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# To sample or not to sample? Detection of African swine fever in wild boar killed in road traffic accidents

Katja Schulz[1] | Franz Josef Conraths[1] | Christoph Staubach[1] | Arvo Viltrop[2] | Edvīns Oļševskis[3,4] | Imbi Nurmoja[2,5] | Kristīne Lamberga[3,6] | Carola Sauter-Louis[1]

[1]Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Epidemiology, Greifswald-Insel Riems, Germany

[2]Estonian University of Life Science, Institute of Veterinary Medicine and Animal Sciences, Tartu, Estonia

[3]Food and Veterinary Service, Riga, Lativa

[4]Institute of Food Safety, Animal Health and Environment - "BIOR", Riga, Lativa

[5]Estonian Veterinary and Food Laboratory (VFL), Tartu, Estonia

[6]Latvian University of Life Sciences and Technologies, Jelgava, Lativa

**Correspondence**
Katja Schulz, Friedrich-Loeffler-Institut, Südufer 10, 17493 Greifswald-Insel Riems, Germany.
Email: katja.schulz@fli.de

**Abstract**

African swine fever (ASF) in wild boar remains a threat for the global pig industry. Therefore, surveillance is of utmost importance, not only to control the disease but also to detect new introductions as early as possible. Passive surveillance is regarded as the method of choice for an effective detection of ASF in wild boar populations. However, the relevance of wild boar killed through road traffic accidents (RTA) for passive surveillance seems to be unclear. Using comprehensive ASF wild boar surveillance data from Estonia and Latvia, the prevalence of ASF-infected wild boar was calculated and the probability of infection as measured by PCR compared for animals that were hunted, found dead, shot sick or killed in a RTA. The number of samples originating from wild boar killed in a RTA was low and so was the ASF prevalence in these animals. However, the reasons for this low number of RTA animals remain unknown. Therefore, we recommend to sample wild boar killed in a RTA to a greater extent, also to explore, if this approach can increase the detection probability, and to avoid missing disease introduction.

KEYWORDS

African swine fever, detection probability, prevalence, road traffic accidents, wild boar

## 1 | INTRODUCTION

African swine fever (ASF) continues to threaten the pig industry globally. The current epidemic of ASF genotype II that started in Georgia in 2007 has now lasted for more than 10 years and has not been brought under control so far (Cwynar, Stojkov, & Wlazlak, 2019; Sanchez-Cordon, Montoya, Reis, & Dixon, 2018).

It is known that the probability of ASF detection in wild boar is highest through passive surveillance, that is the sampling of dead, injured or sick animals (European Food Safety Authority, 2018, 2019; Nurmoja et al., 2017; Schulz, Oļševskis, et al., 2019). Thus, estimating the prevalence of ASFV-positive, that is PCR-positive, wild boar helps evaluating the extent of the disease. Passive surveillance activities are implemented in many countries including member states of the European Union. The European Commission (EC) emphasized in a working document the focus to sample found carcasses and sick wild boar. Sampling wild boar killed in RTA in areas free from ASF and in neighbouring areas of affected zones is also recommended (European Commission, SANTE/7113/2015—Rev 11). The European Food Safety Authority (2019) also stated that it differs from country to country, if wild boar killed in RTA are included in passive surveillance activities or not. In general, wild boar are frequently involved in RTAs (Benten, Balkenhol, Vor, & Ammer, 2019; Jakubas, Rys, & Lazarus, 2018; Kruuse, Enno, & Oja, 2016; Kusta, Keken, Jezek, Hola, & Smid, 2017; Saenz-de-Santa-Maria & Telleria, 2015). It is not clear, however, whether the risk of an ASF-infected wild boar of getting involved in a RTA is increased as compared to an uninfected wild boar. This issue has been intensively discussed, and there are arguments in favour and against the hypothesis, but the number of available datasets has not been sufficient to come to a scientifically sound conclusion. These observations and the recent detection of an ASF case in a wild boar potentially involved in a RTA in a newly affected area in the province Lubusz in Western Poland initiated the present study. To use every chance for improving the efficacy of ASF surveillance and minimize the risk of missing an early ASF case, we aimed to investigate the potential role of wild boar killed through a RTA in the detection of ASF in wild boar.

