Survey of wild cervid bloods for Bovine viral diarrhoea virus (BVDV) across England and Wales

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Key words: Deer, Bovine viral diarrhoea virus (BVDV),

ABSTRACT

BACKGROUND: Bovine viral diarrhoea (BVD) is a production disease commonly found in British cattle herds. Species other than cattle have been shown to be infected with the virus, thereby providing a potential source of infection for livestock. This study surveyed serum samples taken from 596 culled wild deer from England and Wales, between 2009-2010, for the presence of BVD antibodies.

METHODS: 596 samples were tested with the SVANOVIR BVDV p80-Ab ELISA and a subset of 64 were tested with the IDEXX BVDV p80 Antibody ELISA. ELISA results were confirmed using serum neutralisation tests.

RESULTS: 2/596 samples (0.35%) tested positive for BVD antibodies using the Svanova test and one of these tested positive and the other inconclusive using the IDEXX test; both were confirmed positive with serum neutralisation tests. These were both red deer stags, one from Devon and the other from East Anglia.

CONCLUSIONS: The results indicate that it is unlikely that BVDV is widely circulating within the wild deer population and particularly unlikely that persistently infected deer are present in the populations surveyed. These results suggest that wild deer are unlikely to be a significant reservoir of BVD infection in cattle.

INTRODUCTION

Bovine viral diarrhoea virus (BVDV) causes significant economic and production losses in cattle worldwide. In England and Wales it is estimated that approximately 90% of cattle herds have been exposed to BVDV (1) and at any one time approximately 65% of herds are estimated to be undergoing active BVDV infection (1, 2). In a study performed in Scotland over the same period as the samples

collected in this manuscript, 25.8-37.7% farms did not contain sero-positive young stock (3) indicating that the remaining 74.2-63.3% of herds had recent or active infection, however the most recent figures for Scotland suggest that only 10% of farms are currently returning a 'not negative status (4). Nationally, BVDV is estimated to cost the UK cattle industry £39.6 million per year (5) and at the individual animal level economic models indicate that the cost of herd infection in a beef suckler system is in the region of £37 per cow per year (6). The Scottish government estimated that the average dairy herd would be £15,800 better off per annum if it had eradicated BVD (7).

Presently, with a growing drive to eradicate BVDV from many countries worldwide (8), the significance of wildlife vectors for the disease becomes highly relevant. Whilst current control schemes focus on the control of PI animals, BVD infection is not restricted to cattle and, as yet, the potential for wild animal reservoirs of the disease to complicate control measures has not been determined. Various domestic species including sheep, pigs and camelids (9), have been shown to be seropositive for the virus. Additionally, sympatric wild species have been exposed to the virus, including rabbits that had a low seroprevalence (10) and several different cervid species, for example fallow deer (*Dama dama*) (11, 12), mule deer (*Odocoileus hemionus)* (13), white-tailed deer (*Odocoileus virginianus*) (14), black-tailed deer (*Odocoileus hemionus columbianus*), (13), red deer (*Cervus elaphus*) (12, 15-18), Sika deer (*Cervus nippon*) (12) and roe deer (*Capreolus capreolus*) (15, 19). Their role in the dynamics of BVDV, for example whether they are spill-over or maintenance hosts, with subsequent impact on farm elimination programs has yet to be defined. Two deer species, red deer and roe deer, have widespread distributions within the UK, including areas of the country with the most dense cattle populations and in areas with the highest levels of BVDV in cattle. BVDV has not been reported in wild British deer since 1977 when McMartin *et al.* (1977) (20), isolated the virus from red deer in Scotland. Although BVDV-like viruses were isolated from European Bison (*Bison bonasus*), Pére David's deer (*Elaphurus davidianus*), Chinese water deer (*Hydropotes inermis*) and Scimitar Horned Oryx (*Oryx dammah*) at Whipsnade zoo between 1973-1994, origin and strains of virus were not reported (21) (Frolich and Flach, 1998). It has been shown, experimentally, that if deer are infected during early gestation they

can give birth to PI fawns that may act as a reservoir of infection in the same manner as PI cattle (22-24). Furthermore, both PI cattle and deer are capable of infecting susceptible deer (24, 25) therefore the role of deer in the epidemiology of BVDV requires further clarification.

