



The surveillance programme for diseases in wild boars in Norway 2020



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Suggested citation

Grøntvedt, Carl Andreas, Nordstoga, Anne, Hamnes, Inger Sofie, Bergsjø, Bjarne, Urdahl, Anne Margrete, Slette-meås, Jannice Schau, Norström, Madelaine, Wolff, Cecilia, Danielsen, Agathe Vikre, Welde, Hilde, Rolandsen*, Christer Moe, Odde*n, John, Våge, Jørn and Madslie, Knut. The surveillance programme for diseases in wild boars in Norway 2020. Surveillance program report. Veterinærinstituttet 2021. © 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

2021 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: Colourbox

www.vetinst.no

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Summary

Wild boar health surveillance was re-established in August 2018 to gain insight about the incidence of pathogens of importance for animals and humans and to enable early detection of notifiable diseases in this expanding species. In 2020, samples from 203 wild boars were submitted to the Norwegian Veterinary Institute, representing approx. 65% of harvested wild boar, as reported to Statistics Norway (SSB) during the hunting year of 2019/2020. Of these, five wild boars (two found dead and three killed in traffic accidents) 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), with no agents detected.

Furthermore, all samples were negative for antibodies for the following notifiable pathogens: Aujeszky's disease virus (ADV), transmissible gastroenteritis virus (TGEV), porcine respiratory corona virus (PRCV), 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), and *Mycoplasma hyopneumoniae*.

Salmonella Typhimurium was detected in faeces from five hunted wild boars. In addition, two isolates of *S. enterica* subsp. *diarizonae* and one isolate of *S. enterica* subsp. *enterica* were detected. *Campylobacter coli* was not detected.

Parasitological analysis did not demonstrate presence of *Trichinella* larvae or *Alaria alata* mesocercariae.

Susceptibility testing of *Escherichia coli* indicated a low prevalence of resistance with a total of 97.0% of the tested isolates being susceptible to all antimicrobial classes included in the test panel. Multidrug resistance (MDR) (i.e. resistance to three or more antimicrobial classes) was detected in four of the five resistant isolates. In addition, MDR was detected in four out of 43 selectively isolated quinolone resistant *E. coli*. Extended-spectrum cephalosporin-resistant *E. coli* was detected from twelve animals, of which five isolates had an AmpC beta-lactamase phenotype due to chromosomal mutations and seven isolates displayed an ESBL phenotype where four were due to presence of the gene *bla*_{CTX-M-14}, two due to the gene *bla*_{CTX-M-15}, and one due to the gene *bla*_{CTX-M-1}, respectively.

Methicillin-resistant *Staphylococcus aureus*, and carbapenem-resistant *Enterobacteriaceae* were not detected in any of the examined samples.

Sammendrag

Villsvinhelseovervåkning ble reetablert i august 2018 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 2020 ble det sendt inn prøver fra 203 villsvin til Veterinærinstituttet. Dette representerer om lag 65 prosent av antallet felte villsvin som ble rapportert til Statistisk sentralbyrå (SSB) i jaktåret 2019/2020. Fem (to påtrufne døde villsvin og tre trafikkdrepte) av de 203 dyrene ble sendt inn av Mattilsynet som en del av den passive overvåkingen for afrikansk og klassisk svinepest, men disse virussykdommene ble ikke påvist.

Det ble ikke påvist antistoff for de alvorlig meldepliktige svinesykdommene Aujeszky's disease (AD), smittsom gastroenteritt (TGE), porcint respiratorisk korona virus (PRCV), porcint respiratorisk og reproduksjonssyndrom (PRRS), porcin epidemisk diaré (PED) eller influensa A (SI). 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.

Salmonella spp. ble påvist i avføringsprøve fra åtte villsvin, fem av disse var *S. Typhimurium*, to var *S. enterica* subsp. *diarizonae* og en var *S. enterica* subsp. *enterica*. *Campylobacter coli* ble ikke påvist.

Parasittologiske undersøkelser påviste ikke forekomst av *Trichinella* spp. larver eller *Alaria alata* mesocercarier i innsendte prøver.

Undersøkelsene indikerer en lav forekomst av antibiotikaresistens. Totalt 97 prosent av de testede *Escherichia coli* isolatene var fullt følsomme for antibakterielle klasser de ble undersøkt for. Det ble påvist noen få multiresistente (dvs. resistente mot tre eller flere antibakterielle klasser) *E. coli*. Ved bruk av selektive metoder ble det påvist noen få utvidet spektrum cefalosporin-resistente *E. coli* der overførbare plasmider var bakenforliggende årsak.

