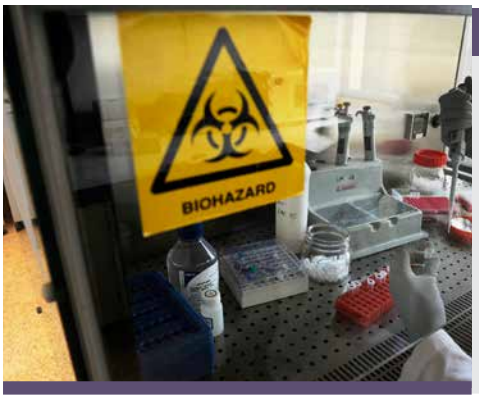

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ADVANCES

Options for non-invasive collection of saliva from wild ungulates for disease surveillance

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INTRODUCTION

In many parts of the world, the risk of wildlife being involved in the transmission of livestock diseases such as foot-and-mouth disease (FMD) is growing, together with wildlife populations (Kittelberger *et al*., 2011; Putman, Apollonio and Andersen, 2011; EFSA, 2012). Experience shows that surveillance in wildlife for early detection of the FMD virus (FMDV) is a crucial part of risk management (EFSA, 2012). The traditional collection of samples from hunter-harvested animals is time-consuming and logistically challenging. Hunting is limited in both time and space, and wildlife authorities and the general public do not always favour the killing of wild animals for sample collection purposes. In addition, the samples collected by hunters are often of poor quality. For these reasons, an early warning system for diseases such as FMD based on serological surveillance in wildlife is almost impossible to implement (EFSA, 2012). Development of a non-invasive (NI) methodology that would allow timely detection of pathogens through the collection of saliva is a viable option for overcoming some of these complications.

Several infectious agents are shed in oral fluid, including viruses such as FMDV, classical swine fever virus (CSFV) and porcine reproductive and respiratory syndrome virus (PRRSV) (Prickett and Zimmerman, 2010). The feasibility of using NI saliva collection from domestic swine on commercial farms (Hoffman *et al*., 2008) and from wild boar (Chichkin *et al*., 2012) for the diagnostics of various diseases has already been demonstrated. Experimental infection of wild boar (Breithaupt *et al*., 2012) showed that FMDV could be found in saliva several days before the detection of antibodies, and was still detectable until at least 27 days post-infection (DPI). FMDV was detectable in the oral fluids of some deer species until 28 to 63 DPI (Forman *et al*., 1974; Gibbs *et al*., 1975). In theory therefore, the testing of saliva enables both earlier detection and more effective monitoring than do traditional serological surveys for FMD and other pathogens in wildlife species.

One of the challenges confronting this approach is how to develop methods for the cost-effective and logistically simple collection

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Bait designs used in the study: 1) maize cobs with six swabs incorporated in each; 2) CSF vaccine bait used as attractant: 2a) vaccine in a blister replaced with a swab and incorporated into the bait; 2b) vaccine bait wrapped in cotton gauze and string; 2c) vaccine bait placed in plastic tubing wrapped in cotton string; 3a) and 3b) blocks of salt with holes to incorporate saliva-trapping swabs

of saliva from wild ungulates. In February 2013, the European Commission for the Control of Foot-and-Mouth Disease (EUFMD) and the Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases (EMPRES) of the Food and Agriculture Organization of the United Nations (FAO) conducted a small pilot field experiment in the framework of a study commissioned by the EUFMD Standing Technical Committee and funded by the European Commission to investigate the options for collecting saliva from three wild ruminant species – wild boar (*Sus scrofa*), red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) – using an NI approach.

EXPERIMENT DESCRIPTION

The study location Bobla Gora in Bulgaria is a 25-km^2 forest near the town of Tutrakan in Silistra Oblast (Alexandrov *et al*., 2011). In spring 2013, there were an estimated 220 red deer, 80 roe deer and 70 wild boar (at 8.8, 3.2 and 2.8 animals per square kilometre respectively). Animals were provided with regular supplementary feed as part of their normal management in this area. Additional experiments at salt licks were conducted at a separate location in Teteven (Lovec Oblast). Three main types of bait were developed incorporating swabs to trap saliva (see page 15). The baits were distributed to seven feeding sites (four for wild boar and three for red deer) and bait uptake (including bait taken by non-target species), consumption by target species and swab recovery rates were recorded for four nights (19 to 23 February 2013).

