

RESEARCH ARTICLE

Challenges and opportunities using hunters to monitor chronic wasting disease among wild reindeer in the digital era

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Abstract

1. Surveillance of wildlife diseases poses considerable logistical challenges compared to that of humans or livestock. Citizen science can enable broader coverage, but building an efficient disease monitoring system that relies on hunters is challenging. Chronic wasting disease (CWD) is a lethal and infectious prion disease of cervids. Improving surveillance is important with the detection of CWD in wild reindeer (*Rangifer tarandus*) in Norway.
2. This study describes the components of an efficient CWD monitoring system utilizing recreational hunters. We report the success of data capture after 6 years of surveillance. We provide an overview of CWD occurrence among the 24 wild reindeer areas and quantify the likelihood of disease absence in areas without detection.
3. Surveillance aimed to test hunted reindeer aged ≥ 1 year. With higher quotas and extended hunting seasons, proactive surveillance was implemented in at-risk areas. There were several challenges of population demarcation and the lack of surveys required for risk-based sampling. Several specific tools for hunters have been developed, including digital apps for rapid reporting and feedback. Laboratory capacity was expanded, and novel statistical tools were developed for the specifics of the sampled tissues.
4. The surveillance (2016–2021) achieved a sample return rate of 61.5% from a maximum of 22,123 harvested reindeer aged ≥ 1 year. Among these, 64.1% included both relevant tissues (retropharyngeal lymph nodes and brain), yielding 9412 (42.5%) complete samples of harvested reindeer. Samples originating from harvest constituted ~84% of total wild reindeer samples.

Atle Mysterud and Hildegunn Viljugrein shared first authorship.
Jørn Våge and Christer M. Rolandsen shared last authorship.

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5. CWD was detected in 2 of the 24 wild reindeer management areas. The remaining populations had a probability of CWD-freedom from 60% to 99% (mean = 77%) at a design prevalence of 0.5%.
6. Utilizing hunters to monitor wildlife disease appears to be the most realistic option for cervid species. However, the logistical and economic constraints are substantial and pose long-term challenges. Considerable uncertainty about disease occurrence remains even after massive surveillance, and whether management should take preventive actions remains a challenge.

KEYWORDS

cervids, citizen science, disease control, disease surveillance, freedom-from-disease, hunters, logistics, tissue sampling

1 | INTRODUCTION

The prevention and control of infectious diseases start with successful surveillance (Gortazar et al., 2014). Estimating wildlife disease prevalence requires demarcation of primary (epidemiological) sampling units, while estimation of population abundance and demographic composition enable using risk-based or weighted surveillance (Jennelle et al., 2018). However, wildlife populations are difficult to demarcate (Meisingset et al., 2018), and estimation of population abundance is a recurrent challenge (Forsyth et al., 2022). Thus, there are considerable practical and methodological challenges in establishing interdisciplinary and appropriate data collection for wildlife diseases (Lawson et al., 2021; Ryser-Degiorgis, 2013; Walton et al., 2016). Citizen science is developing as an important tool in wildlife management in general (Dickinson et al., 2010), and its potential for use in wildlife disease surveillance has been highlighted (Lawson et al., 2015). Hunters are involved in monitoring populations of at least one large mammalian species in 32 of 36 European countries (Cretois et al., 2020). However, obtaining specific tissues from animals for disease testing poses considerable challenges when hunters are used as citizen scientists.