## 2 | MATERIALS AND METHODS

To investigate and to validate the role of samples from wild boar killed in RTA in ASF surveillance and the early detection of the disease, data from ASF-affected countries had to be used. Thus, for the study, ASF wild boar surveillance data from Estonia and Latvia were used. The data originated from the CSF/ASF wild boar surveillance database of the EU Reference Laboratory (https://public.surv-wildboar.eu/Default.aspx). The available dataset consisted of 108,617 data records. Each record included information on a single animal. It comprised information about the date of sampling, the age, the ASF PCR result and the origin of the sample ('hunted', 'found dead', 'shot sick', 'killed in a RTA', these categories are referred to as 'carcass code' in the following). Data with missing information regarding the carcass (nine samples) or the PCR test result (384 samples) were excluded. Therefore, 108,224 data records were used in the analyses. The final dataset covered the period from 25th June 2014 until 30th September 2019 and included only months, in which PCR-positive wild boar were detected.

All statistical analyses were performed using the software package R (http://www.r-project.org). To test for statistically significant associations between the carcass code and the ASF PCR result on animal level, a Fisher's exact test was performed. In the case of a significant association, each carcass code was tested separately against each other using Fisher's exact test. To control the type I error for multiple testing, a Bonferroni adjusted p-value was calculated for each individual test (Dunn, 1961). A $p$-value of $\leq .05$ was considered statistically significant.

The ASF prevalence for each carcass code was determined on the basis of ASF PCR-positive results, and confidence intervals were calculated according to Clopper and Pearson (1935).

## 3 | RESULTS

Most samples that were available and tested by ASF PCR originated from hunted animals, and wild boar found dead. Only 26 samples were investigated from wild boar shot sick, and 99 samples came from animals that had been killed in a RTA (Table 1).

Out of 99 RTA samples in total, 36 were recorded in 2017, whereas in 2015, no sample were registered as originating from animals killed in a RTA. In the remaining years, between 6 and 33 samples came from wild boar involved in RTA. Except for 2014, where no samples were recorded as originating from wild boar killed in RTA in Estonia, the majority of samples of wild boar killed in a RTA came from Estonia (Figure 1).

The estimated ASFV prevalence was highest in wild boar found dead (0.735; CI 0.722–0.747), whereas the prevalence in animals killed in a RTA yielded the lowest value (0.010; CI 0.0003–0.055) (Figure 2). The one ASF-positive wild boar killed through a RTA was found in Estonia in 2017 (infected zone).

The association between the carcass codes and the ASF PCR result was statistically significant ($p < .001$). In the direct comparison of the individual carcass codes vice versa, most differences were significant ($p < .001$; Table 2). Only the difference between the probability to find an ASF-positive PCR result in hunted wild boar and wild boar killed in a RTA was not statistically significant ($p = 1$).

**TABLE 1** African swine fever PCR-positive and negative wild boar samples from Estonia and Latvia and their origin

| | Hunted | Found dead | Shot sick | Road traffic accident |
|---|---|---|---|---|
| PCR negative | 100,627 | 1,372 | 19 | 98 |
| PCR positive | 2,301 | 3,799 | 7 | 1 |