Meticillin-resistent *Staphylococcus aureus*, og karbapenem-resistent *Enterobacteriaceae* ble ikke påvist i noen av de undersøkte prøvene.

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 seen/harvested several hundred kilometres north of this. Hunting statistics (Statistics Norway (SSB), www.ssb.no) document a steadily increasing number of wild boars harvested in the same period. 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 self-funded 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 and also provides free testing for *Trichinella* spp.

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 the parasites *Trichinella* spp. and *Alaria alata*, and AMR in bacteria (*E. coli*, *Campylobacter coli* and *Salmonella* spp.). The national surveillance programme for specific viral infections in domestic swine was launched in 1994, and documents the status of Aujeszky's disease (AD), 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.

The aims of the wild boar health surveillance are to investigate the health status and the prevalence of selected agents, as well as AMR, 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 kits were distributed to hunters, including submission forms that contained questions about the sampled animal, geographic reference to the location where the animal was harvested and estimated population densities. 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 wounded by hunting or traffic

accidents. Before distribution of sample collection material, 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. In addition to the submission forms, the sample collection kits included sterile bacteriological swabs with transport medium, sterile 25 ml screw-cap containers for collection of skeletal muscle, faeces and blood, disposable gloves and an insulated pre-paid return envelope.

Wild boar camera trap data are based on a network of more than 600 cameras that has been active in different periods, mainly since 2010 (SCANDCAM, <https://viltkamera.nina.no/>). Sightings of wild boar are mostly from 2017 and onwards. Originally the system is designed to have one camera per 50 km² in areas selected for lynx (*Lynx lynx*)-monitoring, but that also covers large parts of the current distribution of wild boar. Since 2020 more cameras have been used to monitor wild boar occurrence in more detail in selected areas. Hunting statistics is based on reporting of hunted wild boar to Statistics Norway, and data collection by NVI and the Norwegian Institute for Nature Research (NINA). Incidental mortalities, including vehicle collisions, was obtained from the module for reports of incidental mortality implemented in the national deer register (www.hjorteviltregisteret.no), which also contains such data on wild boar. Species observations was obtained from the Species Observations System, a citizen science project for recording species on maps into a national and freely accessible database, developed by the Norwegian Biodiversity Information Centre.

Laboratory analyses

All serological and bacteriological analyses, including AMR, and analysis for *Alaria alata* were performed at the NVI. Skeletal muscle samples were submitted to the National Veterinary Institute (SVA) in Uppsala, Sweden for *Trichinella* spp. analysis. 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)

A commercial blocking ELISA from Svanova (SVANOVIR® TGEV/PRCV-Ab) was used to detect antibodies against TGEV/PRCV. The ELISA test enables 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 European and American strains of PRRSV. In cases of positive or inconclusive results, the samples were sent to the National Veterinary Institute (DTU-Vet) in Denmark for confirmatory testing using ELISA and immunoperoxidase tests for detection of antibodies against EU- and US-strains of the PRRSV and real-time PCR for PRRSV.

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). In cases of positive or inconclusive results, the samples were sent to the DTU-Vet in Denmark for confirmatory testing using an in-house ELISA.

Mycoplasma hyopneumoniae

Serological examinations for antibodies against *M. hyopneumoniae* were performed with the use of a blocking ELISA produced by Oxoid (*Mycoplasma hyopneumoniae* ELISA).

Bacteriological analyses and antimicrobial resistance

From each wild boar, nose swabs were taken for detection of methicillin-resistant *Staphylococcus aureus* (MRSA) and faecal samples for detection of *Salmonella* spp., *Campylobacter coli*, *Escherichia coli*, extended-spectrum cephalosporin (ESC)-resistant *E. coli*, quinolone-resistant *E. coli* (QREC), colistin-resistant *E. coli* and carbapenem-resistant *Enterobacteriaceae* (CRE).

Methicillin-resistant Staphylococcus aureus (MRSA)

Nasal swabs were analysed for MRSA by incubation in Mueller-Hinton broth (Difco Laboratories, Fisher Scientific, Thermo Fisher Scientific, Waltham, Massachusetts, USA) with 6.5% NaCl (Merck KGaA, Darmstadt, Germany) at $37\pm 1.0^\circ\text{C}$ for 18-24 hours. A loopful of the overnight broth (10 μL) was plated onto Brilliance™ MRSA2 agar plate (Oxoid, Oslo, Norway) (https://www.eurl-ar.eu/CustomerData/Files/Folders/21-protocols/430_mrsa-protocol-final-19-06-2018.pdf). Suspected colonies were subjected to species identification using the MALDI-TOF MS (Bruker Daltonics GmbH, Bremen, Germany) before further phenotypical testing by disc diffusion (EUCAST, www.eucast.org).