The early detection of infectious disease outbreaks is crucial for effective mitigation (Savill et al., 2008). Surveillance relying on submitted carcasses is often used to detect epidemic disease outbreaks, but submitted carcasses for laboratory examination may be limited. Achieving the required sample sizes that enable early detection can be challenging for diseases with extended periods at low prevalence, such as chronic wasting disease (CWD) of cervids. Wild cervids found dead or showing clinical signs may be few due to remoteness or forested habitats. Therefore, surveillance using samples from hunter harvests is the key to monitoring CWD in wild deer populations (Gillin & Mawdsley, 2018; Joly et al., 2009; Samuel et al., 2003). CWD has a long pre-clinical phase when the animals appears healthy, but where prions are being replicated and shed (Tamguney et al., 2009). It may take 2–3 years or more before the death of infected individuals (Johnson et al., 2011). Tissues for CWD detection include post-mortem inspection of either the brain

tissue (obex) or retropharyngeal lymph nodes (RLNs). In the United States, the return rate of deer heads at check stations may be below 50% (Belsare et al., 2020a), and obtaining a sufficient sample size is a recurring challenge (Belsare et al., 2020a; Belsare et al., 2021). Knowledge of collecting, storing and preventing contamination is important in obtaining high-quality samples and poses diagnostic challenges when using hunters.

CWD is a transmissible spongiform encephalopathy (TSE), a prion disease, first described in farmed deer in 1967 and wild deer in 1981 in Colorado, USA (Spraker et al., 1997). CWD has been detected in 30 states in the United States and 4 provinces of Canada, and there have been outbreaks in farmed elk *Cervus canadensis* and deer after an import to South Korea. The disease causes a decline in numbers in the most affected populations of white-tailed deer *Odocoileus virginianus* and mule deer *Odocoileus hemionus* in the United States (DeVivo et al., 2017). Development of CWD prevalence vary between areas (Osnas et al., 2009), and active management have likely contributed to a lack of increase in prevalence in some areas (Conner et al., 2021; Manjerovic et al., 2014). Europe was regarded free of CWD (EFSA Panel on Biological Hazards (BIOHAZ) et al., 2016) until the unexpected discovery of CWD in a wild reindeer *Rangifer tarandus* in the spring of 2016 (Benestad et al., 2016). This led to the extensive expansion of CWD surveillance in Norwegian cervid populations.

The aims of this study are (1) to describe how a system for disease surveillance for CWD using hunters as citizen scientists has been built using and improving existing infrastructure and establishing novel ones, and how logistical difficulties have been solved. We considered the main components and stages of how the surveillance system was built (Table 1). (2) To provide a descriptive analysis of spatial variation and temporal trends in logistics efforts and data capture for different management areas. (3) To report the status of CWD occurrence among wild reindeer in Norway after 6 years (2016–2021) of monitoring and testing 15,369 reindeer from the 24 wild reindeer populations in Norway (Figure 1). To estimate (4a) CWD prevalence in the second (latest) population with detection and (4b) the likelihood of the remaining 22 areas being free from CWD.

TABLE 1 An overview of infrastructure used to build a chronic wasting disease (CWD) surveillance system for reindeer in Norway based on pre-existing and new components. See [Figure 2](#) and Supplementary [Table S2](#) for the direct components of the hunter sampling