The implications of BVDV infection in deer for nationwide control programmes have not yet been determined. If BVDV is present in wild deer populations, it may help it explain new BVDV infections in herds with reasonable biosecurity. Many European countries are currently undertaking BVDV eradication at the national level with Sweden achieving near elimination in 2004 (26). The experimental production of PIs within deer demonstrates the possibility that BVDV may be retained within the deer population and could potentially be transmitted back to cattle (spill-back event). This suggests that wildlife could play a role in BVDV dynamics in cattle and may need to be considered in control plans (9).

BVDV sero-positive deer are found throughout the world yet the role wild deer populations play in the dynamics of BVDV infection in cattle is, currently, unknown. It has yet to be determined whether BVDV in deer is circulating without the need for re-seeding by contact with infectious cattle or whether it is being transmitted from infectious deer to naïve cattle. The aim of this study was to determine whether the disease is present in the deer population in England and Wales by analysing sera from deer culled as part of the annual Forestry Commission deer cull in 2009-2010.

MATERIALS AND METHODS

Serum Samples

Sera have been collected from 596 deer from eight regions across England and Wales. All samples were collected as part of a cull between April 2009 and March 2010 and had previously been analysed as part of a *Borrelia burgdorferi* serosurvey in wild deer (27). Permission was kindly granted by the Forestry Commission, who collected the original samples, for the current study to assess the seroprevalence of BVDV in the sample set. Five hundred and eighteen samples were collected from

Roe Deer and 78 samples from Red Deer (Table 1). Sex, location and approximate age in years (<1, 1, 2, 3, 4 and NA when unknown) were recorded at the time of sampling (Table 1). Blood was collected into plain tubes and then stored at 4°C until transport to the laboratory where samples were centrifuged and sera stored at -20°C (27). The sera was then thawed, vortexed and 90ul were centrifuged at 13,000 revolutions per minute for one minutes and then aliquotted into eppendorfs for ELISAs.

Antibody and Antigen (ELISA)

Detection of specific antibody response was carried out by ELISA according to the manufacturers instructions using bovine validated kits from both Svanova (n=596 deer) and IDEXX (n=64 deer); SVANOVIR BVDV p80-Ab ELISA (Boehringer Ingelheim Svanova). IDEXX BVDV p80 Ab Test (IDEXX Laboratories) respectively. Svanovir kits had previously been used on deer sera by Kirchgessner et al. 2013 and Graham et al. 2017 (12, 14), and validated through serum neutralisation tests. IDEXX kits had been used by Rodriguez-Prieto et al. 2016 in conjunction with reverse transcriptase-polymerase chain reaction (RT-PCR) (18).

The SVANOVIR BVDV p80-Ab ELISA kits were used to test all the deer sera and positive and negative controls from the kit, cattle and deer were used. Deer serum controls from deer experimentally infected with BVDV and uninfected control deer were kindly provided by Dr Julia Ridpath (USDA, Ames, United States). Duplicate wells were used for each sample and mean optical densities (ODs) were obtained. The percentage inhibition for the Svanovir ELISA kit was calculated using equation 1.

$$\text{Percentage Inhibition} = \frac{OD_{Negative\ control} - OD_{Sample\ or\ Positive\ control}}{OD_{Negative\ control}} \times 100 \quad \text{(equation 1)}$$

The manufacturers cut-offs (>45% inhibition) were used to state whether an animal was positive or negative, these were commercially validated in bovines and the control deer serum correlated with these cut off values.

The IDEXX BVDV p80 Ab Test was used to test 64 deer samples to assess the kits ability to detect antibody positive and negative deer. The IDEXX results were reported as signal:noise percentages and were calculated using equation 2.

$$S/N\% = 100 \times \frac{Sample}{Negative\ Control\ \bar{x}} \qquad \text{(equation 2)}$$

This kit is also a bovine validated kit and the cut-off values are as follows: Positive<40%, Inconclusive between 40-50% and Negative>50%.

Levels of BVDV antigen were determined by antigen-capture ELISA using two monoclonal antibodies (Mabs) directed against conserved domains of the NS2/3 non-structural protein (28).