Salmonella spp.

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

Campylobacter coli

Faecal content from the wild boars were plated directly onto mCCDA (Oxoid) agar and incubated under microaerobic conditions at $41.5\pm 0.5^\circ\text{C}$ for 48h. Typical colonies were subcultured on blood agar and confirmed as *Campylobacter coli* using MALDI-TOF MS before susceptibility tested.

Escherichia coli

Antimicrobial resistance in *E. coli* is used as an indicator on AMR levels within a population. For this purpose a random picked *E. coli* per animal was susceptibility tested. Faecal content were plated directly onto MacConkey agar (Difco) and incubated at $44.0\pm 0.5^\circ\text{C}$ for $20\pm 2\text{h}$. Typical colonies were subcultured on blood agar (Heart infusion agar, Difco) containing 5% bovine blood and incubated at $37\pm 1^\circ\text{C}$ for $20\pm 2\text{h}$. Colonies were identified as *E. coli* by typical colony appearance and a positive indole reaction before susceptibility tested.

Extended-spectrum cephalosporin (ESC)-resistant Escherichia coli, quinolone-resistant E. coli (QREC), colistin-resistant E. coli and carbapenem-resistant Enterobacteriaceae (CRE)

The faecal samples were enriched prior to plating onto selective media for detection of ESC-resistant *E. coli*, QREC, colistin-resistant *E. coli* and CRE. A total of $1\pm 0.1\text{ g}$ faecal material was homogenised with 9 mL of BPW-ISO (Oxoid), and incubated at $37\pm 1^\circ\text{C}$ for $20\pm 2\text{h}$ according to the protocol from the European reference laboratory on antimicrobial resistance (EURL-AR, <http://www.eurl-ar.eu/233-protocols.htm>). After incubation, 10-20 μL of the enrichment broth was plated onto each of the different selective media; MacConkey agar (Difco) containing 1 mg/L cefotaxime and MacConkey agar (Difco) containing 2 mg/L ceftazidime for ESC-resistant *E. coli*, MacConkey agar (Difco) containing 0.06 mg/L ciprofloxacin for QREC, CHROMID® Colistin-R (bioMérieux, Marcy l'Etoile, France) for colistin-resistant *E. coli*, and CHROMID® CARBA and CHROMID® OXA-48 agar (bioMérieux) for CRE. The agar plates were incubated at $44.0\pm 0.5^\circ\text{C}$ ($35\pm 2^\circ\text{C}$ for CRE and $37\pm 0.5^\circ\text{C}$ for $21\pm 3\text{h}$ for colistin-resistant *E. coli*) for $20\pm 2\text{h}$. Presumptive colonies were subcultured on both selective media and blood agar and confirmed using MALDI-TOF MS before susceptibility tested.

Susceptibility testing

Isolates were tested for antimicrobial susceptibility using a broth microdilution method. Minimum inhibitory concentration (MIC) values were obtained using plates from Sensititre® (TREK Diagnostic LTD) with different panels depending on the tested bacteria as shown in Table 1. Susceptibility data were recorded and stored in the sample registration system at NVI as discrete values (MIC). Epidemiological cut-off (ECOFF) values recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, accessed 20.05.2021) were used. For antimicrobial agents where ECOFFs are not defined by EUCAST, cut-offs recommended by the European Food Safety Authorities was used. Data management was performed both in SAS-PC System® v 9.4 for Windows (SAS Institute Inc., Cary, NC, USA) and in R version 4.0.3 Copyright (C) 2020 (The R Foundation for Statistical Computing Platform), while the statistical analysis was performed in R. The 95% confidence intervals were calculated using the exact binomial test.

Genotyping

Whole genome sequencing of *E. coli* and *Salmonella* spp. 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/>).

Presumptive MRSA isolates were tested by realtime PCR for the detection of *mecA* and *nuc* genes together with a conventional PCR for detection of the *mecC* gene (Tunsjø et al. 2013, Stegger et al. 2012).

Table 1: Overview of antimicrobial groups and agents used in the susceptibility testing of *E. coli* and *Salmonella* spp. with respective EUCAST epidemiological cut-off values (ECOFF).

