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APPLICATION OF COMMERCIAL ENZYME LINKED IMMUNOSORBENT ASSAYS (ELISA) FOR THE DETECTION OF ANTIBODIES FOR FOOT-AND-MOUTH DISEASE VIRUS IN WILD BOAR AND RED DEER

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For detecting antibodies towards foot and mouth (FMD) virus in sera collected from red deer hinds (Cervus elaphus) and wild boars (Sus scrofa), three commercially available enzyme-linked immunosorbent assays (ELISA) were used. Two ELISA kits (PrioCHECK FMDV NS and CHEKIT FMD-3ABC) were used for the detection of antibodies towards non-structural proteins of FMD virus and one assay was based on the detection of antibodies for serotype O (PrioCHECK FMDV type O). All of the sera tested in our study were negative for antibodies against FMD virus. The aim of this study was to investigate the usefulness of commercially available ELISA kits given for marketing authorization in Croatia in testing the prevalence of FMD antibodies in wild boar and red deer populations. Since the producers of ELISA kits used in our study did not declare wild animals as a target species, we hypothesised that the same kits could be used for serological diagnosis of FMD in red deer and wild boars. Our study confirmed that the kits used are acceptable for detecting antibodies in both species tested, however, the investigation highlighted the problem of validating the kits due to the absence of available positive sera originating from red deer, as well as other susceptible species, especially artiodactyls.

Key words: Croatia, ELISA, foot-and-mouth disease, red deer (Cervus elaphus), wild boar (Sus scrofa)

INTRODUCTION

In many countries worldwide, FMD has caused great losses (Perry and Rich, 2007). The causative agent of the disease is foot-and-mouth virus, a member of the genus *Aphthovirus* within the *Picornaviridae* family. There are seven distinct viral serotypes recognized globally, know as O, A, C, SAT1, SAT2, SAT3, and Asia1 (Alexandersen et al., 2003) and multiple subtypes exist within each serotype. Serotype O is most frequently involved as the causative agent of outbreaks, followed by A, C and Asia 1 (Valarcher et. al., 2008). The last FMD outbreak recorded in Croatia was caused by FMD virus type C in 1968 (Cvetnić, 1997).

FMD has been reported in many zoological families such as *Bovidae, Cervidae, Suidae, Camelidae, Giraffidae, Erinaceidae, Tayasuidae, Muridae, Elephantidae, Tapiridae, Ursidae,* as well as guinea pigs and rabbits (Federer, 1969; Forman and Gibbs, 1974; Hedger 1981; Thompson 2001). The major host species are cloven-hoofed animals including domesticated ruminants and pigs. FMD infection is also potentially dangerous for red deer even though they are considered to be less susceptible than roe deer and wild boars and develop milder lesions than those described in roe deer (Haigh et al., 2002) and wild boars.

Wildlife is an ongoing challenge for many scientific researches. Environment, pollution, migration, climate changes, cohabitation and human influence may be possible threats for wildlife health, and preservation worldwide. Disease control is also an important tool for the protection of wild animal species.

In Europe, as well as in Croatia, the red deer (*Cervus elaphus)* and wild boars (*Sus scrofa)* are the most important game animals. Croatia contains 1067 registered hunting grounds in 21 counties (56.538 km^2) with approximately 57000 active hunters. The majority of hunting grounds are situated in the northern parts of the country between the Sava and Drava rivers. During the hunting season 2010, approximately 2500 deer and 18000 wild boars have been hunted (Anonymous, 2010).

After the FMD outbreak in Macedonia in 1996 Croatia implemented a FMD surveillance program for all imported and susceptible animals. During the last five years 109 795 pig sera and 57 744 bovine sera were investigated in the Croatian Veterinary Institute, National reference laboratory for FMD and serology.

