Annual Report

The surveillance programme for hygiene in organic fertilizers in Norway 2017

Veterinærinstituttet
Norwegian Veterinary Institute

Mattilsynet

Hygiene in organic fertilizers in Norway

Content

Authors
Gro S. Johannessen, Jannice Schau Slettemeås, Madelaine Norström, Anne Margrete Urdahl and Mona Torp

Commissioned by
Norwegian Food Safety Authorities

ISSN 1894-5678

Design Cover: Reine Linjer
Photo front page: Colourbox

Sammendrag

Dette prosjektet ble utført på oppdrag fra Mattilsynet for å undersøke forekomsten av *E. coli* som hygiene-indikator og *Salmonella* spp. i organiske gjødselvarer til hagemarkedet. I tillegg skulle også forekomst av utvalgte antibiotikaresistente bakterier undersøkes. Organiske gjødselvarer er ofte basert på husdyrgjødsel, og det norske regelverket har krav til hygienisering av produktene.

Mattilsynet tok prøver av 49 produkter (norskproduserte og importerte) hos forhandler, importør eller hos produsenter. Prøvene ble undersøkt for *Salmonella* og *E. coli*. I tillegg ble prøvene analysert for forekomst av *E. coli* resistente mot tredje generasjon cefalosporiner (ESBL/AmpC), karbapenemase-produserende *Enterobacteriaceae* (CPE), kinolon-resistente *E. coli* (QREC), vankomycin-resistente *Enterococcus faecium* og *Enterococcus faecalis* (VRE) og methicillin-resistente *Staphylococcus aureus* (MRSA) ved bruk av selektive metoder.

Det ble påvist *E. coli* i antall > 10 kde/g i ni (18,4 %, 95 % KI: 8.8-32.0) av produktene, men kun tre (6,1 %) av produktene hadde *E. coli* >1000 kde/g. Det ble isolert *Salmonella* Okatie fra ett av produktene. Det ble ikke isolert ESBL/AmpC, CPE, QREC, VRE eller MRSA fra noen av produktene.

Selv om det ble isolert *Salmonella* fra ett produkt og tre produkter hadde *E. coli* >1000 kde/g, var den hygieniske kvaliteten på de analyserte produktene generelt god. Ved undersøkelse for forekomst av antibiotikaresistens ved bruk av selektive metoder, ble det ikke påvist noen av resistensformene det ble analysert for. Siden antallet analyserte produkter var lavt, er det behov for et større antall prøver for å kunne si noe sikkert om hygienisk kvalitet og forekomsten av antibiotikaresistente bakterier og patogene bakterier i slike produkter.

Summary

This project was carried out for the Norwegian Food Safety Authority (NFSA) to investigate the occurrence of *E. coli* as hygienic indicator and for *Salmonella* spp. in organic fertilizers for the gardening market. In addition, the occurrence of some selected antimicrobial resistant bacteria was investigated. Organic fertilizers are often based on animal manure, and in the Norwegian regulations there are requirements for hygienization of the products.

A total of 49 products of domestic and imported origin were sampled at retail, importers or producers by the NFSA. The samples were analysed for *Salmonella* and *E. coli*. In addition, the samples were analysed for the presence of *E. coli* resistant to third generation cephalosporins (ESBL/AmpC), carbapenemase-producing *Enterobacteriaceae* (CPE), quinolone-resistant *E. coli* (QREC), colistin-resistant *E. coli*, vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* (VRE), and methicillin-resistant *Staphylococcus aureus* (MRSA).

E. coli above 10 cfu/g was detected in nine (18.4%, 95%CI: 8.8-32.0) of the products, but only three (6.1%) had numbers above 1000 cfu/g. *Salmonella* Okatie was isolated from one of the products. ESBL/AmpC, CPE, QREC, VRE and MRSA was not isolated from any of the products.

Although *Salmonella* was isolated from one product and three products had *E. coli* numbers above 1000 cfu/g, the hygienic quality of the analysed products was in general satisfactory. None of the products were positive for the selected antimicrobial resistances tested for. A larger number of samples is needed to make further conclusions on the hygienic quality and the prevalence of selected resistant bacteria and pathogens in fertilizer products on the Norwegian gardening market.