Serum Neutralisation Assay

To further validate the ELISA results, BVDV neutralisation assays were performed on 13 deer samples with a range of ODs, including two that had been culled in close geographical proximity to animals with positive sera. Levels of neutralizing antibody were established as described previously with minor amendments (29) . Briefly serial two-fold dilutions of sera with minimum essential medium (MEM) were prepared in 96 well microtitre plates and incubated with 100 $TCID_{50}$ of ncpBVDV (strain Ky1203 subgenotype 1a) for two hours. Post incubation $1.5x10^4$ Madin-Darby bovine kidney (MDBK) cells were

added and incubated for four days at 37°C 5% CO2 before fixation of cells and immunostaining for presence of BVDV as described previously (30). Neutralising antibody titres to BVDV type 1a was measured as this is the most prevalent BVDV sub-genotype circulating in the UK (31-33). The results are expressed as the reciprocal of the serum dilution at which 50% of the virus was neutralized.

MAPPING

A map of cattle density obtained from Eurostat 2010 and deer samples was produced using R 3.2.0 (R core development team 2015) and R packages: maptools function readShapeSpatial(34), rworldmap function mapCountryData (35), RColorBrewer (36).

RESULTS

The Svanovir ELISA found two antibody positive deer in the 596 sera tested (0.35% positive; 0/518 (0%) roe deer and 2/78 (2.6%) red deer) (Figure 1). These two positive deer were classed as positive and borderline by the IDEXX ELISA that was used on 64 samples, the remaining 62 samples tested with the IDEXX ELISA were negative which correlated with their results on the Svanovir ELISA. The range of ELISA results for the deer tested with the Svanovir kit is shown in Figure 2 and the results for the IDEXX kit in Figure 3. Thirteen sera samples underwent serum neutralisation tests; these included the two positive samples from the Svanovir ELISA and eleven negative samples across the range of negative results. Serum neutralisation testing confirmed the results of the thirteen sera Svanovir and IDEXX results.

The two antibody positive samples were from male red deer. One was one year of age and originated from East Anglia (Norfolk) and was one of seventeen red deer from that region; the other was two years of age and originated from the Southwest (Devon) and was one of thirty five red deer samples from the South West Peninsula region (Figure 1).

The positive animal from Devon was culled in the same location as two other seronegative deer that were shot at the same time whilst the positive animal in East Anglia was culled in the same location as one other seronegative deer that was shot at the same point. Due to the close grazing proximity of these animals, the three seronegative samples were tested for BVD virus via antigen ELISA in order to determine that the seropositive deer were not grazing alongside PI deer; all three seronegative samples were antigen negative.

DISCUSSION

In this study, few deer were BVDV antibody positive (0.35% of the population tested) indicating little potential for disease spill-over between cattle and deer in the populations studied at the time-point and geographical areas from which the specimens were obtained. The positive samples were found in regions of both low and high cattle density. The low number of seropositive animals suggests that it was highly unlikely that a PI deer was within those herds sampled and this is supported by the negative antigen ELISA results obtained from the three animals culled in proximity to the seropositive deer. More significantly, the proportion of seronegative results (99.66%) in the deer sampled indicates that infection of deer via infected cattle populations does not occur frequently. Antibodies to BVDV in cattle can be present for three or more years post-exposure (37); on this basis, the majority of the deer sampled would have been too young for BVDV antibodies to have waned, indicating no exposure in the lifetime of the animals. The two positive animals were estimated to be one year and two years of age. If maternal colostral antibodies had been present within these animals, it would be anticipated that, like calves, they should have declined by the time they were a year old (38, 39) thus these positive results probably indicate contact with either infected cattle or other infected deer. Given the BVDV low seroprevalence and unlikely presence of PI deer in the population sampled, it is most likely that the seropositive deer are a result of seemingly rare contact with infected cattle.

The samples were obtained primarily from areas associated with forest or woodland cover. This is typical woodland grazing in England and Wales where cattle are at very low densities, rarely grazed all year round and the land is primarily used for conservation purposes (40), thus limiting exposure between wildlife and livestock populations. Most cattle in the UK are not kept on pasture during the winter, which reduces the likelihood of pathogen transmission between deer and cattle. Studies have shown that red deer that use pasture after cattle (41) increase their exposure to pathogens shed by those cattle. However, when considering BVDV, Niskanen et al 2003 (42) demonstrated that, in cattle, the time between the individuals using a contaminated area needed to be short (two hours) to cause infection of naïve stock introduced into areas previously occupied by a PI. Low rates of BVD transmission from cattle to deer via contaminated pasture could be an additional reason for the low seroprevalence seen in the deer investigated in this manuscript. .