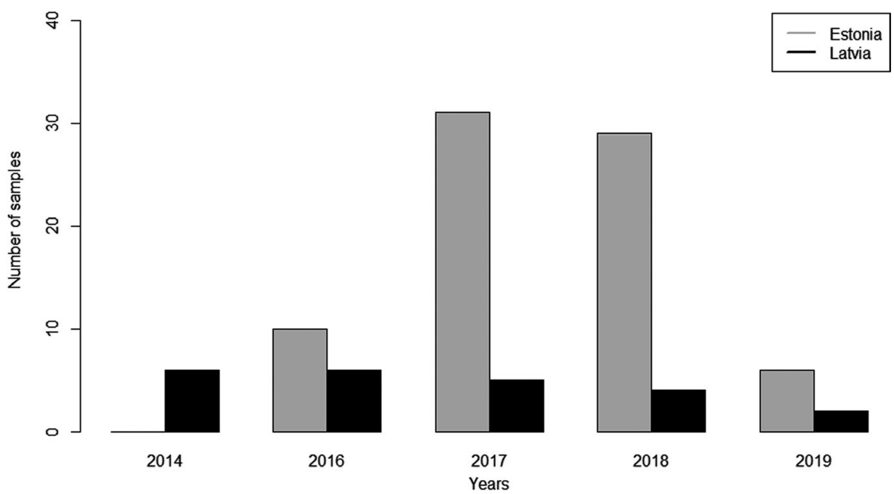


**FIGURE 1** Number of samples originating from wild boar killed in road traffic accidents (RTA) by country and year. In 2015, no samples came from animals killed in a RTA

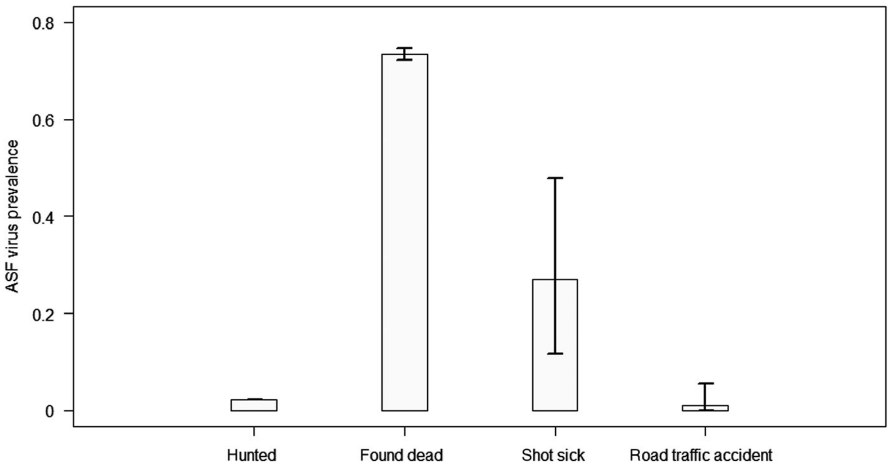


**FIGURE 2** African swine fever virus prevalence in hunted, found dead, shot sick wild boar and animals killed in a road traffic accident. The whiskers indicate 95% confidence intervals

## 4 | DISCUSSION

Recommendations regarding passive surveillance have so far focused mostly on the sampling of wild boar found dead. However, a large number of road traffic accidents including wild life are caused by wild boar (Kruuse et al., 2016; Kusta et al., 2017; Saenz-de-Santa-Maria & Telleria, 2015). ASFV-infected wild boar may be altered in their behaviour, and their risk of becoming involved in a RTA may be increased. Therefore, the chance of detecting ASF-positive wild boar killed in a RTA may also be heightened, particularly in the case of external manipulations like driven hunts. However, there is no scientific evidence so far of an increased probability for ASF-diseased wild boar to become involved in a RTA.

The plenitude of surveillance data originating from the Baltic States allowed to investigate the ASF prevalence in wild boar killed in RTA and to compare the probabilities of detecting ASF-positive wild boar in hunted, found dead, shot sick wild boar and animals killed in a RTA. Despite of the huge number of investigated samples, only 99 samples were obtained from animals registered as killed in a RTA. This small number might be due to several reasons. Wild boar killed in a RTA has not always been recorded in the laboratory database as such, because the information was sometimes missing in the sample submission form as the main source of epidemiological information. Therefore, there may be a reporting bias. Specifically, in Estonia, a field, where pre-formed information on RTA or shot sick as the cause of death could be entered, was missing on the sample submission form until February 2017. In Estonia, hunters have been obliged to report wild boar involved in RTA to the veterinary authority since 2015. Thus, all earlier reports of RTA originate from additional remarks made by veterinary officers or hunters on the sample submission forms. In all other cases, we may suppose that the animals killed in a RTA, were reported as animals 'found dead'. Also, it is conceivable that animals, which were originally involved in RTA and not immediately killed and could retract, are not easily identifiable as such and therefore wrongly reported as 'found dead'. Therefore, an underestimation of samples coming from wild boar killed by RTA is very likely.