Introduction

Organic fertilizers and soil improvement products are commonly used in gardens, sold in gardening centres and often based on manure from animals, such as cattle, poultry, horses and sheep. The products can be composted, milled/grinded and sold as compost, meal, pelleted, mixed with peat etc., and may be imported or of Norwegian origin. According to the Norwegian Food Safety Authority (NFSA), in 2017 243 products of organic fertilizer/organic-mineral fertilizer/soil improvement/growth media with animal manure as raw material was registered (Anne Bøen, pers comm.). In 2012, the NFSA conducted a survey on wastebased fertilizers (1). In this project, processing facilities were visited and these included among others biogas facilities, drying facilities, different composting facilities (windrows and reactors) and facilities with long time storage.

The hygienic quality of organic fertilizers in Norway is regulated by FOR 2003-07-04 nr 951, "Forskrift om gjødselvarer mv. av organisk opphav" (2). Here it is stated that the products and the use of them, including probable misuse, shall not lead to risk of transmission of infectious agents to humans and animals. This means that *Salmonella* spp. and infective parasite eggs should not be detected in the product and the level of thermotolerant coliforms should be below 2500 cfu/g dry matter. However, this regulation is currently under revision, and it is considered to change from thermotolerant coliforms to *E. coli* (Anne Bøen, pers. comm.). Another central regulation is FOR-2016-09-14-1064, "Forskrift om animalske biprodukter som ikke er beregnet på konsum (animaliebiproduktforskriften" (3). In this regulation there are requirements to treatment, including time-temperature and there are hygienic requirements depending on which raw materials and treatment that are used. However, a general requirement is that *Salmonella* spp. should not be detected in the end product.

EU has taken an initiative to make a common set of regulations for fertilizers, soil improvements and growth media. In the Annexes (4) to the new proposal it is proposed absence of *Salmonella* spp. in a 25 g sample, and <1000 cfu/g of *E. coli* or *Enterococcaceae* as limits.

Antimicrobial resistance (AMR) is one of the major public health challenges and a possible route of transmission could be through manure into the environment and further to vegetables and the consumers. It is well documented that faeces from animal husbandary is a reservoir of AMR bacteria (5), and finding of AMR resistant bacteria in fertilizer products of such origin should therefore to some degree be expected. In this project it was thus decided to focus on resistance to some of the critically important antimicrobials as defined by WHO (6). So far, the data is sparse on the occurrence of antimicrobial resistance in organic fertilizers in Norway. Thus, it is desirable to obtain knowledge of AMR in such products.

Aims

The aim of this limited study was to investigate the occurrence of *E. coli* as hygiene-indicator and *Salmonella* spp. in organic fertilizers aimed for the gardening market in Norway. In addition, the study aimed at investigating the occurrence of some selected antimicrobial resistant bacteria in the products.

Materials and methods

Sampling

A collection of 50 samples from different parts of the country was planned. The samples were collected from producers, importers or retailers, and were of domestic and imported origin. The sampling period was from March 15th to June 15th 2017. The samples were collected by NFSA and sent to the laboratory. One sample consisted of a composite sample of 500 – 1000 g. The sampling was carried out to make the final sample, to the largest extent possible, representative for the sampled lot. The samples were taken from products ready for sale. Composts sold in bulk are of various stability and the degradation process may continue after sampling. Thus, samples taken from compost heaps were analysed immediately after arrival at the laboratory, while samples of stabilised products were stored up to one week prior to analysis.

Analytical methods

Hygiene analysis

The samples were analysed for *Salmonella* spp. and *E. coli*, and five parallels were analysed from each sample. For *Salmonella*, the samples were analysed using the procedure for primary production stage described in ISO 6579-1:2017 (7). Briefly, 25 g sample was enriched with buffered peptone water (BPW-ISO) at 37°C for 18 24 hrs followed by selective enrichment on modified semi-solid Rappaport-Vassiliadis (MSRV) agar incubated at 41,5°C for 24 hrs. After incubation, suspect MSRV plates will show a grey-white turbid zone surrounding the inoculated drop. If the MSRV plates were negative after 24 hrs, they were incubated for another 24 hrs. From suspect MSRV plates, a loop was dipped in the opaque growth farthest from the inoculation point and the surface of an XLD plate (Xylose Lysine Deoxycholate agar) was inoculated. The procedure was repeated with Brilliant Green Agar (BGA). The plates were incubated at 37°C for 24 hrs. Typical and suspicious colonies were confirmed using matrix-assisted laser desorption/ionization time of flight (Maldi-TOF) (Bruker Daltronics) and the serotype was determined using traditional serotyping.

