

The surveillance programme for diseases in wild boars in Norway 2023



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Authors

Carl Andreas Grøntvedt, Anne Nordstoga, Inger Sofie Hamnes, Sokratis Ptochos, Bjarne Bergsjø, Anne Margrete Urdahl, Christer Moe Rolandsen*, John Odden*, Jørn Våge, Knut Madslien and Attila Tarpai *Norwegian Institute for Nature Research (NINA)

Suggested citation

Grøntvedt, Carl Andreas, Nordstoga, Anne, Hamnes, Inger Sofie, Ptochos, Sokratis, Bergsjø, Bjarne, Urdahl, Anne Margrete, Rolandsen*, Christer Moe, Odden*, John, Våge, Jørn, Madslien, Knut and Attila Tarpai. The surveillance programme for diseases in wild boars in Norway 2023. Surveillance program report. Veterinærinstituttet 2024. © Norwegian Veterinary Institute, copy permitted with citation

Quality controlled by

Merete Hofshagen, Director of Animal Health, Animal Welfare and Food Safety, Norwegian Veterinary Institute

Published

2024 on www.vetinst.no
ISSN 1890-3290 (electronic edition)

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Commissioned by / In collaboration with

Norwegian Food Safety Authority



Colophon

Cover design: Reine Linjer

Cover photo: ©Martin Blom/Runhild Gjelsvik

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Summary

Wild boar health surveillance was re-established in August 2018 to gain insight into the incidence of pathogens of importance for animals and humans and to enable early detection of notifiable diseases in this expanding species.

In 2023, samples from 373 wild boars were submitted to the Norwegian Veterinary Institute, representing approx. 90% of harvested wild boar, as reported to Statistics Norway (SSB) during the partially overlapping hunting year of 2022/2023. In addition, one harvested wild boar with bruising and subcutaneous bleeding, and one wild boar found dead on the shoreline of a small lake were submitted by the Norwegian Food Safety Authority (NFSA) as a part of passive surveillance for African swine fever virus (ASFV) and classical swine fever virus (CSFV), with no agents detected.

Furthermore, all samples were negative for antibodies against the following notifiable pathogens: Aujeszky's disease virus (ADV), transmissible gastroenteritis virus (TGEV), porcine respiratory and reproductive syndrome virus (PRRSV), porcine epidemic diarrhoea virus (PEDV) and swine influenza virus (SIV) (these are part of the surveillance programme for specific viral diseases in domestic pigs). Antibodies against *Mycoplasma hyopneumoniae*, an agent that was eradicated from the domestic pig population in 2008 and has not been detected since, were not detected.

Serum from a single male wild boar was positive for antibodies against porcine respiratory corona virus (PRCV). It was concluded that the seroreaction most likely was a false positive, as no other wild boar from the same area had antibodies against PRCV.

The following species and serotypes (n) of Salmonella spp. were detected in faeces from eight hunted wild boars: Salmonella Abony (2), Salmonella Typhimurium (1), Salmonella Hessarek (1), S. enterica subsp. diarizonae serovar 42:r:z (1) and S. enterica subsp. diarizonae serovar 38:r:z (3). All isolates were fully susceptible to all tested antimicrobial agents included in the test panel.

Parasitological analysis did not demonstrate presence of *Trichinella* larvae.

Sammendrag

Villsvinhelseovervåkning for 2018 ble initiert og gjennomført av Veterinærinstituttet, og fra 2019 reetablert som et løpende overvåkningsprogram i regi av Mattilsynet for å øke kunnskapen om forekomst av patogene mikroorganismer med betydning for dyre- og folkehelse, og for tidlig å kunne oppdage meldepliktige dyresykdommer hos en art på fremmarsj i Norge.

I 2023 ble det sendt inn prøver fra 373 villsvin til Veterinærinstituttet. Dette representerer om lag 90 prosent av antallet felte villsvin som ble rapportert til Statistisk sentralbyrå (SSB) i jaktåret 2022/2023, men periodene er ikke fullstendig overlappende. Ett villsvin felt under jakt med bloduttredelser og blødning, og ett villsvin påtruffet dødt langs vannkanten av en innsjø ble sendt inn av Mattilsynet som en del av den passive overvåkningen for afrikansk og klassisk svinepest, men disse virussykdommene ble ikke påvist.

