transmission of wild-type bluetongue virus serotype 8 in cattle after experimental infection, Vet. Microbiol. 138, 235-243.

Breard, E., Sailleau, C., Nomikou, K., Hamblin, C., Mertens, P.P.C., Mellor, P.S., El Harrak, M., Zientara, S., 2007. Molecular epidemiology of bluetongue virus serotype 4 isolated in the Mediterranean Basin between 1979 and 2004. Virus Res. 125, 191-197.

Ellis, J.A., Coen, M.L., Maclachlan, N.J., Wilson, W.C., Williams, E.S., Leudke, A.J., 1993. Prevalence of bluetongue virus expression in leukocytes from experimentally infected ruminants. Am. J. Vet. Res. 54, 1452-1456.

Enserink, M., 2008. Exotic disease of farm animals tests Europe's responses. Science 319, 710-711.

Eschbaumer, M., Hoffmann, B., Moss, A., Savini, G., Leone, A., König, P., Zemke, J., Conraths, F., Beer, M., 2009. Emergence of bluetongue virus serotype 6 in Europe-German field data and experimental infection of cattle. Vet- Microbiol. DOI:10.1016/j.vetmic.2009.11.040

European Commission, 2009. Bluetongue. In: Food Safety – From the Farm to the Fork, Animal Health and Welfare, on line. Consulted on 12 October 2009. http://ec.europa.eu/food/animal/diseases/controlmeasures/bluetongue_en.htm

Fernández-Pacheco, P., Fernández-Pinero, J., Agüero, M., Jiménez-Clavero, M.A., 2008. Bluetongue virus serotype 1 in wild mouflons in Spain. Vet. Rec. 162, 659-660.

Ferrari, G., De Liberato, C., Scavia, G., Lorenzetti, R., Zini, M., Farina, F., Magliano, A., Cardeti, G., Scholl, F., Guidoni, M., Scicluna, M.T., Amaddeo, D., Scaramozzino,

268 P., Autorino, G.L., 2005. Active circulation of bluetongue vaccine virus serotype-2
269 among unvaccinated cattle in central Italy. Prev. Vet. Med. 68, 103-113.
270
271 García, I., Napp, S., Casal, J., Perea, A., Allepuz, A., Alba, A., Carbonero, A., Arenas,
272 A., 2009. Bluetongue epidemiology in wild ruminants from Southern Spain. Eur. J.
273 Wildlife Res. 55, 173-178.
274
275 Gerdes, G.H., 2004. A South African overview of the virus, vectors, surveillance and
276 unique features of bluetongue. Veter. Ital. (2004) 40:39-42.
277
278 Hadorn, D.C., Racloz, V., Schwermer, H., Stärk, K.D.C., 2009. Establishing a cost-
279 effective national surveillance system for Bluetongue using scenario tree modelling.
280 Vet. Res. 40, 57.
281
282 Hamers, C., Rehbein, S., Hudelet, P., Blanchet, M., Lapostolle, B., Cariou, C., Duboeuf,
283 M., Goutebroze, S., 2009. Protective duration of immunity of an inactivated bluetongue
284 (BTV) serotype 2 vaccine against a virulent BTV serotype 2 challenge in sheep.
285 Vaccine 27, 2789-2793.
286
287 Linden, A., Mousset, B., Grégoire, F., Hanrez, D., Vandenbussche, F.,
288 Vandemeulebroucke, E., Vanbist, T., Verheyden, B., de Clerck, K., 2008. Bluetongue
289 virus antibodies in wild red deer in southern Belgium. Vet. Rec. 162, 459.
290

Lovari S., Herrero J., Conroy J., Maran T., Giannatos G., Stubbe M., Aulagnier S., 2009. *Cervus elaphus*. In: IUCN 2009, IUCN Red List of Threatened Species. Version 2009.1, on line. Consulted on 11 September 2009. http://www.iucnredlist.org/details/41785/0.

Luedke, A.J., 1969. Bluetongue in sheep: Viral assay and viraemia. Am. J. Vet. Res. 30, 499-509.