E. coli was enumerated using 3M™ Petrifilm™ Select *E. coli* according to the manufacturer's instructions. Briefly, 10 g sample was homogenized with Peptone Saline (0.85% NaCl and 0.1% peptone), and tenfold dilution series were prepared. Appropriate dilutions were inoculated on the Petrifilm™ and incubated at 42°C for 24 hrs. Colonies with typical appearance were counted.

Antimicrobial resistant bacteria

The samples were analysed for the presence of *E. coli* resistant to third generation cephalosporins (ESBL/AmpC), carbapenemase-producing *Enterobacteriaceae* (CPE), quinolone-resistant *E. coli* (QREC), colistin-resistant *E. coli*, vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* (VRE), and methicillin-resistant *Staphylococcus aureus* (MRSA).

ESBL/AmpC, QREC, CPE and colistin-resistant E. coli

For analysis of ESBL/AmpC, QREC, CPE and colistin-resistant *E. coli*, the methods described in NORM-VET 2016 were applied (8). Briefly, 25 g sample was enriched in BPW-ISO, incubated at 37°C and plated on selective agar plates as described in the NORM-VET report. Colonies were confirmed using Maldi-TOF.

Vancomycin-resistant Enterococcus faecium *and* Enterococcus faecalis

Analysis for VRE was carried out by enrichment of 10 g sample in 90 mL BBL™ Enterococcosel™ Broth (Becton, Dickinson and Company, Maryland, USA**)** containing 4 mg/L vancomycin and 0.25 mg/L clindamycin at 37°C for 24 hrs. After incubation, aliquots of the overnight broths were plated on Slanetz and Bartley agar (Oxoid) containing 4 mg/L vancomycin and incubated at 41.5°C for 24-48 hrs. Colonies were confirmed using Maldi-TOF.

Methicillin-resistant Staphylococcus aureus

Analysis for MRSA was carried out by enrichment of 5 g sample in 45 mL Mueller Hinton broth containing 6.5% NaCl at 37°C for 24 hrs. After incubation, aliquots of the overnight broths were plated on Brilliance™ MRSA2 Agar (Oxoid) and incubated at 37°C for 24 hrs. Colonies were confirmed using Maldi-TOF.

Results and Discussion

In total, 49 samples were collected in the period March 15th to June 15th 2017. An overview of the samples is listed in Annex 1. The origin of the manure was from poultry (hens) in 22 of the 49 samples (43%), while ten, eight, seven and two samples were from cattle, unknown origin, horse and mixed origin, respectively. The samples also included bulk products of compost (samples collected at the production facility), and five such products were collected.

Hygiene parameters

All samples were analysed for *Salmonella* (qualitative analysis) and *E. coli* (quantitative analysis) in five parallels of each sample. *E. coli* was enumerated in nine of 49 samples (18.4%, 95%CI: 8.8-32.0) with varying numbers (Table 1). *Salmonella* Okatie was isolated from one of five parallels from one sample. The parallels from the *Salmonella* positive samples had *E. coli* numbers below 10 cfu/g.

Table 1. Samples positive for *E. coli*. Prøver positive for *E. coli*.

ID	Sample material / Prøvemateriale	Origin of manure/ Opphav gjødsel	Import/ domestic Import/ norsk	No. of positive parallels/ Antall pos. paralleller	No. of *E. coli* in positive samples - median (range) (cfu/g)/Antall *E. coli* i pos. prøver
2017-22-246	Fertilizer	Poultry	Import	5/5	1200 (1080 – 1720)
2017-22-247	Compost	Cattle	Import	5/5	3400 (2500 – 5300)
2017-22-248	Compost	Cattle	Norwegian	5/5	100 (60 – 150)
2017-22-387	Compost, bulk	Horse	Norwegian	3/5	10[1]
2017-22-401	Compost	Cattle	Norwegian	5/5	2800 (1800 – 3900)
2017-22-402	Fertilizer, bulk	Horse	Norwegian	3/5	10[1]
2017-22-406	Fertilizer	Poultry	Unknown	4/5	20 (10 – 220)
2017-22-494	Compost, bulk	Horse	Norwegian	2/5	(10 – 30)
2017-22-578	Fertilizer meal	Unknown	Norwegian	1/5	10

[1] All three parallels had 10 cfu/g.