Det ble ikke påvist antistoff mot de alvorlig meldepliktige svinepatogenene Aujeszky's disease virus (ADV), smittsom gastroenterittvirus (TGEV), porcint respiratorisk og reproduksjonssyndromvirus (PRRSV), porcin epidemisk diarévirus (PEDV) eller influensa A virus (SIV). Dette er smittestoff som også er gjenstand for overvåking i overvåknings- og kontrollprogrammet for spesifikke virussykdommer hos tamsvin. Det ble heller ikke påvist antistoff mot *Mycoplasma hyopneumoniae*, et agens som forårsaker smittsom grisehoste hos tamsvin og som har vært gjenstand for systematisk bekjempelse i den norske svinepopulasjonen. Siste påvisning av smittsom grisehoste i Norge var i 2008.

I prøver fra ett hanndyr av villsvin felt under jakt i Marker ble det påvist antistoff mot porcint respiratorisk koronavirus (PRCV). Det vurderes som mest sannsynlig at antistoffpåvisningen skyldes en falsk positiv reaksjon, da det ikke har vært påvist antistoff mot PRCV i prøver fra andre villsvin felt i samme område.

Salmonella spp. med følgende serotyper (n) ble påvist i avføringsprøve fra åtte villsvin felt under jakt: Salmonella Abony (2), Salmonella Typhimurium (1), Salmonella Hessarek (1), S. enterica subsp. diarizonae serovar 42:r:z (1) og S. enterica subsp. diarizonae serovar 38:r:z (3).

Parasittologiske undersøkelser påviste ikke forekomst av *Trichinella* spp. larver.

Background

During the last decade wild boar (*Sus scrofa*) populations have been established mainly in a core area, the south-eastern part of Norway, bordering Sweden. A few solitary animals have also been observed or shot by hunters several hundred kilometres north of this. Hunting statistics (Statistics Norway (SSB), www.ssb.no) documented a steadily increasing number of wild boars harvested since 2014/2015, however the number of harvested wild boars seems to have stabilised since 2020/2021. The Norwegian Veterinary Institute (NVI) initiated a comprehensive wild boar health surveillance in 2018. This was based on a surveillance in 2011-2014, financed by the Norwegian Food Safety Authority (NFSA), discontinued because of low sample submission rate. From 2019, the NFSA included parts of the wild boar health surveillance in their surveillance programmes for terrestrial animals, and the surveillance is now run in collaboration with the NVI. Furthermore, additional pathogens were included through project-based financing and self-funding by the NVI. Specifically, the serological investigation for antibodies against *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) was financed by Animalia (The Norwegian Pig Health Service) and the NVI funded the analyses for antimicrobial resistance (AMR).

To promote the submission of samples for testing, the NFSA, since July 2020, pays a compensation to hunters for the submission of samples. From October 2023, land-owners of properties were wild boar are harvested, are also eligible for compensation by the NFSA. Free-of-charge testing for *Trichinella* spp. has also been included since 2018.

The wild boar health surveillance includes the same pathogens as the national surveillance programme for specific viral infections in domestic pigs, with additional analyses for for *Mycoplasma hyopneumoniae*, the parasite *Trichinella* spp., and bacteriological analyses for *Salmonella* spp. The national surveillance programme for specific viral infections in domestic swine was launched in 1994, and documents Norway's disease-free status for Aujeszky's disease, and the status of transmissible gastroenteritis (TGE), porcine respiratory corona virus (PRCV), porcine respiratory and reproductive syndrome (PRRS), porcine epidemic diarrhoea (PED) and swine influenza (SI) in the Norwegian swine population.

Aims

The aims of the wild boar health surveillance are to investigate the health status, the prevalence of selected agents, and the early detection of disease in the expanding wild boar population in Norway. The surveillance is designed with a particular focus on notifiable diseases, zoonoses, agents under active surveillance in the domestic pig population and agents with a potential for transmission between wild and domestic pigs.

Material and methods

Sampling and data collection

Purpose-built sample collection kits were distributed to hunters, including sample collection and submission information forms. These forms also had a printed QR-code that linked to the Norwegian Cervid Register (Hjorteviltregisteret, the national database for data from hunted cervids and wild boar), were data on the sampled animal (incl. gender, age group, dressed weight and carcass weight), geographic reference to the location where the animal was harvested and number of wild boars observed in the immediate vicinity of the harvested wild boar was collected. Distribution of kits was done via municipal wildlife managers, the local offices of the NFSA and also upon request directly to hunters and personnel involved in searching for animals injured by hunting or traffic accidents. Before distribution of sample collection kits, the NVI hosted an open seminar in August 2018 to provide wildlife management personnel and hunters with background information about wild boars and health surveillance, and to demonstrate sampling of wild boar carcasses. The forms also included contact information to the NVI and NFSA. In addition to the written information, the sample collection kits included, sterile 25 ml screw-cap containers for collection of skeletal muscle, faeces and blood, disposable gloves and an insulated pre-paid return envelope.