The diagnosis of FMD in wildlife is more complicated than in domestic stock because the variation in severity of presenting signs is greater than in domestic animals. Laboratory tests such as virus isolation, antigen ELISA, PCR and antibody ELISA are essential for an accurate diagnosis. By using the nonstructural protein (NSP) commercially available ELISA tests offer a potential to identify seropositive animals for any of the seven serotypes of FMD virus in a single test (Broonsvoort et al., 2008).

The aim of this study was to investigate the wild boar and red deer population in Croatia for the presence of FMD antibodies for the non structural protein of FMD virus. Even though the producers of ELISA kits used in our study did not declare wild animals as a target species we investigated whether these tests could be acceptable for the use in serological diagnosis of FMD in red deer and wild boars. Moreover, these are the first published results of such an investigation of FMD in wildlife species in Croatia.

MATERIAL AND METHODS

Animals and serum sampling

Samples from 52 red deer hinds, 1 to 11 years old (75 to 90 kg weight) kept in restricted hunting grounds, were collected. Hinds showed no signs of disease and were in good health condition. Most of the deer were imported from Hungary

while some of them originated from Croatia. Blood samples were collected by venepuncture after sedation (Rompun, Bayer AG).

Blood samples collected by heart puncture from 25 wild boars immediately after they were shot, were transferred to the laboratory in a refrigerator at 4ºC. Serum was eluted by centrifugation and frozen at -20ºC until analysis. Only sera without hemolysis were selected for testing.

Enzyme linked immunosorbent assays

Three commercially available FMD ELISA's were used: PrioCheck FMDV NS (Prionics Lelystadt B.V., The Netherlands), CHEKIT FMD-3ABC bo-ov (Dr. Bommeli AG, IDEXX Laboratories, Bern, Switzerland) and PrioCheck FMDV type O (Prionics Lelystadt B.V., The Netherlands) according to the manufacturer's instructions.

The PrioCheck FMDV NS (Prionics Lelystadt B.V., The Netherlands) and CHEKIT FMD-3ABC bo-ov (Dr. Bommeli AG, IDEXX Laboratories, Bern, Switzerland) detect antibodies against highly conserved non-structural (NS) proteins of FMD virus, whereas PrioCheck FMDV type O (Prionics Lelystadt B.V., The Netherlands) is a blocking ELISA and detects antibodies (Ab) against foot-and-mouth disease virus serotype O.

The optical density (OD) was measured by an automatic microplate reader TECAN Sunrise–Magellan (TECAN, Austria) at a wavelength of 450 nm.

STATA 6.0 software was used for descriptive statistic analysis of our results.

RESULTS

All of the tested sera were FMD antibody negative.

Table 1. Mean and standard deviation of corrected optical density (OD) in red deer and wild boars

ELISA kits	Species	Number	Mean of corrected OD	Standard deviation
PrioCheck FMD NS (>50% OD is considered as a positive result)	red deer	52	12.25	9.37
Checkit FMD 3ABC bo-ov (>30% OD is considered as a positive result)	red deer	52	2.37	3.76
PrioCheck FMD O (>50% PI is considered as a positive result)	red deer	52	8.28	8.52
PrioCheck FMD NS (>50% OD is considered as a positive result)	wild boar	25	1.61	10.72
PrioCheck FMD O (>50% PI is considered as a positive result)	wild boar	25	1.88	0.29

In our study the corrected optical density (OD) values for wild boars sera, as well as for red deer, did not show a large dispersion. OD values in red deer sera