The results from the present study show that *E. coli* was present in quantifiable numbers in some of the samples. According to the proposed EU regulations, samples from three (6.1%) of the products analysed in this study would be exceeding the limit of *E. coli* (1000 cfu/g) in organic fertilizers of acceptable hygienic quality. Two of these products were imported and based on poultry and cattle manure, respectively, while the third product was domestically produced and based on cattle manure.

From the other products where *E. coli* was enumerated, only two samples from two different products had *E. coli* numbers above 100 cfu/g. The remaining *E. coli* positive samples had numbers below 100 cfu/g. The number of positive parallels in a sample varied from one to five positives.

To the best of our knowledge, there is not much information available on the hygienic quality of organic fertilizer neither in Norway nor Europe. In Finland, organic fertilizers are tested yearly and the results from 2015 and 2016 are in agreement with the results from this study. In 2015 and 2016 in Finland, *Salmonella* was not detected in any of the 10 and 12 samples of organic fertilizer, while *E. coli* was enumerated in six samples, but below the unacceptable limit (>1000 cfu/g). For soil improvement products, *Salmonella* was detected in two of 23 samples (compost samples) in 2015, and in one of 33 samples in 2016. *E. coli* was enumerated in numbers below the limit in 11 of 31 samples in 2016 and in 11 of 23 samples in 2015. In 2015, one sample had *E. coli* in numbers above the limit (9, 10; Ossi Ala-Mantila, pers com.).

In this study, *Salmonella* Okatie was isolated from one of five parallels in one sample of imported organic fertilizer based on horse manure. According to the current Norwegian regulations and also the proposed new EU regulations, *Salmonella* should not be detected. From the results from Finland, it can be observed that *Salmonella* occasionally might be isolated from organic fertilizers and soil improvement products. Since the intestines of warm-blooded animals are the reservoir of *Salmonella*¸ it is not unexpected that *Salmonella* can be recovered from such products. For manure based fertilizer products, the raw material may contain *Salmonella* or the product might have been re-contaminated at some stage during the production process.

Antimicrobial resistance

None of the samples analysed in this project were positive for the selected antimicrobial resistances tested for; indicating a low prevalence of *E. coli* resistant to third generation cephalosporins (ESBL/AmpC), carbapenemase-producing *Enterobacteriaceae* (CPE), quinolone-resistant *E. coli* (QREC), colistin-resistant *E. coli*, vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* (VRE), and methicillin-resistant *Staphylococcus aureus* (MRSA).

To the best of our knowledge there is little information available on the occurrence of AMR bacteria in organic fertilizers and soil improvement products. However, as mentioned above it is well documented (5) that faeces from animal housbundary is a reservoir of AMR bacteria, and finding of AMR resistant bacteria in fertilizer products of such origin should therefore to some degree be expected.

The current programme included selective methods for detecting AMR solely, and other AMR bacteria may have been present, including among the hygiene parameters *E. coli* and *Salmonella* isolates that were not tested for AMR. Moreover, the programme included only a very limited number of samples and more extensive sampling is needed for more precise estimations of AMR occurrence in fertilizer products.

Conclusion

Although *Salmonella* Okatie was isolated from one product, and samples from three products had numbers of *E. coli* above the proposed hygiene criterium for *E. coli*, the hygienic quality of the products tested in this study was generally satisfactory. None of the samples were positive for the selected antimicrobial resistances tested for. However, this project was a limited study and included a rather small number of samples and products tested. A larger number of samples is needed to make further conclusions on the hygienic quality and the prevalence of selected resistant bacteria and pathogens in fertilizer products on the Norwegian gardening market.

