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Content

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 2021, samples from 294 wild boars were submitted to the Norwegian Veterinary Institute, representing approx. 80% of harvested wild boar, as reported to Statistics Norway (SSB) during the hunting year of 2021/2022. Of these, one wild boar found dead was 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 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*.

Serum from a single young female wild boar was positive for antibodies against porcine respiratory and reproductive syndrome virus (PRRSV) type 1 and 2, but PRRSV was not detected on PCR-testing. It was concluded that the seroreaction most likely was a false positive one, as no other wild boar from the same area had antibodies against PRRSV.

The following serotypes (n) of *Salmonella* spp. were detected in faeces from 13 hunted wild boars: *S. enterica* subsp. *diarizonae* (7), *S.* Typhimurium (2), *S.* Duesseldorf (1), *S.* Hessarek (1), *S.* Newport (1) and *S. enterica* subsp. *enterica* (1).

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

Methicillin-resistant *Staphylococcus aureus* was not detected in any of the examined samples.

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 2021 ble det sendt inn prøver fra 294 villsvin til Veterinærinstituttet. Dette representerer om lag 80 prosent av antallet felte villsvin som ble rapportert til Statistisk sentralbyrå (SSB) i jaktåret 2021/2022. Ett påtruffet dødt villsvin 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 for de alvorlig meldepliktige svinesykdommene Aujeszky's disease (AD), smittsom gastroenteritt (TGE), porcint respiratorisk korona virus (PRCV), 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.

I prøver fra ett ungt hunndyr av villsvin felt under jakt i Aremark ble det påvist antistoff mot porcint respiratorisk og reproduksjonssyndromvirus (PRRSV) type 1 og 2, men virus ble ikke påvist ved PCR undersøkelse. Det vurderes som mest sannsynlig at antistoffpåvisningen skyldes en falsk positiv reaksjon, da det ikke har vært påvist antistoff mot PRRSV i prøver fra andre villsvin felt i samme område.

Salmonella spp. med følgende serotyper (n) ble påvist i avføringsprøve fra 13 villsvin felt under jakt: *S. enterica* subsp. *diarizonae* (7), *S.* Typhimurium (2), *S.* Duesseldorf (1), *S.* Hessarek (1), *S.* Newport (1) og *S. enterica* subsp. *enterica* (1).

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

Meticillin-resistent *Staphylococcus aureus* 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 bacteriological analyses for *Salmonella* spp. and methicillin-resistant *Staphylococcus aureus* (MRSA). 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, the prevalence of selected agents as well as AMR, 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 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 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. 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.

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 in the 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. 2022).

Laboratory analyses

All serological and bacteriological analyses 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. TGEV positive or inconclusive samples were tested with a confirmatory test at the NVI (Swinecheck®TGEV/PRCV, Biovet).

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.

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, nose swabs were taken for detection of methicillin-resistant *Staphylococcus aureus* (MRSA) and faecal samples for detection of *Salmonella* spp.

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±1.0°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. 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.

Genotyping
Whole genome sequencing of *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).

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. The ISO 18743:2015 is the global standard for detection of detection of *Trichinella* spp. muscle stage larvae in meat of individual animal carcasses intended for human consumption, and this method has been implemented as the *Trichinella* reference method within EU (https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32020R1478&qid=1660823245563&from=EN).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 precence 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 sets and completed submission forms from a total of 293 hunted wild boars, and the carcass from one found-dead wild boar were submitted to the NVI during 2021 (Figure 1) for inclusion in the health surveillance programme and passive surveillance, respectively. These samples were submitted from 16 municipalities (Figure 2a), and corresponds well with the estimated distribution of wild boar in 2021 (Figure 2b).