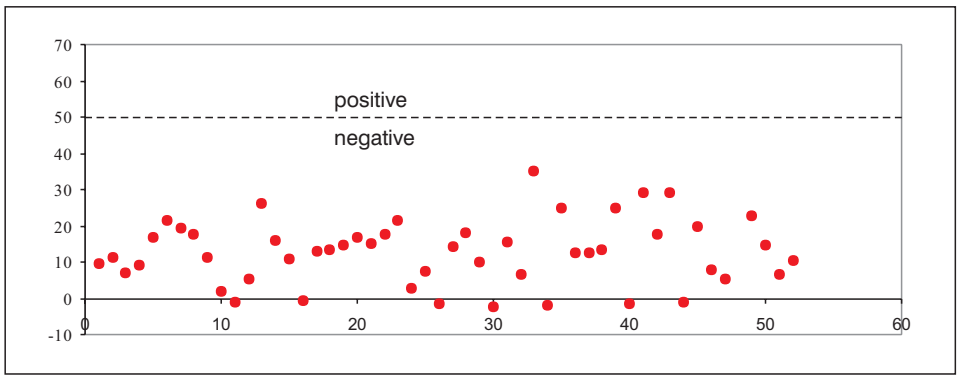

Figure 1. Red deer sera investigated by PrioCheck FMD NS

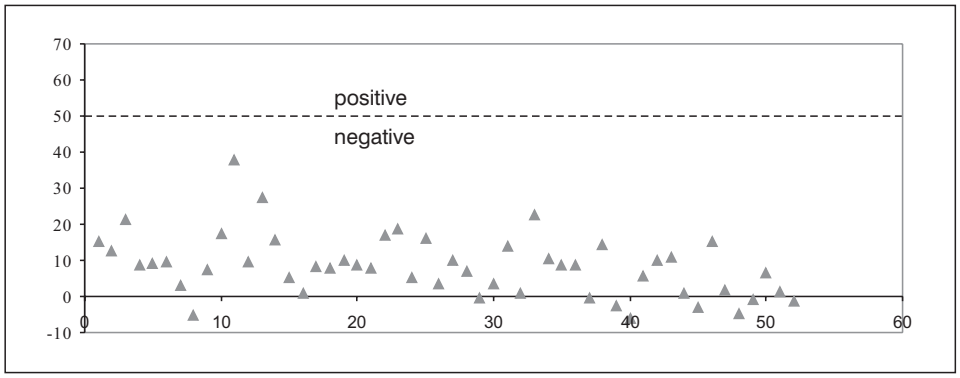

Figure 2. Red deer sera investigated by PrioCheck FMD O

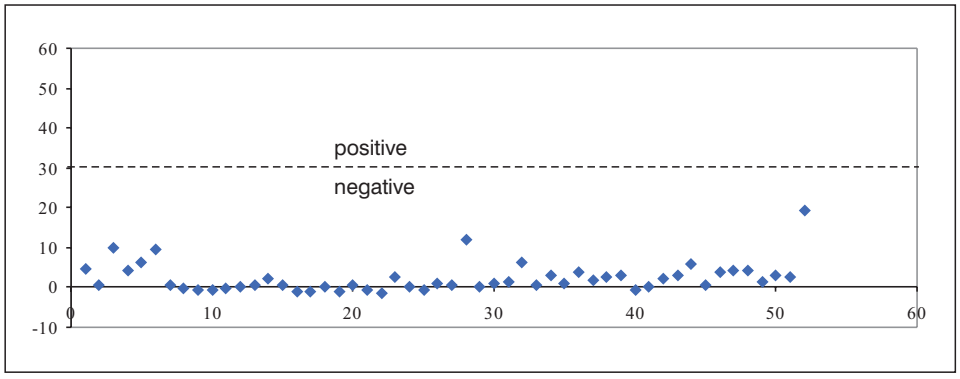

Figure 3. Red deer sera investigated by PrioCheck FMD 3ABC bo-ov

were distributed in the area of negative results (standard deviation was 9.37 for PrioCHECK FMD NS and 8.52 for PrioCHECK FMD O). Corrected OD values of red deer sera investigated by PrioCheck FMD 3ABC bo-ov also showed a small dispersion in the negative result area.