We used observations from the SCANDCAM camera trap network, the species observation system ("Artsobservasjoner"), individuals dying from other causes than hunting ("fallviltregisteret"), and the location of harvested wild boar reported to NINA and NVI to estimate the wild boar distribution range. The main distribution of wild boar is today found along the Swedish border from Halden municipality in the south to Elverum municipality approx. 300km further north. The majority of observations are found in the far south in the municipalities of Aremark and Halden. Dispersing individuals can be expected to occur over larger parts of Southern Norway (Odden et al. 2023).

Laboratory analyses

All serological and bacteriological analyses were performed at the NVI. Skeletal muscle samples were submitted to the National Veterinary Institute (SVA) in Uppsala, Sweden for *Trichinella* spp. analysis. Samples with positive or inconclusive results on serological analysis were retested in duplicate with the same test method. Samples were concluded as negative if the retests gave a negative result.

Serological analyses

Aujeszky's disease/pseudorabies virus (ADV/PRV)

All serum samples were tested for antibodies against ADV using a commercial blocking ELISA from Svanova (SVANOVIR® PRV gB-Ab).

Transmissible gastroenteritis virus (TGEV) and porcine respiratory coronavirus (PRCV) Samples were tested with either one or two commercial blocking ELISAs (SVANOVIR® TGEV/PRCV-Ab, Svanova and Swinecheck®TGEV/PRCV, Biovet. The ELISA tests enable discrimination between antibodies to TGEV and PRCV in serum samples.

Porcine reproductive and respiratory syndrome virus (PRRSV)

All serum samples were tested for antibodies against PRRSV using a commercial indirect ELISA from IDEXX (IDEXX PRRS X3), which detects the most (pre)dominant type 1 and type 2 strains of PRRSV. PRRSV positive or inconclusive samples were tested with a confirmatory ELISA at the NVI (ID Screen® PRRS Indirect).

Swine influenza virus (SIV)

A commercial competitive ELISA from IDvet (ID Screen® Influenza A Antibody Competition, Multi-species) was used to screen serum samples from swine for antibodies against influenza A virus. In cases of positive or inconclusive results, the serum samples were retested using the haemagglutination inhibition (HI) test, for the detection of antibodies against the A/Swine/California/07/09 (A/H1N1/pdm09), A/Swine/Belgium/1/98 (H1N1), A/Swine/Gent/7623/99 (H1N2) and A/Swine/Flanders/1/98 (H3N2) subtypes as described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (1). The antigens for the tests were produced at the NVI.

Porcine epidemic diarrhoea virus (PEDV)

All serum samples were tested for antibodies against PEDV using a commercial indirect ELISA from IDvet (ID Screen® PEDV Indirect).

Mycoplasma hyopneumoniae

Serological examinations for antibodies against *M. hyopneumoniae* were performed with the use of an indirect ELISA produced by IDvet (IDScreen *Mycoplasma hyopneumoniae* Indirect).

Bacteriological analyses and antimicrobial resistance

From each wild boar, faecal samples were taken for detection of Salmonella spp.

Salmonella spp.

Faecal content from the wild boars were analysed according to ISO 6579-1:2017, Detection of *Salmonella* spp. Serotyping was performed by seroagglutination, ISO 6579-3:2017.

Antimicrobial susceptibility testing was performed using EUVSEC3 plates from Sensititre® (TREK Diagnostic LTD). Epidemiological cut-off (ECOFF) values recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, accessed 16.02.2022) were used for classification of resistance.

Whole genome sequencing

Whole genome sequencing of *Salmonella* spp., though with the exception of *S. enterica* subsp. *diarizonae* isolates, was performed at the NVI on an Illumina® MiSeq (Illumina, San Diego, California, USA). Paired end reads were subjected for analysis using ResFinder V.4.1 for both acquired genes and chromosomal mutations (PointFinder) using the online tool at the Centre for Genomic Epidemiology web site (https://cge.cbs.dtu.dk/services/ResFinder/).

Parasitological analyses

Trichinella spp.

Muscle samples from the front legs of wild boars were examined for the presence of *Trichinella* spp. larvae. The samples were packed with cooling elements and shipped overnight to the Swedish Veterinary Institute (SVA).