Component	Opportunity	Challenge	Infrastructure, resource or reference
Institutions	Combine veterinary and ecological expertise required for improved disease management	Collaboration across disciplines and institutes are challenging with own cultures. Overcoming institutional competition and personal conflicts	Supplementary Table S1 The Norwegian Food Safety Authority The Norwegian Veterinary Institute (NVI) The Norwegian Environment Agency The Norwegian Institute for Nature Research (NINA)
Disease and CWD surveillance	Develop laboratory capacity and build competence	Capacity; up to 1600 samples a day in season; seasonal labour	Pre-existing: Cervid Health Monitoring of NVI; CWD monitoring; prion-lab NVI: significant experience with testing for scrapie and BSE. March 2018: NVI was designated as the 3rd reference laboratory for CWD testing in the world and the 1st in Europe by the World Organization of Animal Health (OIE)
Population monitoring	Allow estimation of population abundances and composition; age-determination	Not funded for all areas. Conditions may not always allow for annual surveys	Pre-existing: Population monitoring of NINA (Solberg et al., 2017); age determination lab at NINA (Veiberg et al., 2020)
Demarcation of epidemiological units	Obtain higher correspondence management borders and epidemiological units	Considerable effort to obtain data from specific local region; required establishment of new management area	Figure 1 and Supplementary Table S2 New management area: Raudafjell Differentiate between Nordfjella zone 1 and Nordfjella zone 2 Include small northern part of Hardangervidda in Nordfjella zone 2 Lack of ability to separate Rondane into southern and northern subunits
Hunting regulations (quota, season)	Increase sample sizes, obtaining samples increasing detection or lower detrimental population impact	Getting public understanding of novel quota settings	Supplementary Table S3 Ordinary quotas ('calves', 'adult females and yearlings' and 'free licences'). New: quota composition and size; new type of licence (small, <50 kg male, Nordfjella zone 2) Annual hunting season is 20 August–30 September. New: extension of seasons in Nordfjella and Hardangervidda
Statistical estimation tools	Develop tools as a basis for evidence-based management; prevalence estimation. Account for imperfect detection of CWD during infection with samples at hand	Data limitations, data not available for all populations, data incoming without age or sex. Uncertainty about disease progression; sample quality due to autolysis. Implementation unpopular	Pre-existing: Population estimation model (Nilsen & Strand, 2018) New: Disease detection model (Viljugrein et al., 2019); scenario-tree model, freedom-from-CWD (Viljugrein et al., 2019); Harvest strategy simulation model (Mysterud et al., 2020)

2 | MATERIALS AND METHODS

An overview of the various steps performed to build the current CWD surveillance system is presented in [Table 1](#) and [Figure 2](#). The CWD surveillance in Norway involves four institutions (Supplementary [Table S1](#)). Their pre-existing infrastructure in the form of wildlife disease surveillance and ecological population monitoring of cervids was used and modified to fit the new context. We therefore highlight that many critical components for efficient surveillance was in place, and also identify lacking components (see sections 'After CWD detection').

2.1 | Disease and CWD surveillance in cervids

The Norwegian Veterinary Institute (NVI) operates several surveillance programmes for wildlife diseases, including the National Wildlife Health Surveillance Program (ViltHOP), focussing on cervid health. NVI is also the National Reference Laboratory for TSE in animals, and Norway follows the European surveillance program for TSE (European Food Safety Authority (EFSA), 2019). Norwegian CWD surveillance has been reported annually since 2003, although with a low number of wild reindeer tested prior to 2016. The program was