MacLachlan, N.J., Jagels, G., Rossito, P.R., Moore, P.R., Heidner, H.W., 1990. The pathogenesis of experimental bluetongue infection of calves. Vet. Pathol. 27, 223-229.

MacLachlan, N.J., Crafford, J.E., Vernau, W., Gardner, I.A., Goddard, A., Guthrie, A.J., Venter, E.H., 2008. Experimental Reproduction of Severe Bluetongue in Sheep. Vet. Pathol. 45, 310-315.

Menzies, F.D., McCullough, S.J., McKeown, I.M., Forster, J.L., Jess, S., Batten, C., Murchie, A.K., Gloster, J., Fallows, J.G., Pelgrim, W., Mellor, P.S., Oura, C.A.L., 2008. Evidence for transplacental and contact transmission of bluetongue virus in cattle. Vet. Rec. 163, 203-209.

Murray, J.O., Trainer, D.O., 1970. Bluetongue Virus in North American Elk, J. Wildlife Dis. 6, 144-148.

Puentes, E., Fernández-Pinero, J., Fernández-Pacheco, P., Sánchez, M.A., Varo, I., Salguero, J., Pinilla, M.J., Villalba, R., San Miguel, E., Zamora, M.J., Arias, M.,

316 Agüero, M., Gómez-Tejedor, C., Jiménez-Clavero, M.A., 2008. Inactivated vaccines
317 against bluetongue: protection of cattle and sheep from experimental challenge with
318 bluetongue virus serotypes 1, 4 and 8. In: EPIZONE 2008 Bluetongue Satellite
319 Symposium Abstract book, Brescia, Italy, p. 68.
320
321 Purse, B.V., Mellor, P.S., Rogers, D.J., Samuel, A.R., Mertens, P.P.C., Baylis, M.,
322 2005. Climate change and the recent emergence of bluetongue in Europe. Nature Rev.
323 Microbiol. 3, 171-181.
324
325 Rodríguez-Sánchez, B., Iglesias-Martín, I., Martínez-Avilés, M., Sánchez-Vizcaíno,
326 J.M., 2008. Orbiviruses in the Mediterranean basin: updated epidemiological situation
327 of Bluetongue and new methods for the detection of BTV serotype 4. Transbound.
328 Emerg. Dis. 55, 205-214.
329
330 Rodríguez-Sánchez, B., Gortázar, C., Ruiz-Fons, F., Sánchez-Vizcaíno, J.M., 2010.
331 Bluetongue virus serotypes 1 and 4 in red deer, Spain. Emerg. Infect. Dis. 16, 518-520.
332
333 Ruiz-Fons, F., Reyes-García, A.R., Alcaide, V., Gortázar, C., 2008. Spatial and
334 temporal evolution of bluetongue virus in wild ruminants, Spain. Emerg. Infect. Dis. 14,
335 951-953.
336
337 Stallknecht, D.E., Luttrell, M.P., Smith, K.E., Nettles, V.F., 1996. Hemorrhagic disease
338 in white-tailed deer in Texas: A case for enzootic stability. J. Wildlife Dis. 32, 695-700.
339

Thomas F.C., Trainer D.O. Bluetongue virus in white-tailed deer. 1970. Am. J. Vet. Res. 31, 271-278.

Toussaint, C., Breard, E., Zientara, S., De Clercq, K., 2007. Bluetongue virus detection by two real-time RT-qPCRs targeting two different genomic segments. J. Virol. Methods. 140, 115-123.

Vosdingh, R.A., Trainer, D.O., Easterday, B.C., 1968. Experimental Bluetongue Disease in White-Tailed Deer. Can. J. Comp. Med. Vet. S. 32, 382-387.

Wilson, A., Darpel, K., Mellor, P.S., 2008. Where does bluetongue virus sleep in the winter? PLoS Biol. 6, e210.

Figure captions

Figure 1.- Viral load (Ct=Cycle at which specific amplification starts to be detectable in the real-time RT-PCR technique) determined by generic BTV RRT-PCR on whole blood samples from 0 dpi to the end of the experiment (98-112 dpi). Serotype was confirmed by serotype-specific RRT-PCRs of the serotype inoculated in randomly selected samples for each deer. Upper panel: BTV1; Central panel: BTV8; Lower panel: control.