Feeding sites were 2 to 3 km apart. At each of the four wild boar feeding sites, five baits of type 1, three of type 2a, and two of type 2b were distributed every night (ten baits per site); four baits of type 2c were distributed once. At two of the red deer feeding locations baits of types 1 and 3 were used every night (five maize cobs and one block of salt with three swabs). The third red deer site was baited with ten maize cobs for one night. Three salt licks for roe deer were also established in Teteven, with blocks of salt wrapped in cotton gauze. Four camera traps on infrared photo (at 15-second intervals) or video mode were set up to monitor site attendance 24 hours a day in Bobla Gora. Animal tracks were recorded at the sites without camera observation.

All baits and data from camera traps were checked daily. Baits that were consumed or disappeared were replaced. All the recovered baits or parts of baits were recorded. Recovered swabs and cotton material visibly contaminated with saliva were collected into individually labelled sealable plastic bags for further testing for the presence of species-specific desoxyribonucleic acid (DNA). The statistical significance of differences in bait performance was assessed with the Fisher Exact Test (FET) using the GraphPad Software (2013) online calculator. All P values are two-tailed.

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Collection of saliva samples from the baits taken by wild boar for DNA testing

RESULTS

Site attendance: Over the 20 bait nights at different locations there were a total of seven wild boar and six red deer visits (35 and 30 percent success rates respectively). Newly established salt licks were visited twice by red deer. Wild boar feeding sites were attended three times by a family group of five wild boar; once by a group of six; once by a group of unknown size; and twice by a single male wild boar. The red deer herd sizes were two, 17, three and 12 animals, with two cases when it was not possible to identify the herd size. Based on the observations and bait uptake results, this implies that a minimum of three family groups of wild boar totalling at least 11 animals, and six herds of red deer totalling at least 32 animals were sampled – 15.7 and 15.4 percent of their respective population estimates. Two salt lick locations in Teteven (with 44 bait days from 17 March to 10 April 2013) were attended 28 times by a total of 84 wild boar in groups of one to six animals (a 63.6 percent attendance rate), and 13 times by a total of 22 roe deer in groups of one to three (a 29.5 percent attendance rate). Wild boar visited sites from 21:00 to 24:00, while deer attended mostly in the morning and evening.

Bait performance: Of the 210 exposed baits of all types, 54.8 percent were taken by both target and non-target species, 39.0 percent by target species only, and 30.5 percent were recovered with swabs intact (Table 1) – an average of 3.3 baits were needed to collect one swab sample.

Type 1: Maize cobs were consumed by both wild boar and red deer. Most of the six swabs (5.0 + 1.1) remained inside the cobs and could be recovered. Of the 125 maize cobs exposed, 49.6 percent were taken, 44 percent were consumed by target species (24 percent by wild boar and 20 percent by red deer, difference non-significant). A few cobs were eaten or destroyed by jay (*Garrulus glandarius*) and possibly badger (*Meles meles*). Swabs were recovered from 37.6 percent of the exposed cobs. Sites attended by wild boar only (five bait nights, 25 cobs exposed) and red deer only (four bait nights, 25 cobs exposed) were compared. No significant difference in the swab recovery rate between species was found (P = 0.1963).

Types 2: All of these bait subtypes were consumed by wild boar. The survey team found no significant differences in the performance of different subtypes, which means that the placement of swabs (inside the blister or simply wrapped around the bait) does not significantly influence their performance. The blisters containing swabs were recovered close to the exposal site (within a 10-m radius) and could be recognized by signs of chewing. Vaccine baits wrapped in cotton (2b) were often swallowed by animals. All four baits with vaccine inside plastic tubes (2c) were recovered on the site. Overall, 67.5 percent of the three bait subtypes were taken, but the target species accounted for only about half of this uptake. The other half was taken by non-target animals, which might include (based on traces, as no camera evidence is available) jays, badgers, jackals (*Canis aureus*), domestic dogs and possibly rodents. In summary, 32.5 percent of all exposed baits were consumed by wild boar and 20.8 percent were recovered with swabs.