Although the majority of sera sampled were from roe deer, both positive samples were from red deer, a similar finding to other studies (16, 17, 43, 44). Red deer have a greater dietary overlap with cattle than roe deer (45, 46) and, as a result, are more likely to come into contact with cattle and their associated pathogens by spending more time on pasture (47) than roe deer. The home range of a red deer is larger (2-67 ha, Isle of Rhum, (48)) than that of a roe deer (3.3 +/- 0.24 ha Thetford forest UK, (49)) and may have a greater chance of overlapping with sympatric cattle or multiple herds of cattle. The home ranges of deer in the UK are generally smaller than in other countries in Europe (50-52), which may alter the likelihood of exposure of populations in different countries. Roe deer tend to be more solitary and live in small family groups compared with red deer that herd together (49) and therefore have higher frequency of contact and enhance disease transmission.

Limitations

Whilst the authors have concentrated the discussion on BVDV within this manuscript, both antibody ELISA kits and the SNT used in this work may return positive results in Border Disease Virus (BDV)

infected deer. Due to the low level of seroconversion seen in the deer within this study, the conclusion remains that likelihood of infection of wild deer with either pestivirus appears to be very low and thus wild deer do not appear to pose a significant reservoir of infection for either disease.

The quality of a small number of sera samples in this study was variable with some noted to be haemolysed at the point of testing. This is likely due to the time lag between culling and receiving the samples that for a few samples was up to 30 days. Due to the strong agreement between the test methods employed in this manuscript and the previous successful use of these samples by Alonso et al 2012 (27) the authors do not believe that this issue would have a significant effect on the results presented here but highlight it for transparency.

The deer sera came from a small proportion of animals from two deer species found in England and Wales that were opportunistically sampled as a result of a cull. Other species for example Sika and Fallow deer have been identified with BVDV positive sera in Ireland (12). Fallow deer are a deer species that congregate in herds and are predominately grazers leading to dietary overlap with cattle through using fields adjacent to woodland (53). They can also be found at high densities in many different areas of the country (53) therefore it would be valuable to examine sera for BVDV in this species.

Implications

Deer in the UK have been found to have many pathogens that are concurrently present within livestock (54, 55). With the few BVDV antibody-positive deer in this wide study, it indicates that the disease is not widespread within the deer study populations. This implies there is little likelihood of spillback of BVD infection from the deer to cattle; thus control of the disease within the cattle population should be focussed on cattle to cattle transmission and good farm biosecurity and

biocontainment measures. With the current emphasis to control BVD in cattle there may be a reduced likelihood of identifying positive deer from current or future sampling.

Conclusions

This study shows that the majority of infections within cattle herds are likely to have originated from the cattle and not from deer populations. This highlights the importance of the identification of PIs, biosecurity and biocontainment at a farm level. However, further studies with more deer species and wider geographical scale would be needed to provide greater insight into the role of deer species in the transmission of BVD.

ACKNOWLEDGEMENTS

We would like to thank the Forestry Commission for collecting deer bloods during their culls and for permission to conduct the analysis of BVDV seroprevalence. We would like to thank Dr Alonso for providing us with the deer bloods enabling us to carry out this study. The authors would also like to thank Dr Julia Ridpath (USDA, Ames, United States) for kindly providing the deer control sera. The authors are grateful for funding provided by the Royal Veterinary College Internal Grant Scheme, without which the study could not have taken place.

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Table 1. Population characteristics of culled deer.

Population characteristics		Species	
		Red	Roe
Sex	Male	35	258
	Female	43	260
Age (years)	<1	20	104
	1	24	73
	2	18	113
	3	-	22
	4	-	50
	Not known	16	156
Area	East Anglia	17	118
	Forest of Dean	-	21
	Kielder	-	120
	New Forest	26	80
	North West England	-	28
	South West Peninsula	35	85
	South East England	-	60
	Wales (Coed Y Gororau)	-	6

Figure Legends

Figure 1. Red crosses = roe deer, yellow circles=red deer, green circles – positive deer, background: cattle density - white 0/km^2, very light grey 0-20/km^2, light grey 20-40/km^2, grey 40-80/km^2 and darkest grey 80-120/km^2. 591 deer locations are mapped as coordinates were not recorded for five animals (these were all seronegative).