Antimicrobial group	Antimicrobial agents	ECOFF for <i>E. coli</i> *	ECOFF for <i>Salmonella</i> spp.
Tetracyclines	Tetracycline	>8	>8
	Tigecycline	>0.5	>1**
Amphenicols	Chloramphenicol	>16	>16
Penicillins with extended spectrum	Ampicillin	>8	>8
	Temocillin	(>16)	
2 nd generation cephalosporins	Cefoxitin	(>8)	
3 rd generation cephalosporins	Cefotaxime	>0.25	>0.5
	Ceftazidime	>0.5	>2
Combinations of 3 rd generation cephalosporins and clavulanic acid	Cefotaxime/clavulanate	(>0.25)	
	Ceftazidime/clavulanate	(>0.5)	
4 th generation cephalosporins	Cefepime	(>0.25)	
Carbapenems	Meropenem	>0.125	>0.125
	Ertapenem	(>0.03)	
	Imipenem and enzyme inhibitor	(>0.5)	
Trimethoprim and derivatives	Trimethoprim	>2	>2
Sulfonamides	Sulfamethoxazole	>64**	>256**
Macrolides	Azithromycin	>16	>16**
Other aminoglycosides	Gentamicin	>2	>2
Fluoroquinolones	Ciprofloxacin	0.064	0.064
Other quinolones	Nalidixic acid	>8	>8
Polymyxins	Colistin	>2	>2**

*(ECOFF) = only ESC-resistant *E. coli* and CRE suspected isolates are tested with these antimicrobial agents.

**ECOFF defined by European Food Safety Authority.

ND = ECOFF not defined

Parasitological analyses

Trichinella spp.

Muscle samples from front leg of wild boars were examined for the presence of muscle larvae of *Trichinella* spp. Muscle samples was packed with cooling element and shipped as express-over-night parcel to SVA in Sweden. For samples arriving at the NVI on Thursday evening and Friday the samples were refrigerated until Monday morning and shipped to SVA. Five grams of muscle per sample was examined using the magnetic stirrer method for the detection of *Trichinella* larvae in muscle samples. This is the international accepted reference method (<https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32015R1375>; https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.01.20_TRICHINELLOSIS.pdf). This method is considered the gold standard for *Trichinella* testing of meat and can be used for single or pooled muscle samples.

Alaria alata

Mixed soft-tissue samples (from front leg, around the mandible, tongue) from wild boar were examined for the presence of *Alaria alata* mesocercaria by a modified *A. alata* mesocercariae migration technique, AMT (Riehn et al 2010).

Results

Samples and locations of wild boar

Sample kits and completed submission forms from a total of 198 hunted wild boars, and carcasses from two found-dead and three traffic killed wild boars were submitted to the NVI during 2020 for inclusion in the health surveillance programme and passive surveillance, respectively. The municipalities where wild boars were harvested in 2020, as reported by the hunters, are shown in Figure 1 (which also includes wild boars harvested from 2014 onwards).