Figure 2.- Kinetics of the BTV-neutralizing antibody response measured by VNT in BTV-inoculated and control red deer. Upper panel: sera from BTV-1 inoculated deer 1-

364 4 and control deer 9 and 11 tested against BTV-1. Lower panel: sera from BTV-8
365 inoculated deer 5-8 and control deer 10 and 11, tested against BTV-8.

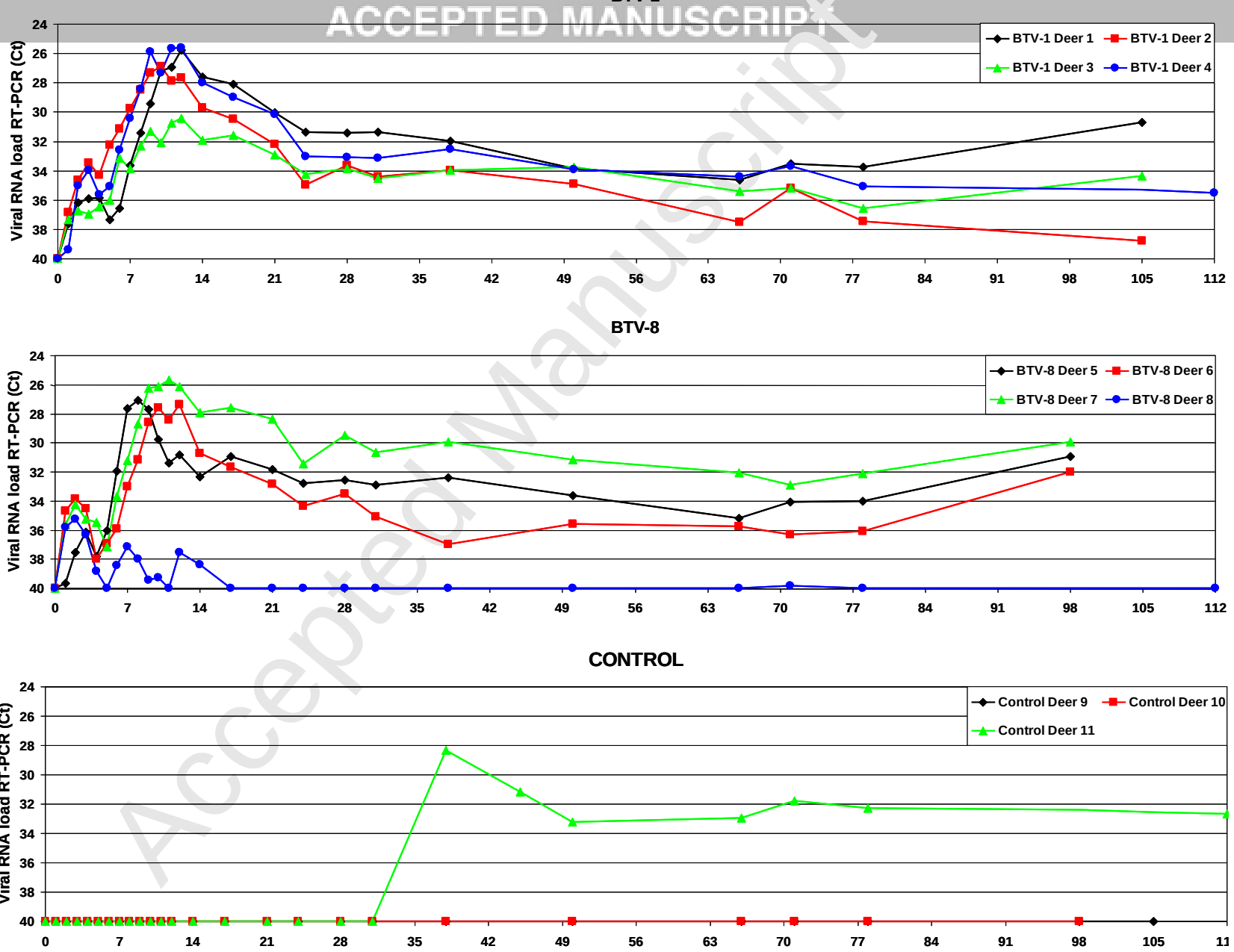

FIGURE 1

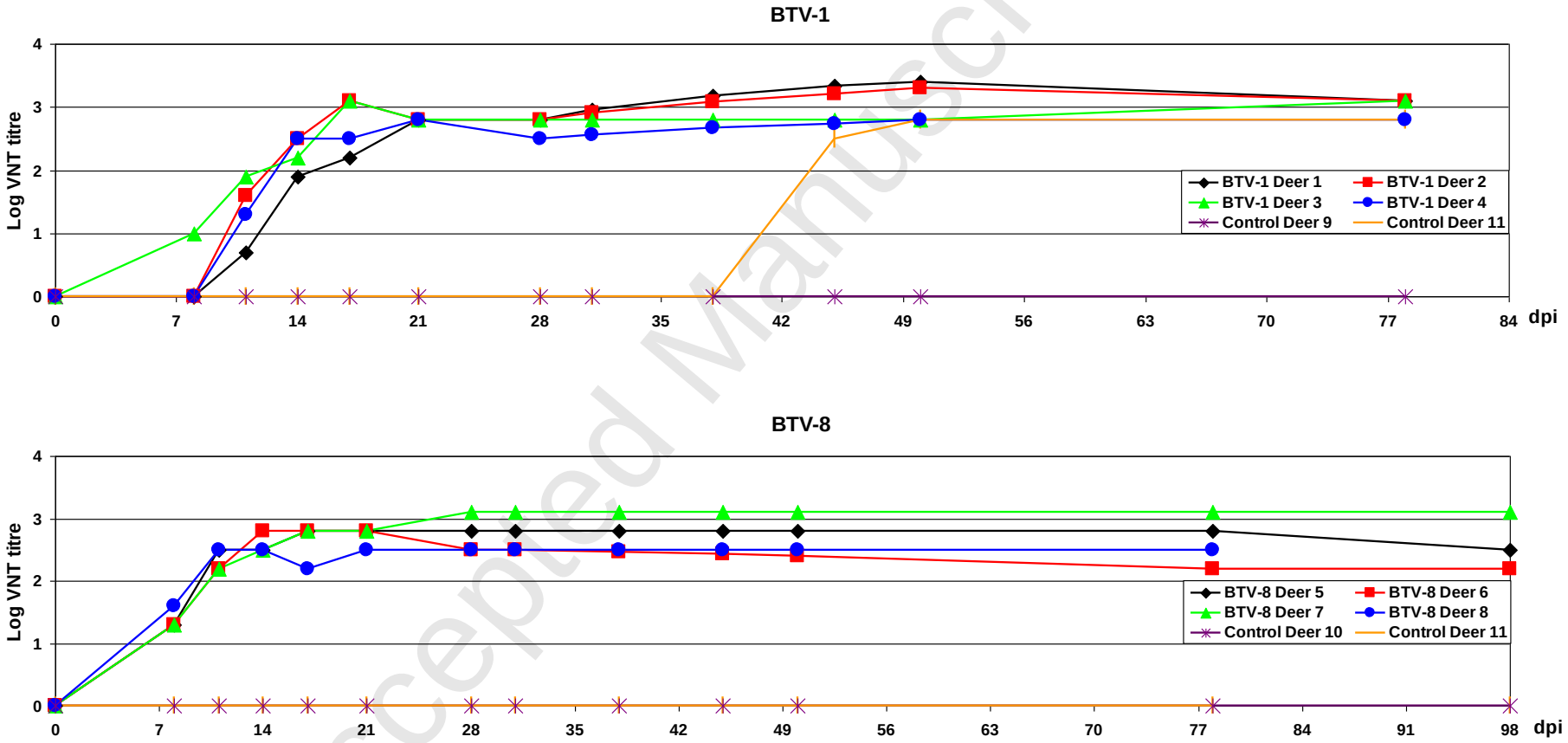

FIGURE 2