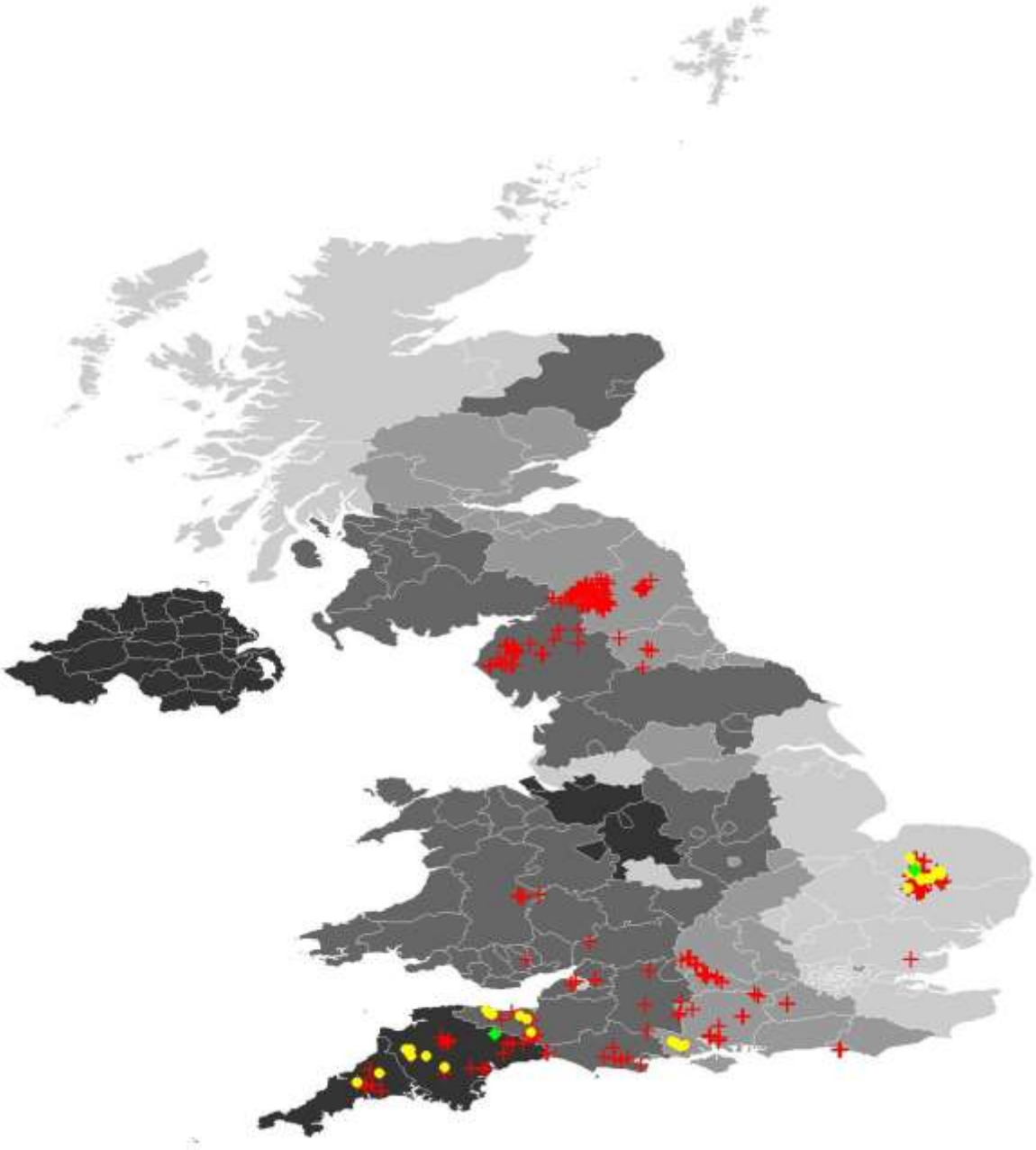

Figure 2. ELISA results for the 596 deer tested using the SVANOVIR BVDV p80-Ab ELISA Test. Individual deer results are shown as a black dot and the positive/negative cut off is represented by a line at 45% Inhibition where samples above this value are antibody positive.