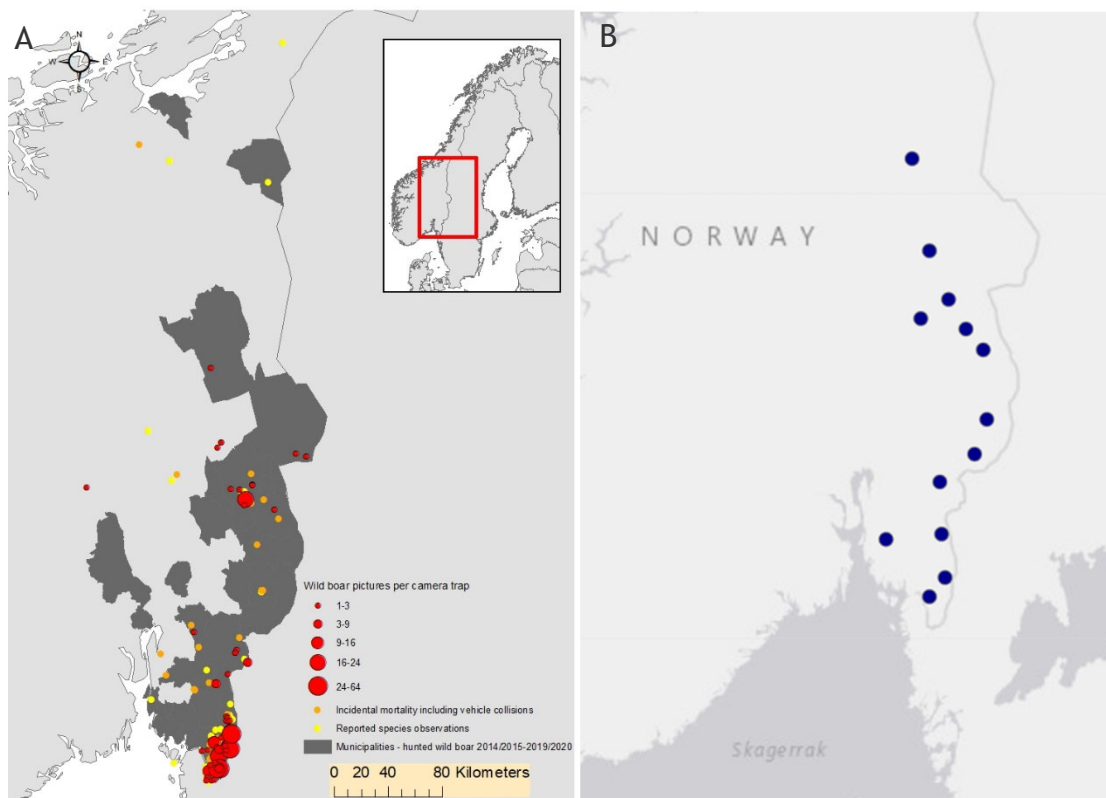


Figure 1 A: Geographical distribution of wild boar based on camera trap monitoring, hunting statistics, incidental mortality including vehicle collisions, and reported species observations in Norway in the period 2008-2021. A few observations that are believed to have been incorrectly reported were removed. The map is prepared by NINA.

B: Municipalities from where wild boars were reported harvested through hunting during 2020. Blue dots indicate the centre of each municipality. The map is prepared by NVI.