References

1. Mattilsynet. Er avfallsbaserte gjødselvarer trygge å bruke? Resultat frå Mattilsynets nasjonale tilsynsprosjekt. Mattilsynet, Norge, 2012.
2. Anonymous. FOR 2003-07-04 nr 951, "Forskrift om gjødselvarer mv. av organisk opphav". 2003
3. Anonymous. FOR 2016-09-14-1064, "Forskrift om animalske biprodukter som ikke er beregnet på konsum (animaliebiproduktforskriften)". 2016. https://lovdata.no/dokument/SF/forskrift/2016-09-14-1064
4. Anonymous. Proposal for a regulation of the European Parliament and of the Council laying down rules on the making available on the market of CE marked fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009.
5. EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control). EU Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2013. EFSA Journal 2015;13(2):4036, 178 pp., doi:10.2903/j.efsa.2015.4036
6. WHO (World Health Organization). Critically important antimicrobials for human medicine. 2017.
7. International Standardization Organization. Microbiology of the food chain – Horizontal method for the detection and enumeration and serotyping of Salmonella – Part 1: Detection of Salmonella. ISO 6579-1:2017.
8. NORM/NORM-VET, NORM/NORM-VET 2016. Usage of antimicrobial agents and occurrence of antimicrobial resistance in Norway. 2017: Tromsø/Oslo. ISSN:1502-2307 (print)/1890-9965 (electronic).
9. EVIRA. Tillsynsresultat över gödselfabrikat. Lannoitevalmisteiden toutevalvonnan analyysitulokset 2016. https://www.evira.fi/globalassets/tietoa-evirasta/julkaisut/raportit/lannoitevalmisteiden-tuotevalvonnan-analyysitulokset-2016.pdf, accessed 20.11.2017). EVIRA, Finland, 2017.
10. EVIRA. Tillsynsresultat över gödselfabrikat. Lannoitevalmisteiden toutevalvonnan analyysitulokset 2015. EVIRA, Finland, 2016.

Annex 1. Samples analysed in the present project.

ID	Fertilizer/gjødsel*	Material / Materiale	Land/ Origin**	Manure / Gjødsel**
2017-22-165	Growth	Blomsterjord	U	U
2017-22-166	Fertilizer	Plengjødsel	Imp	U
2017-22-167	Fertilizer	Hestegjødsel	Imp	Horse/Hest
2017-22-168	Fertilizer	Hønsegjødsel	Imp	Poultry/Høns
2017-22-169	Fertilizer	Hønsegjødsel	Imp	Poultry/Høns
2017-22-170	Growth	Kugjødselkompost	U	Cattle/Storfe
2017-22-171	Growth	Kugjødsel	Imp	Cattle/Storfe
2017-22-172	Growth	Naturgjødsel	U	U
2017-22-186	Fertilizer	Kompostert hønsegjødsel+kjøttbenmel (pelletert)	U	Poultry/Høns
2017-22-187	Fertilizer	Kompostert hønsegjødsel+kjøttbenmel (knust)	U	Poultry/Høns
2017-22-246	Fertilizer	Hønsegjødsel	Imp	Poultry/Høns
2017-22-247	Fertilizer	Kugjødselkompost	Imp	Cattle/Storfe
2017-22-248	Growth	Kompost	NO	Cattle/Storfe
2017-22-323	Fertilizer	Hønsegjødsel	U	Poultry/Høns
2017-22-324	Fertilizer	Kugjødsel	U	Cattle/Storfe
2017-22-387	Growth	Kompost	Nor	Horse/Hest
2017-22-388	Fertilizer	Ukjent	U	U
2017-22-401	Growth	Kugjødselkompost	NO	Cattle/Storfe
2017-22-402	Fertilizer	Hestegjødsel	U (NO?)	Horse/Hest
2017-22-403	Fertilizer	Hønsepellets	U	Poultry/Høns
2017-22-404	Fertilizer	Kupellets	U	Cattle/Storfe
2017-22-405	Fertilizer	Ukjent	U	U
2017-22-406	Fertilizer	Hønsegjødsel	U	Poultry/Høns
2017-22-410	Growth	Kompost	NO	Cattle+sheep/Storfe+sau
2017-22-411	Fertilizer	Naturgjødsel	NO	Poultry/Høns
2017-22-412	Fertilizer	Plengjødsel	U	Poultry/Høns
2017-22-413	Fertilizer	Hønsegjødsel	U	Poultry/Høns
2017-22-414	Fertilizer	Helgjødsel	U	Poultry/Høns
2017-22-415	Fertilizer	Gjødsel	U	Cattle/Storfe
2017-22-416	Fertilizer	Blomstergjødsel	U	Poultry/Høns
2017-22-417	Fertilizer	Gjødsel	U	Poultry/Høns
2017-22-421	Growth	Gjødsel	U	Poultry/Høns
2017-22-437	Fertilizer	Kompostert fjørfegjødsel	U	Poultry/Fjørfe
2017-22-438	Fertilizer	Ukjent	U	U
2017-22-439	Fertilizer	Ukjent	U	U
2017-22-461	Fertilizer	Gjødsel	DK	Poultry/Høns
2017-22-462	Growth	Kugjødsel	NO	Cattle+Poultry/Storfe+høns
2017-22-463	Growth	Kugjødselkompost	NO	Cattle/Storfe
2017-22-464	Fertilizer	Naturgjødsel	NO	Poultry/Høns
2017-22-465	Growth	Naturgjødsel med mineralgjødsel	SE(?)	Poultry/Høns
2017-22-493	Fertilizer	Kompostjord	NO	Horse/Hest
2017-22-494	Fertilizer	Kompostjord	NO	Horse/Hest
2017-22-495	Fertilizer	Naturgjødsel	NO	Horse/Hest
2017-22-496	Growth	Jordforbedring	NO	Horse/Hest
2017-22-577	Fertilizer	Hønsegjødsel	SE	Poultry/Høns
2017-22-578	Fertilizer	Gjødselmel (blodmel)	NO	U
2017-22-579	Fertilizer	Kugjødsel	SE	Cattle/Storfe
2017-22-580	Fertilizer	Plantejord	SE	Poultry/Høns
2017-22-581	Fertilizer	Kompostert jord	NO	Poultry/Høns