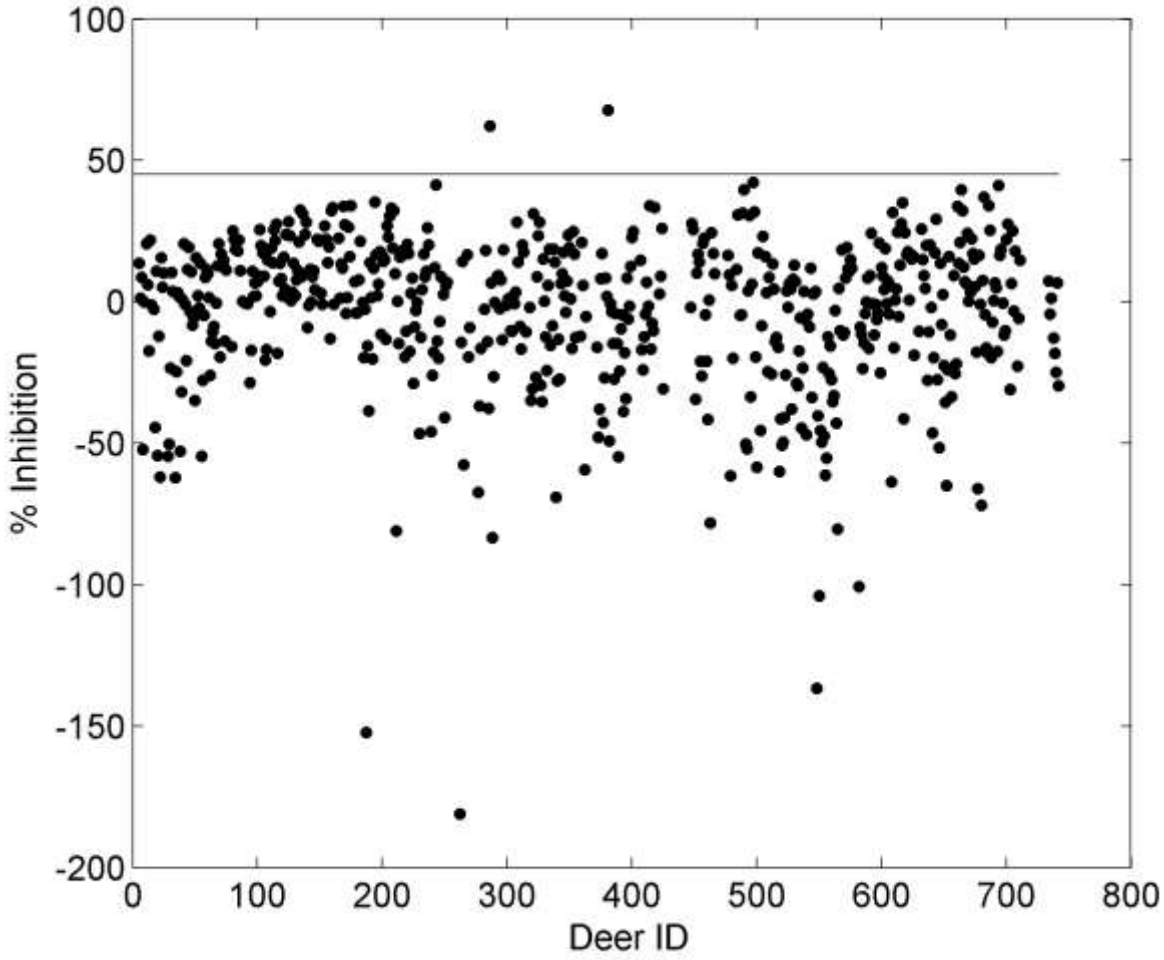

Figure 3. ELISA results for the 64 deer tested using the IDEXX BVDV p80 Antibody ELISA Test. Signal: Noise % (S/N %) is shown on the y axis. Individual deer results are shown as a black dot and the positive/inconclusive cut off is represented by a line at S/N 40% and the inconclusive/negative cut off is represented by a line at S/N 50% where results below S/N 40% are classed as positive, those between S/N 40% and S/N 50% are classed as inconclusive and those above S/N 50% are classed as negative.