Five grams of each muscle sample were examined using the magnetic stirrer method to detect *Trichinella* larvae. This method follows the ISO 18743:2015 standard, the global benchmark for detecting *Trichinella* spp. larvae in meat from individual animal carcasses intended for human consumption. This standard has also been adopted as the *Trichinella* reference method within the EU, as outlined in regulation (EU) 2020/1478. This method, considered the gold standard for *Trichinella* testing, can be used for both individual and pooled muscle samples.

Results

Samples and locations of wild boar

Sample sets and completed submission forms from a total of 373 hunted wild boars were submitted to the NVI during 2023 (Figure 1) for inclusion in the health surveillance programme. In addition, one harvested wild boar with bruising and subcutaneous bleeding, and one wild boar found dead on the shoreline of a small lake were submitted by the Norwegian Food Safety Authority (NFSA) as a part of passive surveillance for African swine fever virus (ASF) and classical swine fever virus (CSFV).

The samples were submitted from 14 municipalities, and corresponds well with the estimated distribution of wild boar in 2021 (Figure 2).

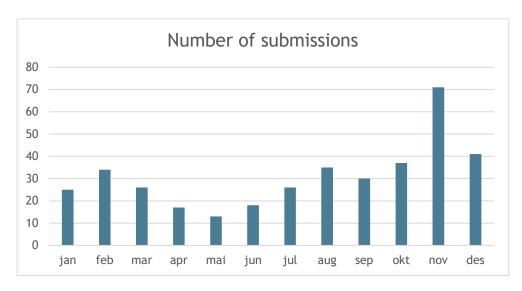


Figure 1: Number of submissions from wild boar per month during 2023.



Figure 2. Estimated distribution of wild boar in 2021. The map shows the probability that wild boar is present in a blue (low probability) to red (high probability) scale. The model is based on observations of wild boar from SCANDCAM, species observations and locations of wild boar shot during hunting and reported dead from other causes (Odden et al. 2023). The map is prepared by NINA.

Serological analyses

Blood samples from 373 wild boars were included in the serological analyses. In some cases, samples were unsuitable for one or more specific serological tests, hence not all samples were subject to every serological analysis. The results of the serological analyses are shown in Table 1.

Antibodies against the notifiable infectious diseases included in the analyses and *Mycoplasma hyopneumoniae* were not detected.

Table 1: Overview of serological results from samples submitted from wild boar hunted in Norway during 2023.

Agent-specific antibodies	Number of positive / analysed samples
SuHV1/ADV/PRV	0 / 350
TGEV	0 / 343
PRCV	1* / 343
PRRSV	0 / 352
SIV	0 / 348
PEDV	0 / 351
MHYO	0 / 352

^{*}Serum from a single male wild boar was positive for antibodies against porcine respiratory corona virus (PRCVV). It was concluded that the seroreaction most likely was a false positive, as no other wild boar from the same area had antibodies against PRCV.

Bacteriological analyses and antimicrobial resistance

Out of 363 available fecal samples, the following species and serotypes (n) of Salmonella spp. were detected in faeces from eight hunted wild boars: Salmonella Abony (2), Salmonella Typhimurium (1), Salmonella Hessarek (1), S. enterica subsp. diarizonae serovar 42:r:z (1) and S. enterica subsp. diarizonae serovar 38:r:z (3). All isolates were fully susceptible to all tested antimicrobial agents included in the test panel. Although phenotypically sensitive to aminoglycosides, whole genome sequencing detected presence of the gene aac(6')-laa that confer resistance to aminoglycodides in the four sequenced Salmonella spp. isolates.

Parasitological analyses

Trichinella spp. larva were not detected in muscle samples from 366 wild boars.

Discussion

Wild boar populations have been establishing in south-eastern Norway, with several solitary animals also being observed or shot by hunters far north and west of this (Figure 2). Wild boar health surveillance focusing on viral diseases and *Trichinella* spp. was conducted from 2011 to 2014, but was discontinued from 2015 due to very few samples being submitted. Numbers of wild boars harvested by hunters have increased from approx. 70 in the hunting year 2014/2015 to 415 in the hunting year 2022/2023 (Statistics Norway, www.ssb.no).