* Fertilizer= Organic fertilizer/organisk gjødsel, Growth = Growth media/soil improvement / Dyrkningsmedier/jordforbedringsmidler
**U = Unknown/ukjent, NO = Norway/Norge, Imp = Import, SE = Sweden/Sverige, DK = Denmark/Danmark

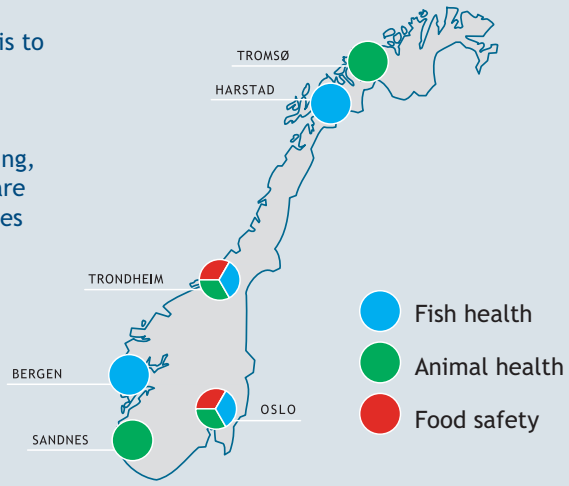
Scientifically ambitious, forward-looking and cooperatively oriented – for integrated health

The Norwegian Veterinary Institute is a national research institute that operates in the fields of animal and fish health, food safety and feed hygiene; its primary task is to provide the authorities with independently generated knowledge.

Emergency preparedness, diagnostic services, monitoring, reference functions, consulting, and risk assessments are all important areas of activity. Our products and services include research results and reports, analyses and diagnoses, studies and advice.

The Norwegian Veterinary Institute's central laboratory and administration lie in Oslo, and we operate regional laboratories in Sandnes, Bergen, Trondheim, Harstad and Tromsø.

The Norwegian Veterinary Institute collaborates with a large number of national and international institutions.



Fish health

Animal health

Food safety

Oslo	Trondheim	Sandnes	Bergen	Harstad	Tromsø
postmottak@vetinst.no	vit@vetinst.no	vis@vetinst.no	post.vib@vetinst.no	vih@vetinst.no	vitr@vetinst.no

www.vetinst.no

Veterinærinstituttet
Norwegian Veterinary Institute