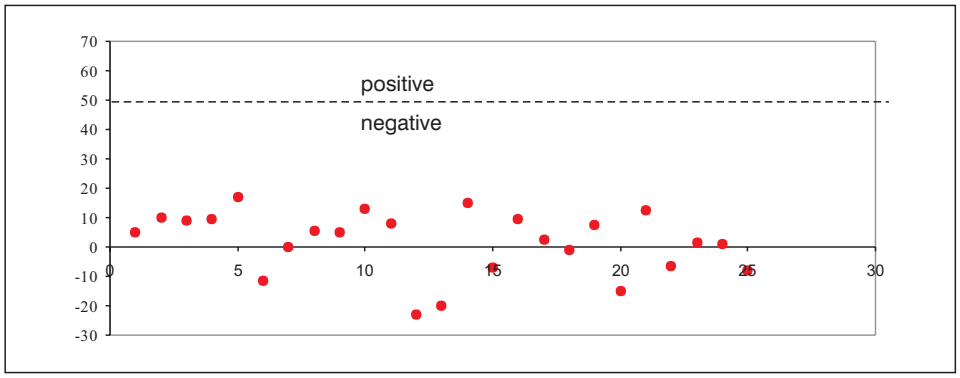

Figure 4. Wild boars sera investigated by PrioCheck FMD NS

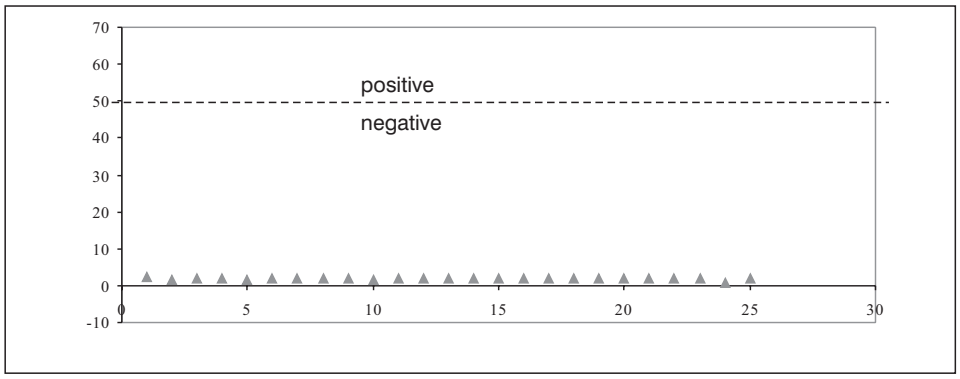

Figure 5. Wild boars sera investigated by PrioCheck FMD O

DISCUSSION

Nowadays the ELISA technology is widely applied in veterinary diagnostics. With the availability to be used routinely, it became a very reliable diagnostic tool. This study has been conducted in the Croatian Veterinary Institute in Laboratories that are responsible and accredited for the serological diagnosis, control and monitoring of FMD, as well as evaluation of documentation for marketing authorisation of veterinary medicines and diagnostics. PrioCHECK FMDV NS is an ELISA test authorized in Croatia and it has previously been used for trade and monitoring purposes in wild boars (unpublished data). However, red deer sera have never been investigated for FMD antibodies in Croatia.

Economically, FMD is one of the most important animal diseases worldwide (Domingo et al., 2002). Apart from domestic animals, wild animals have also been known as susceptible (Anderson et al., 1993). Infected red deer can spread the virus to cattle and sheep (Gibbs et al., 1975), however, Mouchantat et al. (2005) observed that FMDV has not been transmitted to free-ranging roe deer in adjacent areas of Germany during the FMD virus outbreak in the Netherlands in 2001 therefore the spread could be limited. Moreover, persistently infected wild animals very rarely transmit the infection (Thomson et al., 2003). Even so, survey and prediction of FMD virus transmission between wild and domestic animals (wild deer and feral pigs as reservoirs) is important for the eradication of FMD (Ward et al., 2007). The last FMD outbreak in Europe of serotype O was notified in January 2011 in Bulgaria (OIE Communication portal). The infection seemed to have been spread from infected wild boars to domestic pigs through contact with contaminated meat and meat products. Among different diagnostic methods that have been used, ELISA was used for the detection and control of the disease in cattle and pigs. The detection of antibodies to structural proteins confirms current or previous infections with FMD virus in unvaccinated animals, whereas antibodies towards non-structural proteins can be indicators of the disease in vaccinated animals.