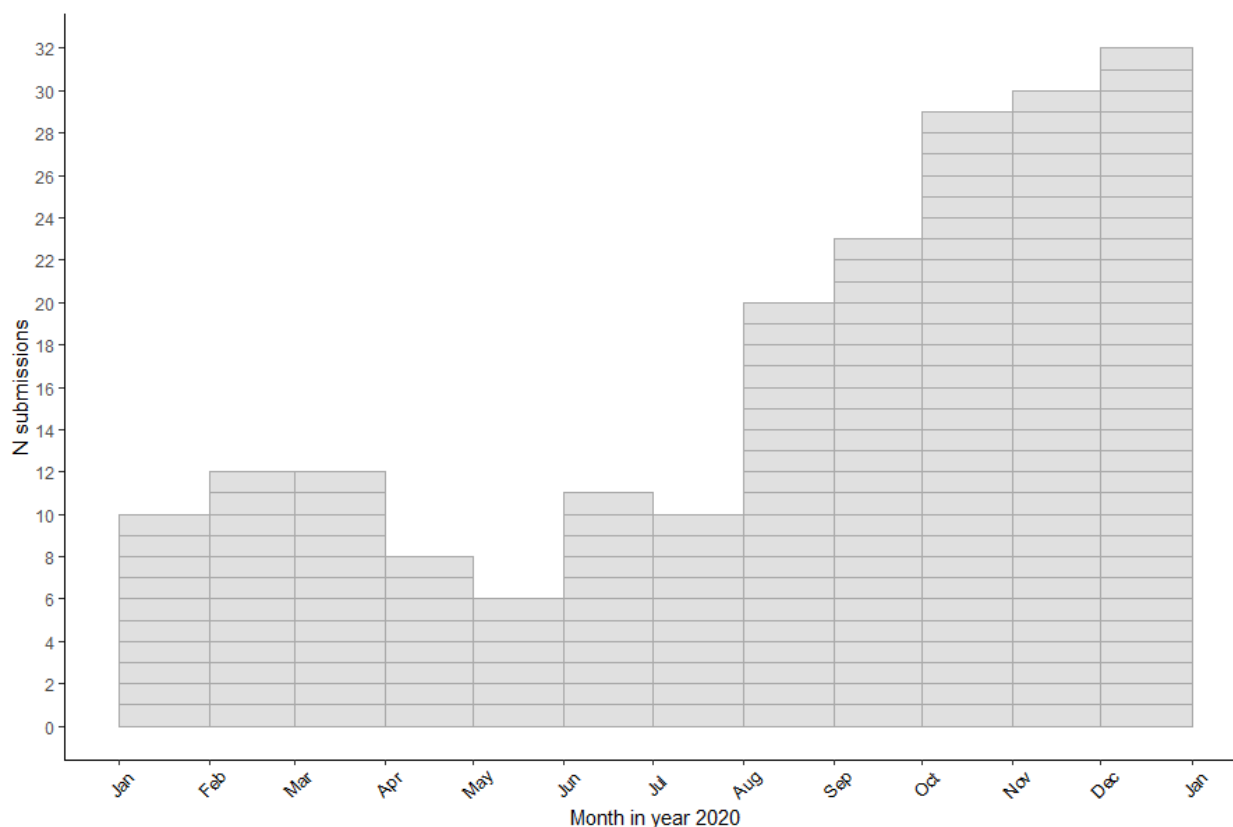


Figure 2: Number of submissions from wild boar per month during 2020.

Serological analyses

Blood samples from 186 wild boars were included in the serological analyses. In a few 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 2. Antibodies against the notifiable infectious diseases included in the analyses and *Mycoplasma hyopneumoniae* were not detected.

Table 2: Overview of serological results from samples submitted from wild boar hunted in Norway during 2020.

Agent-specific antibodies	Number of positive / analysed samples
SuHV1/ADV/PRV	0 / 184
TGEVPRCV	0 / 183
PRRSV	0 / 186
SIV	0 / 184
PEDV	0 / 186
MHYO	0 / 184

Bacteriological analyses and antimicrobial resistance

Samples from a total of 187 wild boars were screened for the presence of MRSA. MRSA was not detected from any of the samples [95%CI: 0.0-2.0].

Out of 183 investigated animals, *S. Typhimurium* was detected from five samples, two isolates of *S. enterica* subsp. *diarizonae* and one isolate of *S. enterica* subsp. *enterica* were also detected. The five *S. Typhimurium* isolates showed reduced susceptibility to colistin. However, whole genome sequencing did not detect any acquired genes nor point mutations regarded responsible for these observations.

No *Campylobacter coli* was detected among the 183 samples that were investigated.

Altogether, 167 indicator *E. coli* isolates originating from 183 samples were susceptibility tested. Of these, 3.0% showed reduced susceptibility to the antimicrobials included in the test panel showed in Table 1. One isolate (0.6%) was resistant to tetracyclines, while three (1.8%) were resistant to three antimicrobial classes (penicillins with extended spectrum, trimethoprim and sulphonamides) and three (1.8%) were resistant to four antimicrobial classes (penicillins with extended spectrum, tetracyclines, sulfonamides and either trimethoprim or fluoroquinolones), and thereby regarded as multidrug resistant (Figure 3).

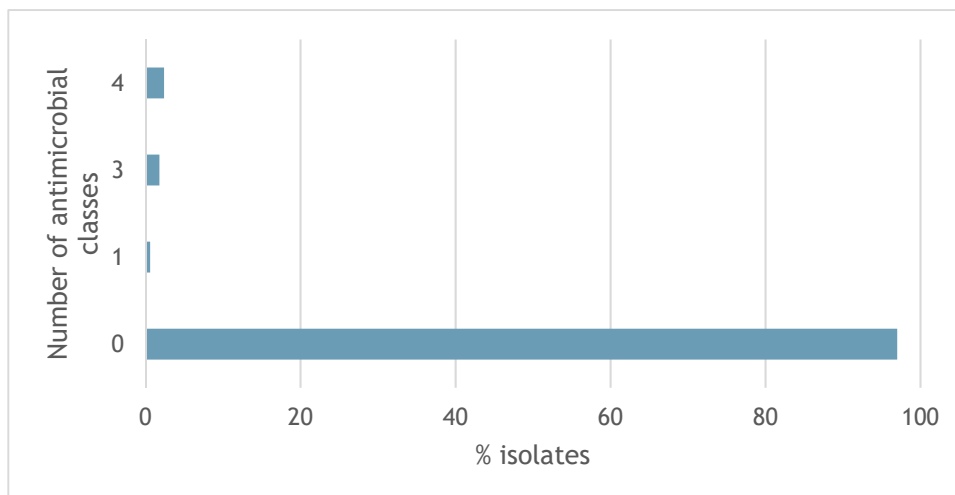


Figure 3: Antimicrobial resistance profile for *Escherichia coli* from faecal samples from wild boars in 2020. Percentage of isolates susceptible to all (0) or resistant to one (1), three (3) and four (4) antimicrobial classes are illustrated.