With an increasing number of animals being harvested annually it is feasible and important to gain insight regarding the presence of notifiable diseases, as well as zoonoses and AMR. Additionally, knowledge is needed about agents with a potential to transmit between wild boar and domestic pigs. Hence, wild boar health surveillance was reinitiated by the NVI during 2018. In the years from 2018 up to and including 2020, samples from a total of 295 wild boars were submitted. In 2020 the NFSA also implemented a financial incentive to report sick or dead wild boars and to submit samples from hunted wild boar. During October 2023, this financial incentive was increased to 3000 NOK for male wild boars and 5000 NOK for female wild boar, and expanded to include eligibility for the land-owner to receive 4000 NOK per wild boar harvested on their property. These incentives seem to have motivated sample submissions as samples were submitted from 373 wild boars during 2023. This constitutes about 90% of hunted wild boar as reported to Statistics Norway (SSB) during the hunting year of 2022/2023. Although these periods do not completely overlap, and as such are not entirely comparable, it indicates that hunters are highly motivated to collect and submit samples. Moreover, the locations of sampled wild boar coincides with areas where wild boar was registered based on other data, such as road kills, species observations and camera traps, indicating that the availability of sample kits and geographical coverage of surveillance was adequate.

As the re-establishment (absent for about 1000 years) of wild boar in Norway is fairly recent, collecting health information from this species is important to be able to monitor changes over time and for early detection of notifiable diseases. Specifically, ASF has emerged as a major cause of disease and death in affected wild boar populations across several European

countries during the last decade, proven very hard to control and eliminate. The most effective and efficient method for early detection of ASF in wild boar is passive surveillance (More, Miranda et al. 2018), where diseased and "found-dead"-wild boars are subjected to notification to the competent authority (i.e. NFSA) and tested for ASF. Two notifications and submissions for passive surveillance of ASF and CSF were made during 2023. One was a harvested wild boar with bruising and subcutaneous bleeding, and the other a wild boar found dead on the shoreline of a small lake, both with subsequent negative laboratory analyses for ASF and CSF.

In September 2023, the Swedish Board of Agriculture and the Swedish Veterinary Agency, reported the first case of African swine fever in the municipality of Fagersta, Sweden (https://jordbruksverket.se/djur/djurskydd-smittskydd-djurhalsa-och-folkhalsa/aktuellt-lage-for-smittsamma-djursjukdomar/afrikansk-svinpest-asf (in Swedish)). African and classical swine fever was not detected through passive surveillance in wild boar in Norway during 2023.

Since the present wild boar population in Norway originate from Sweden, it is of interest to compare the status of infectious agents between these populations, building on data from research and surveillance in Sweden. Although not entirely comparable, the results presented here indicate a lower incidence of *Salmonella* spp., *Mycoplasma hyopneumoniae* and swine influenza virus than what has been recently reported in Sweden (Malmsten, Magnusson et al. 2018, Sanno, Rosendal et al. 2018). Nonetheless, the detection of zoonotic *Salmonella* spp. in faecal samples of wild boar hunted in Norway highlights the importance of maintaining strict hygiene during carcass and meat handling. Furthermore, in Sweden, *Salmonella* Choleraesuis were detected in domestic pigs and wild boar during the fall of 2020 and onwards (https://www.sva.se/djurhalsa/smittlage/overvakning-av-salmonella-choleraesuis-hos-vildsvin/ (in Swedish)). This important pig pathogen with zoonotic potential was not detected in samples from wild boar in Norway during any years after 2020, including 2023.

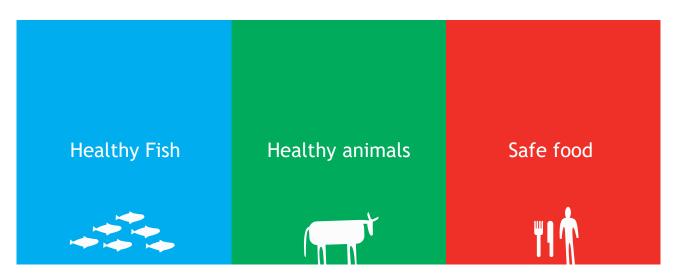
Maintaining a focus on notifiable agents and other pathogens in wild boar is important to recognise their potential significance as a reservoir of transmission to domestic animals and humans, and further facilitate early detection of emerging (e.g. ASF) and re-emerging (e.g. S. Choleraesuis) diseases. This information is important for biosecurity evaluations and risk-mitigation measures, like population management.

Acknowledgement

The authors would like to thank the technical staff for preparing the sample submission kits and performing the analyses with excellence. We would also like to acknowledge all the municipal wildlife managers and personnel from the Norwegian Food Safety Authority for distributing sample submission kits and the hunters for collecting and submitting data and samples from wild boars.

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