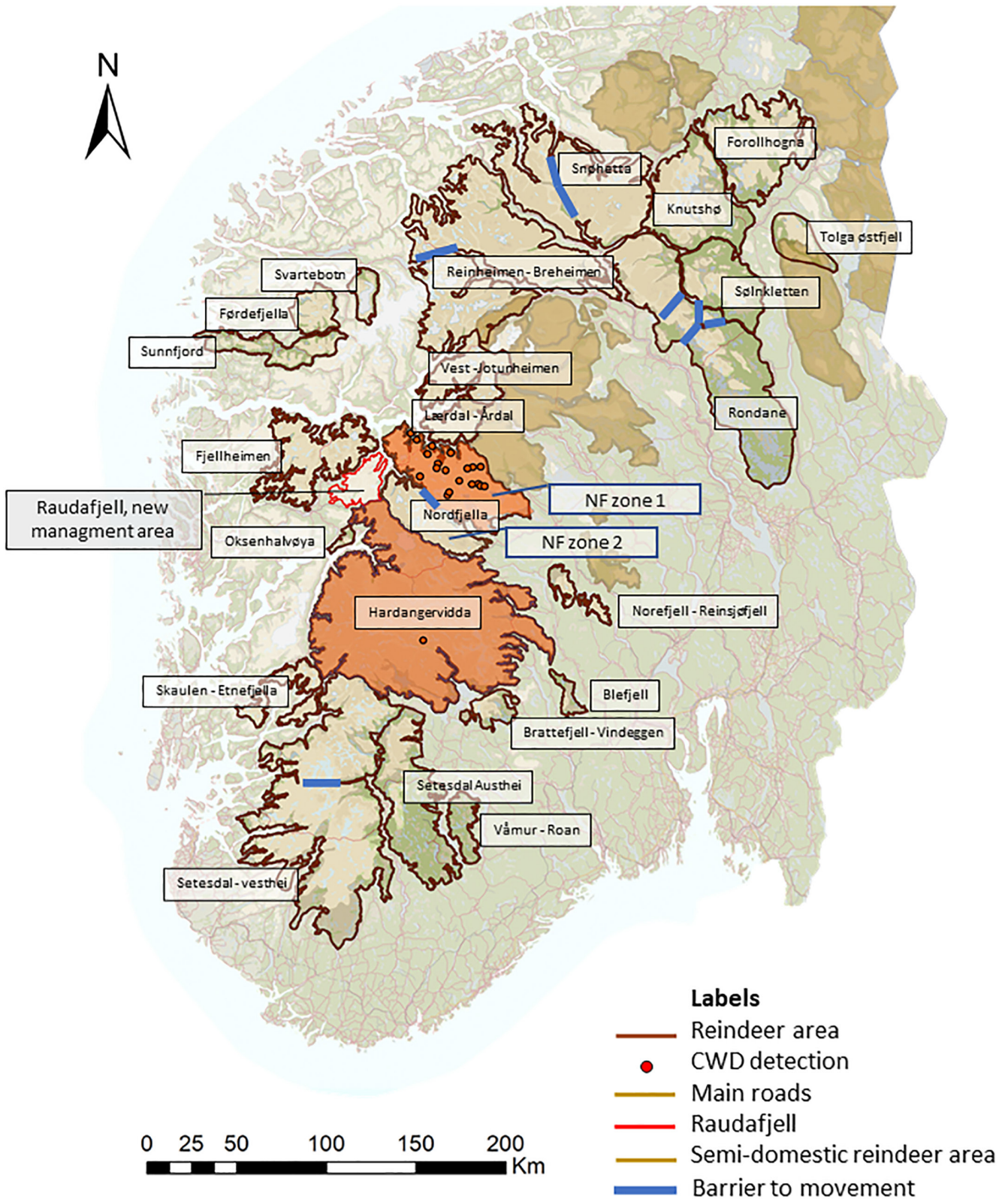


FIGURE 1 An overview of the current demarcation of wild, alpine reindeer populations in southern Norway. One new area was established due to chronic wasting disease (CWD) management. There is ongoing fragmentation into subunits indicated with blue lines for barriers. CWD has only been detected in Nordfjella management zone 1 ($n = 19$) and on Hardangervidda ($n = 1$).