Data from the four wild boar sites baited with only vaccine (n = 77) or only maize (n = 75) made it possible to compare their respective performances in wild boar more

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Red Deer attending feeding location at Bobla Gora as recorded by the camera trap

Table 1: Performances of different baits at Bobla Gora, 19 to 24 February 2013

Bait type	Exposed bait/nights	Total bait uptake		Bait uptake by target species		Baits recovered with swabs	
		n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
1. Maize cobs (all)	**125**	**62**	**49.6 (40–58)**	**56**	**44.8 (36–54)**	**47**	**37.6 (29–47)**
1. Wild boar sites	75	37	49.3 (38–61)	20	26.7 (17–38)	17	22.7 (13–33)
2. Vaccine (all)	**77**	**52**	**67.5 (56–78)**	**25**	**32.5 (22–44)**	**16**	**20.8 (12–32)**
2a. Blisters	45	30	66.7 (51–80)	12	26.7 (15–42)	10	22.2 (11–37)
2b. Cotton	22	18	81.8 (60–95)	10	45.5 (24–68)	3	13.6 (3–35)
2c. Plastic	10	4	40.0 (12–74)	3	30 (7–65)	3	30 (7–65)
3. Salt licks	**8**	**1**	**12.5 (0–53)**	**1**	**12.5 (0–53)**	**1**	**12.5 (0–53)**
Total	**210**	**115**	**54.8 (48–62)**	**82**	**39.0 (32–46)**	**64**	**30.5 (24–37)**

CI = confidence interval. See Figure 1 for a description of bait types. Wild boar sites are wild boar feeding sites baited with both maize and vaccine.

accurately. Wild boar consumed the vaccine baits slightly more frequently than the maize baits (32.5 and 26.7 percent respectively), but this difference was not statistically significant (P = 0.4799). Swab recovery rates did not differ significantly (20.8 for vaccine and 22.8 percent for maize baits, P = 0.8452).

Type 3: Of the eight bait nights with this type of bait, the two newly established salt licks were attended only once by red deer, and saliva could be collected from all the swabs drilled into the salt block. At the location in Teteven, two salt licks were regularly attended by wild boar and roe deer. Blocks of salt were wrapped in a single layer of cotton gauze to collect saliva. This method seemed to work well, and visibly contaminated samples of cotton were collected.

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CONCLUSION

At Bobla Gora, with a total site attendance rate of 65 percent, samples of saliva were collected using NI methods from as much as 15 percent of the local population of wild boar (three family groups) and red deer (six herds) in just four days. One group of wild boar was sampled repeatedly. Twenty-two days of camera trap observations in Teteven showed that wild boar and roe deer attended salt licks nearly every day, thus providing opportunities for the continuous collection of saliva samples using this approach.

Overall, the bait types at Bobla Gora achieved an average uptake rate of 39.0 percent (ranging from 32 to 46 percent) and a swab recovery rate of 30.5 percent (24 to 37 percent). Maize cobs allowed saliva collection from two species, and had better target species uptake and swab recovery rates. Wild boar and red deer ate maize cobs equally frequently (20 to 25 percent). Generally, maize cobs appeared to be the most efficient bait for NI saliva collection, as they could cover two species and required an average exposure of 2.7 baits for each recovered sample from either wild boar or red deer. Vaccine baits targeted only one species (wild boar) and approximately half of them were taken by non-target species. Swab recovery rates from vaccine baits were close to those achieved with maize cobs at all maize-baited locations and at those baited for only wild boar. Collection of saliva at salt lick sites appears to be a feasible method for both deer species and wild boar, which occasionally lick the salt. More observations are needed to quantify these findings more accurately.

This NI sampling approach will be developed further through follow-up on experiments and laboratory tests, including species DNA and pathogen genome detection. However, several advantages can already be identified. First, the NI sampling does not involve the killing of animals, and no dispersal of potentially infected individuals occurs. Second, the sampling scheme can be adjusted according to the pathogen's stability in the environment, the level of disease risk, the season, and the composition, population density and susceptibility to disease of host species; the approach is easy to incorporate into existing game management practices worldwide, and samples can easily be collected by non-professionals such as hunters, gamekeepers and wildlife specialists. Third, the method is relatively inexpensive and available in developing countries, and also has the potential for application in extensive farming systems, such as for monitoring FMD in small ruminants attending salt licks or for sample collection from pigs (V. Milicevic, personal communication). The production of baits with swabs could be commercialized using existing wild boar attractants or salt licks for wild ruminants. 360