The FMD antibody status in red deer and wild boars is unknown in our region. However, the biological and physiological similarities among wild and domestic animals, cohabitation and potential threat of viral spread encourages us to test the possibility of using commercial ELISA kits for this purpose. Serological studies for FMD antibody detection in wild animals usually is performed according to Hamblin et al. (1986). In accordance with the preliminary outbreak investigation carried out in Bulgaria in 2011 (Department for Environment, Food and Rural Affairs, DEFRA), Liquid-phase-blocking ELISA (LPBE) and virus neutralisation test (VNT) are prescribed for the detection of FMD antibodies in livestock. Similar procedures can be applied for antibody detection in wildlife, as well (Anonymous, 2011). During the Bulgarian outbreak seropositive roe deer reactors were not detected during the Bulgarian outbreak.

The results of sensitivity and specificity of the commercial ELISA tests in wildlife are limited and there are only few reported studies (Broonsvoort et al., 2008; Mohamed et al., 2011). Currently, it is recommended to combine enzyme-linked immunosorbent assays for FMDV antibody detection in wild animals with alternative tests such as the virus neutralisation test which is still considered the "gold standard" (Polichronova et al., 2010).

In regards the epizootical situation in Croatia and laboratory monitoring results our study results are in accordance with our expectation. The obtained negative results indicate the absence of any contact with FMD virus in the tested animals. ELISA kits that were used for FMD antibody detection in cattle and pigs are recommended by the OIE for routine diagnostic, especially for serological monitoring of FMD.

On the basis of the result distribution obtained in our study, we can assume that negative results of red deer sera obtained by all three different commercial ELISA kits are reliable. The PrioCHECK tests were proven to be useful tools for the

diagnosis and screening of wild boar populations towards FMD exposure. These assays have also the advantage of identifying all FMD serotypes and differentiating between vaccinated and naturally infected animals, therefore they are recommended by OIE, especially for screening purposes. However, there is no information about using these ELISAs in detecting antibodies in red deer. PrioCHECK FMD NS and PrioCHECK FMD O can be used to test cattle, pig, sheep and goat sera. Unfortunately, the validation of the kits for red deer is not fully possible due to the absence of referral positive samples. Therefore, further investigation is needed. We consider that this study also highlights the problem of validation of commercial ELISA kits due to the absence referral FMD antibodies originating from wild animals.

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ODREĐIVANJE PROTUTIJELA ZA VIRUS SLINAVKE I ŠAPA U DIVLJIH SVINJA I JELENA KOMERCIJALNIM IMUNOENZIMNIM TESTOVIMA (ELISA)

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SADRŽAJ

Serumi jelena (*Cervus elaphus*) i divljih svinja (*Sus scrofa*) pretraženi su na prisutnost protutijela za virus slinavke i šapa (SiŠ) pomoću tri komercijalna imunoenzimna testa (ELISA). Dva ELISA kompleta (PrioCHECK FMDV NS i CHEKIT FMD-3ABC) korišćena su za otkrivanje protutijela za nestrukturne proteine virusa SiŠ, a jedan komplet (PrioCHECK FMDV tip O) za dokazivanje protutijela za serotip O. Svi pretraženi serumi su bili negativni na prisutnost protutijela za virus slinavke i šapa.

Primijenjeni ELISA kompleti nisu deklarirani za primjenu u navedenih vrsta ali pretpostavka je bila da bi mogli poslužiti u istraživanju i serološkoj dijagnostici SiŠ u divljih svinja i jelena.

Provedena studija potvrdila je da su ELISA kompleti korišteni u našem istraživanju prihvatljivi za serološku dijagnostiku u obje istraživane vrste, međutim, svrha naše studije također je bila ukazati na potrebu validacije ELISA kompleta s pozitivnim uzorcima seruma jelena koji za sada nisu dostupni.