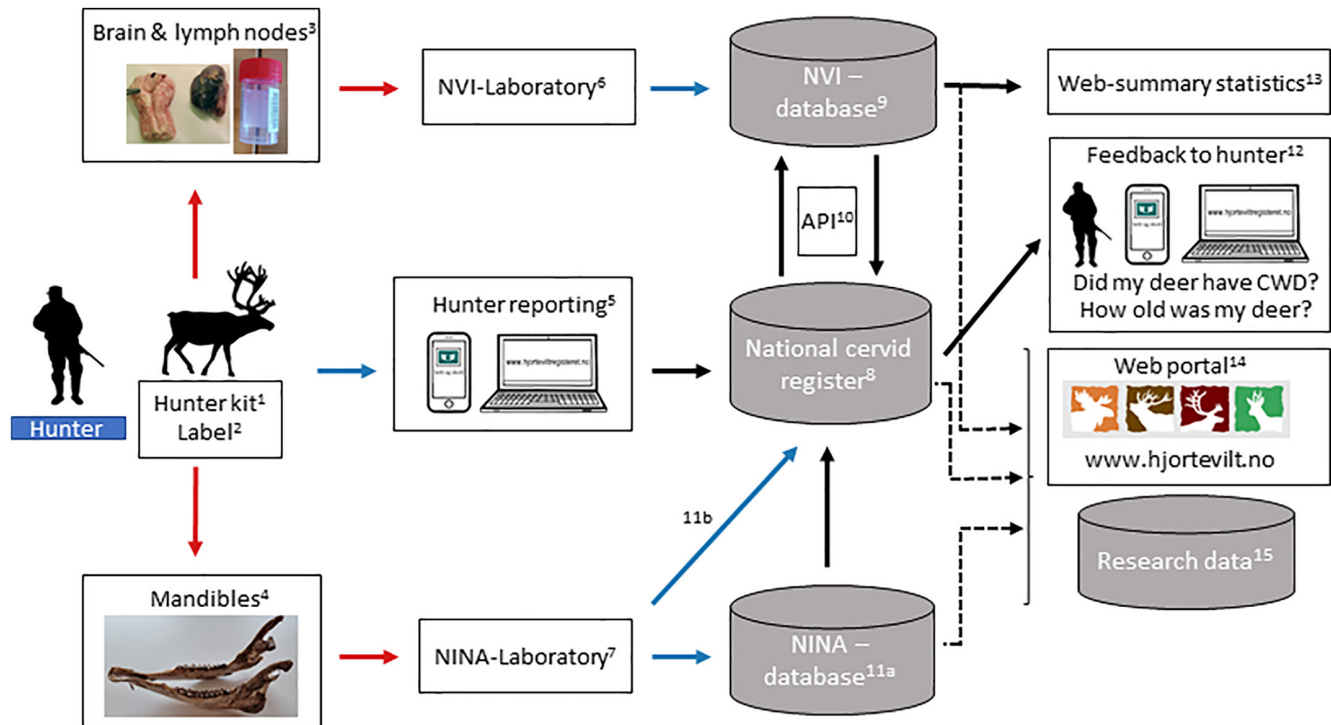


FIGURE 2 An overview of the surveillance system combining population monitoring and chronic wasting disease (CWD) monitoring. Red arrows indicate physical shipment by post, blue arrows manual data entry and black arrows indicate either fully automatic data exchange (solid black) or a combination of automatic, script based and manual data flow (black dashed line).

boosted temporarily from 2006 to 2010 due to an EU CWD survey with 600 cervids tested per country.

2.1.1 | After CWD detection

The NVI TSE laboratory rapidly expanded the analysis capacity to test for CWD from the extensive surveillance program of all cervids being initiated, resulting in 10,152 samples being analysed in 2016 and 25,659 in 2017. In 2018, the NVI was designated a World Organization for Animal Health (OIE) Reference Laboratory for CWD, the first in Europe.

2.2 | Population monitoring of wild reindeer

The Norwegian Institute for Nature Research (NINA) and local management boards conduct population surveillance in eight management areas consisting of (1) minimum counts from aeroplanes or helicopters during winter, (2) calving counts from aeroplanes mainly of female herds during summer and (3) demographic composition counts from the ground during the rutting season, when sexes are aggregated (Nilsen & Strand, 2018). In addition, harvest data in fall were available from all areas, down to sex and age (calves, yearlings and adults). The estimation model was run per population and accounts for variable effort in surveys across years. Calves and yearlings were separated from adult reindeer by tooth eruption patterns (Veiberg et al., 2020).

2.2.1 | After CWD detection

The likelihood of CWD detection varies depending on age, sex and type of sample (e.g. from hunted vs. animals found dead/sick/hurted) and such heterogeneous risk groups form the basis for weighted surveillance (Jennelle et al., 2018). Estimates of population abundance and demographic composition provide data that can be used in weighted surveillance. Detailed monitoring data were available in 8 of the 24 management areas, typically areas with larger populations. For the smaller populations, only rough population estimates were available (VKM et al., 2021). We assumed a similar demographic composition to the areas surveyed. Demographic information form the basis for heterogeneous risk groups for CWD used in estimation of freedom from infection.