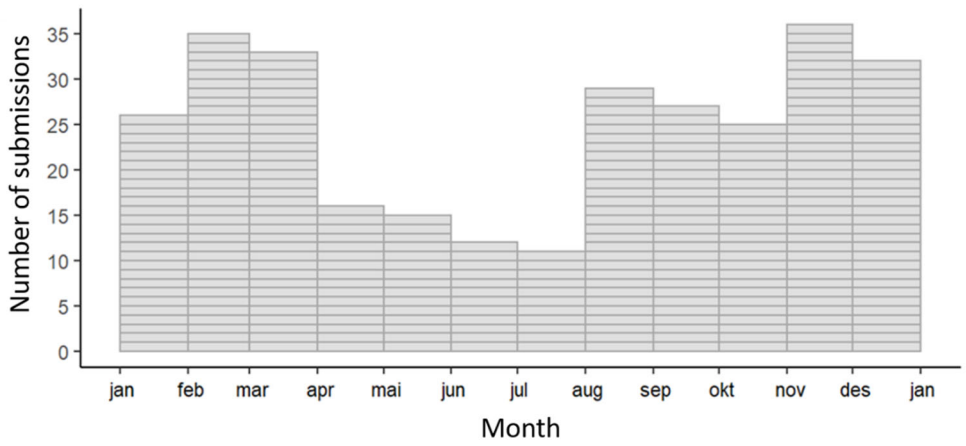

***Figure 1**: Number of submissions from wild boar per month during 2021.*

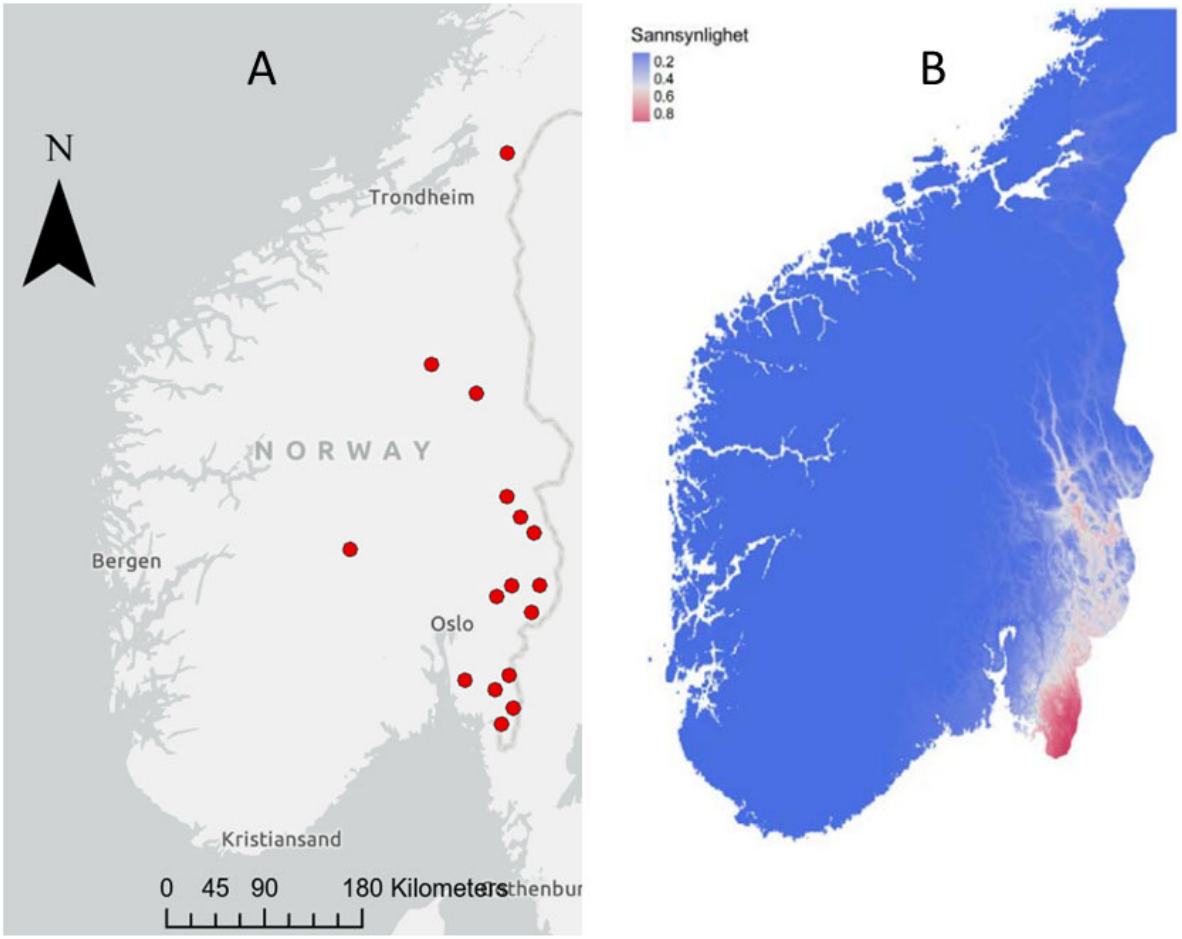

Figure 2. A: *Municipalities from where samples of wild boar were submitted in 2021. Red dots indicate the centre of each municipality.* ***B****: Estimated distribution of wild boar in 2021. The map shows the probability that wild boar is present in a blue (low probability) to red (high probability) 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. 2022). The map is prepared by NINA.*

Serological analyses

Blood samples from 283 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, except serum from one young female wild board which was positive for antibodies against both PRRSV-1 and -2. PRRSV was, however, not detected on PCR-testing blood from this animal and it was concluded that the seroreaction most likely was a false positive one. In addition, no other wild boar from the same area had antibodies against PRRSV.