Samples from a total of 171 wild boars were investigated for occurrence of ESC-resistant *E. coli* by selective media. ESC-resistant *E. coli* was detected from twelve animals (7.0% [95% CI: 3.7-11.9]). The twelve isolates displayed resistance to the beta-lactams (i.e. ampicillin, and cefotaxime and/or ceftazidime). Additional resistance was found in two isolates as shown in Table 3 together with an overview of the genotypes. Five of the isolates had an AmpC beta-lactamase phenotype and the resistance was due to mutations in the promoter and attenuator region of the chromosomally encoded *ampC* gene causing an up-regulation of the gene. In addition, seven isolates displayed an ESBL phenotype and four was genotyped as *bla*_{CTX-M-14}, two as *bla*_{CTX-M-15}, and one as *bla*_{CTX-M-1}, respectively.

Table 3: The genotypes and antimicrobial resistance of the twelve ESC-resistant *Escherichia coli* detected from wild boars in 2020.

Genotype	Cefo- taxime	Genta- micin	Cipro- floxacin	Cefta- zidime	Tetra- cycline	Ampi- cillin	Number of isolates
ESBL <i>bla</i> _{CTX-M-14}	1	0	0	0	0	1	4
ESBL <i>bla</i> _{CTX-M-15}	1	0	0	1	0	1	1
Chromosomally up-regulated <i>ampC</i>	1	0	0	1	0	1	5
ESBL <i>bla</i> _{CTX-M-1}	1	1	0	0	0	1	1
ESBL <i>bla</i> _{CTX-M-15}	1	0	1	1	1	1	1

Samples from a total of 165 wild boars were investigated for the occurrence of QREC by selective media, and 43 QREC (26.1%, [95% CI: 19.5-33.5]) were detected (i.e. *E. coli* displaying resistance to ciprofloxacin and/or nalidixic acid). Of these, 39 showed resistance only to quinolones, while four isolates showed additional resistance to penicillins with extended spectrum, tetracyclines and sulfonamides.

Samples from a total of 165 wild boars were investigated for colistin-resistant *E. coli* by selective media and colistin-resistant *E. coli* was detected from ten samples (6.1%, [95% CI: 2.9-10.9]). However, whole genome sequencing did not detect any known acquired genes nor chromosomal point mutations responsible for this reduced susceptibility to colistin.

Analyses for detecting CRE by selective media was performed on samples from a total of 170 wild boars. No CRE were detected (0%, [95% CI: 0.0-2.1]).

Parasitological analyses

Trichinella spp. larva were not detected in muscle samples from 197 wild boars. Mixed soft tissue samples from 157 animals were investigated for *A. alata* mesocercariae, all were negative.

Discussion

Wild boar populations are establishing in south-eastern Norway, with several solitary animals also being observed and harvested north and west of this (Figure 1). 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. Number of wild boars harvested through hunting have increased from 70 in the hunting year 2014/2015 to 310 in the hunting year 2019/2020 (data from SSB). 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. During 2018 and 2019, samples from a total of 92 wild boars were submitted, increasing to 203 (samples or entire carcasses)

in 2020. This constitutes about 65% of hunted wild boar as reported by SSB during the hunting year of 2019/2020. Although these periods do not completely overlap, and as such are not entirely comparable, it indicates that hunters are willing to 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 was adequate. During July 2020, the NFSA also implemented a financial incentive to report sick or dead wild boars and to submit samples from hunted wild boar. The increased submission rate of samples seems to be a response to this incentive (Figure 2).

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 such notifications with subsequent negative laboratory analyses for ASF and CSF were made during 2020. In addition, three traffic-killed wild boars were included for ASF and CSF laboratory analyses through strengthened passive surveillance, with negative results.