**TABLE 2** Results of the Fisher's exact tests, testing the ASF prevalence within each carcass code separately against each other

| | Found dead | Shot sick | Road traffic accident |
|---|---|---|---|
| Hunted | $p < .001$ | $p < .001$ | $p = 1$ |
| Found dead | | $p < .001$ | $p < .001$ |
| Shot sick | | | $p < .001$ |

Moreover, the disposal of wild boar carcasses associated with RTA is often organized through road agencies, which may dispose carcasses before sampling. Even in Estonia, where hunters are responsible for the disposal of wild boar killed in RTA, reporting of these animals has not been compulsory. It can be assumed that wild boar density, wild boar habitats, road conditions and traffic volume differ between countries, so that our study, which was conducted with data from Estonia and Latvia, may have yielded results that cannot necessarily be generalized. However, as mentioned earlier, several studies from different countries contain a large proportion of wild boar killed in RTA (Benten et al., 2019; Jakubas et al., 2018; Kusta et al., 2017). A study from Estonia reported an increasing number of wild boar and as a consequence also an increasing number of wild boar killed in a RTA during the years 2004–2013 (Kruuse et al., 2016). This study was conducted before the wild boar density in Latvia and Estonia had decreased due to ASF and measures to control the disease had been implemented (Nurmoja et al., 2017; Schulz, Staubach, et al., 2019). Therefore, the number of wild boar killed in RTA might has decreased after 2015. Due to regulations, in Estonia, no samples were recorded as originating from wild boar killed in a RTA in 2014 and 2015, making an evaluation of ASF results from animals killed in RTA before 2016 impossible. Nonetheless, the numbers of samples originating from wild boar killed in a RTA probably represent only a small proportion of wild boar that were in fact killed in a RTA. The ASFV prevalence in wild boar killed in RTA was very low; however, the low sample size and the very low number of positive cases have to be considered. Following these findings, sampling those animals might be considered as unnecessary and uneconomical. Although the detection, sampling and disposal of wild boar found dead usually requires huge personal and financial efforts, the significantly higher probability of detecting a new ASF case clearly justifies this. If wild boar killed by a RTA is sampled, however, the benefits compared to the efforts, also for the traffic authorities, which often have to be involved in many countries, are questionable.

Yet, additional efforts to sample wild boar killed in a RTA should still be considered because it is not clear, whether the risk for an ASF-infected wild boar to be killed in a RTA is increased as compared to an uninfected animal. Also, neither the true number of wild boar involved in RTA nor the true proportion of sampled animals is known. To clarify this, more samples from wild boar killed in RTA are necessary.

In our study, the probability to detect an ASF-positive animal among hunted wild boar was not significantly higher than among wild boar involved in a RTA. Therefore, our results suggest that there is no additional benefit in sampling wild boar involved in RTA compared to sampling hunted wild boar. However, the results have to be interpreted within the framework of this study and the available data. More data with more reliable reporting of the origin of the samples might support the recommendations to sample wild boar killed through RTA. Also, samples originating from wild boar involved in RTA are not dependent on hunters, hunting preferences and hunting season and might therefore be better and more reliably available, especially in countries with high wild boar density and traffic volume.

Along with the current ASF strategy of the EC (European Commission, SANTE/7113/2015 - Rev 11), we recommend, as far as feasible and economically reasonable, to sample every wild boar found dead, no matter if killed through a RTA or died due to any other reason, and test it for ASF. This applies for affected areas, in which testing and removal also wild boar that were killed in an RTA can reduce the number of ASF-positive cadavers in the environment and thus contribute to mitigating the risk of new infections. Also, in risk areas, particularly in the case of a high risk of disease introduction, any delayed detection of ASFV introduction could thus be avoided. At the same time, meticulous recording of the cause of death of the animals (hunted, found dead, shot sick or killed in a RTA) is needed to allow further analyses.

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## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

## ETHICAL APPROVAL

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as no animal experiments were conducted.

## DATA AVAILABILITY STATEMENT

The original data used for the analyses can be obtained from the author after approval by the responsible institution in Estonia and Latvia.

## ORCID

*Katja Schulz* https://orcid.org/0000-0003-1816-6554
*Carola Sauter-Louis* https://orcid.org/0000-0002-6440-1933

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