***Table 1**: Overview of serological results from samples submitted from wild boar hunted in Norway during 2021.*

Agent-specific antibodies	Number of positive / analysed samples
SuHV1/ADV/PRV	0 / 283
TGEV	0 / 276
PRCV	0 / 258
PRRSV	1* / 281
SIV	0 / 271
PEDV	0 / 281
MHYO	0 / 282

* Serum from a single young female wild boar was positive for antibodies against porcine respiratory and reproductive syndrome virus (PRRSV) type-1 and -2, but PRRSV was not detected on PCR-testing. It was concluded that the seroreaction most likely was a false positive one, and no other wild boar from the same area had antibodies against PRRSV.

Bacteriological analyses and antimicrobial resistance

Samples from a total of 277 wild boars were screened for the presence of MRSA. MRSA was not detected from any of the samples [95% CI: 0.0-1.3].

Out of 287 investigated animals, the following serotypes (n) of *Salmonella* spp. were detected in faeces from 13 hunted wild boars: *S. enterica* subsp. *diarizonae* (7), *S.* Typhimurium (2), *S.* Duesseldorf (1), *S.* Hessarek (1), *S.* Newport (1) and *S. enterica* subsp. *enterica* (1).

Resistance to quinolones was detected in one of the tested 11 isolates, while the other isolates were fully susceptible to all tested antimicrobial agents included in the panel.

Parasitological analyses

Trichinella spp. larva were not detected in muscle samples from 292 wild boars. Mixed soft tissue samples from 268 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 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 through hunting have increased from approx. 70 in the hunting year 2014/2015 to 365 in the hunting year 2021/2022 (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. This incentive seems to have motivated sample submissions as samples or entire carcass was submitted from 294 wild boars during 2021. This constitutes about 80% of hunted wild boar as reported by SSB during the hunting year of 2021/2022. 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 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. One such notification with subsequent negative laboratory analyses for ASF and CSF were made during 2021.

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 2021.

Serum sampled from a young female wild boar harvested in the municipality of Aremark tested positive for antibodies against both PRRSV-1 and -2, however PRRSV was not detected through PCR. Although we cannot completely rule out the possibility of a true-positive serological reaction, we regard it more likely that it was a false positive. We base this assumption on the lack of seropositivity against PRRSV in other wild boars sampled in the same municipality and hunting area, and no recent history of PRRSV in wild boar or domestic pigs in neither Norway nor Sweden.

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.

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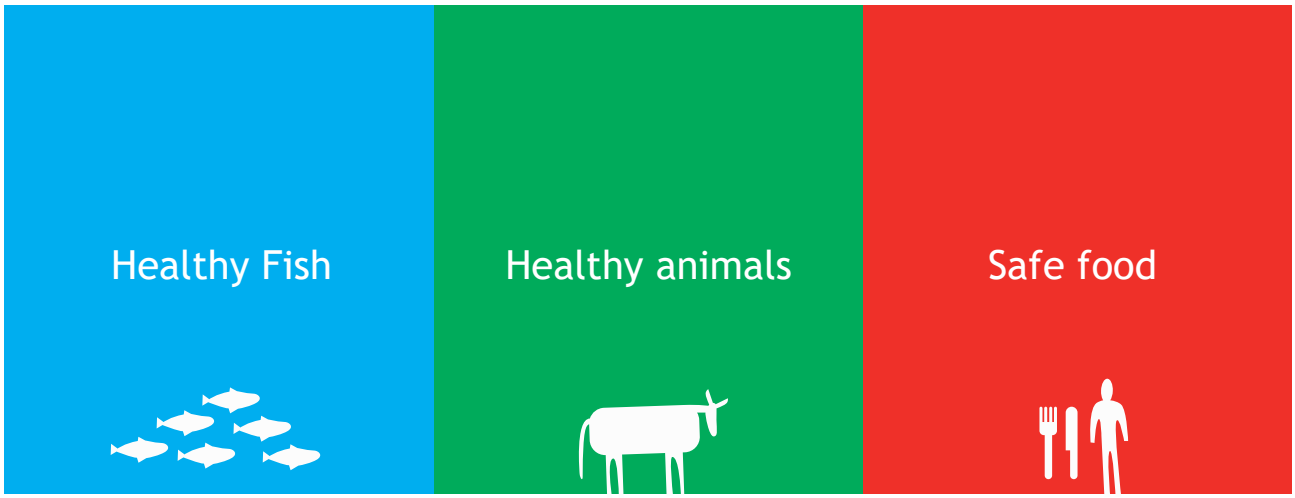

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