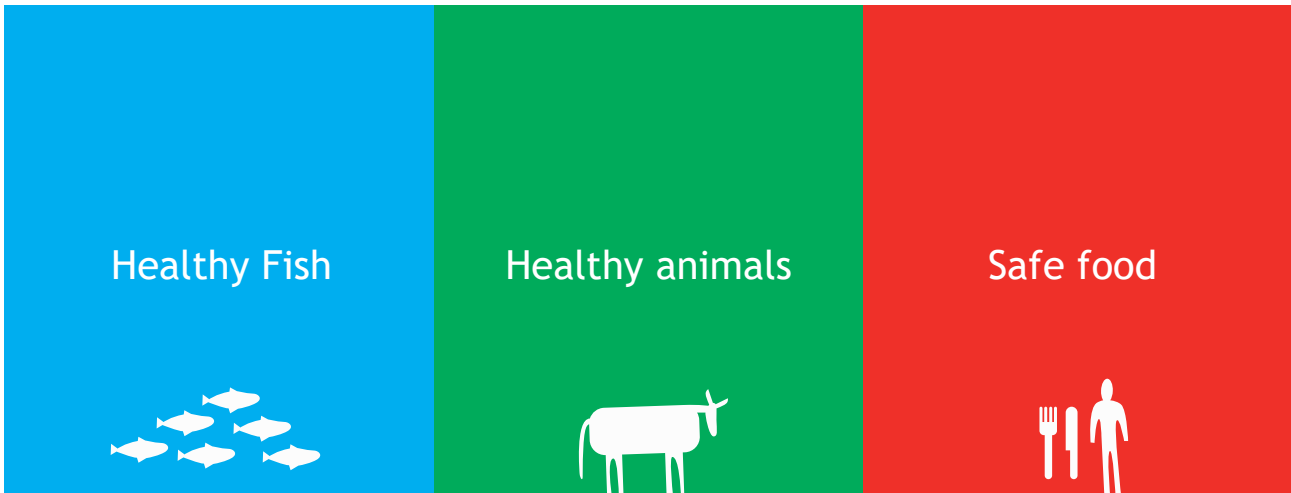
Since the present wild boar population in Norway originate from Sweden, it is of interest to compare the status of infectious agents between these populations, building on data from research and surveillance in Sweden. Although not entirely comparable, the results presented here indicate a lower incidence 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 (<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 2020.

With regard to AMR, the results indicate an overall low occurrence of AMR. However, multidrug resistant isolates (i.e. resistant to three or more antimicrobial classes) were identified, as well as isolates with an ESBL phenotype, showing that wild boar may contribute in dissemination of such AMR bacteria.

Maintaining a focus on notifiable agents and other pathogens, including AMR bacteria, 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.

References

1. Agersø Y, Aarestrup FM, Pedersen K, Seyfarth AM, Struve T, Hasman H. Prevalence of extended-spectrum cephalosporinase (ESC)-producing *Escherichia coli* in Danish slaughter pigs and retail meat identified by selective enrichment and association with cephalosporin usage. *J Antimicrob Chemother.* 2012 Mar;67(3):582-8.
2. Tunsjø HS, Follin-Arbelet B, Clausen NM, Ness Y, Leegaard TM, Bemanian V. A rapid, high-throughput screening method for carriage of methicillin-resistant *Staphylococcus aureus*. *APMIS.* 2013 Sep;121(9):865-70.
3. Stegger M, Andersen PS, Kearns A, Pichon B, Holmes MA, Edwards G, Laurent F, Teale C, Skov R, Larsen AR. Rapid detection, differentiation and typing of methicillin-resistant *Staphylococcus aureus* harbouring either *mecA* or the new *mecA* homologue *mecALGA251*. *CMI.* 2011 18, 395-400.
4. Malmsten, A., U. Magnusson, F. Ruiz-Fons, D. González-Barrio and A.-M. Dalin (2018). "A SEROLOGIC SURVEY OF PATHOGENS IN WILD BOAR (*SUS SCROFA*) IN SWEDEN." *Journal of Wildlife Diseases* 54(2): 229-237.
5. More, S., M. A. Miranda, D. Bicout, A. Bøtner, A. Butterworth, P. Calistri, S. Edwards, B. Garin-Bastuji, M. Good, V. Michel, M. Raj, S. S. Nielsen, L. Sihvonen, H. Spooler, J. A. Stegeman, A. Velarde, P. Willeberg, C. Winckler, K. Depner, V. Guberti, M. Masiulis, E. Olsevskis, P. Satran, M. Spiridon, H.-H. Thulke, A. Vilrop, G. Wozniakowski, A. Bau, A. Broglia, J. Cortiñas Abrahantes, S. Dhollander, A. Gogin, I. Muñoz Gajardo, F. Verdonck, L. Amato and C. Gortázar Schmidt (2018). "African swine fever in wild boar." *EFSA Journal* 16(7): e05344.
6. Sanno, A., T. Rosendal, A. Aspan, A. Backhans and M. Jacobson (2018). "Distribution of enteropathogenic *Yersinia* spp. and *Salmonella* spp. in the Swedish wild boar population, and assessment of risk factors that may affect their prevalence." *Acta Vet Scand* 60(1): 40.
7. Riehn, K., Hamedy, A., Grosse, K., Zeitler, L., Lückner, E. (2010). "A novel detection method for *Alaria Alata mesocercariae* in meat." *Parasitol Res* Jun;107(1):213-20.



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