2.3 | Populations and demarcation of epidemiological sampling units

Demarcation of populations, and hence epidemiological and sampling units, is challenging for wildlife (Belsare et al., 2020b; Joly et al., 2009). The wild reindeer in Norway are mainly alpine and historically consist of northern and southern metapopulations with limited gene flow due to natural topographic barriers (Kvie et al., 2019). Today, wild reindeer are managed within distinct sub-populations due to an increasing degree of fragmentation caused by human-made infrastructure and disturbances (Figure 1). Within these units, wild reindeer are mainly nomadic with limited site fidelity (Panzacchi

et al., 2015), that is, close to full mixing of individuals, as assumed in standard statistical tests. Therefore, these formal management sub-populations comprised our main epidemiological and spatial sampling units.

2.3.1 | After CWD detection (overview in Supplementary Table S3)

Due to new infrastructure limiting connectivity, a further division into sub-populations is observed in some areas, but they do not represent formal management entities. Hence, some management borders no longer reflect epidemiological units, as evidenced by the GPS tracking of reindeer. In addition, local management and researchers identified herds of reindeer using geographical areas outside the current management units. We identified and solved the following mismatches between epidemiological and previous management units: (1) The reindeer in the CWD-infected population in Nordfjella are managed in two zones separated by a road (FV50 Hol-Aurland), comprising semi-separate epidemiological units (Figure 1). Contact with local management was initiated to obtain harvest data separated by zone (zones 1 and 2). (2) Local management and researchers identified herds of wild reindeer living outside Nordfjella zone 2 towards the west, outside any official management region. As a result, a new formal management area of Raudafjell was established in 2019 as the 24th wild reindeer area of Norway (Figure 1). These reindeer, in particular males, often move between Raudafjell and Nordfjella zone 2, as documented with GPS-marked reindeer. Therefore, despite being another legal unit, we considered these data part of Nordfjella Zone 2. (3) The legal and biological divisions between Hardangervidda and Nordfjella zone 2 are different. The biological (and epidemiological) population unit is separated by a road (RV7 Hardangervidda), severely limiting connectivity. However, part of the hunting quota is given by Hardangervidda on the north side of the road (Figure 1). Therefore, we included all individuals harvested north of the road as part of Nordfjella zone 2.

2.4 | Quotas and hunting regulations

Recreational hunters regulate populations within wild reindeer management areas by annual harvests. Quotas are given down to sex and age groups ('calves', 'adult females and yearlings' and 'free licences') in the form of physical licence cards. The annual hunting season is from 20 August to 30 September.

2.4.1 | After CWD detection (overview in Supplementary Table S4)

The decision to depopulate the Nordfjella zone 1 population for reindeer was accompanied by an extended hunting season (10 August–31 October), a high quota of free licences in 2017, helicopter aid with

transport and information of herd whereabouts to increase offtake (Mysterud, Strand, et al., 2019). As part of proactive CWD surveillance, a largely increased and male-biased quota was given in 2019 in Nordfjella zone 2 and Hardangervidda to enable early detection or establish freedom-from-CWD (Mysterud et al., 2020). In zone 2, a special licence card for younger adult males (up to 50 kg) was introduced, while Hardangervidda introduced free 'adult male only' licences from 2019. After the subsequent detection of CWD in Hardangervidda, the season of 2021 was extended (10 August–7 October), aiming to increase the harvest to mitigate the possible outbreak.

2.5 | The CWD sample collection: Information, tools and feedback

An overview of the 15 steps in the CWD surveillance system is provided and marked with numbers in Figure 2. The system was built by modifying pre-existing systems and introducing new ones. Important changes were made during the first years of establishing the system after the first lessons learned.

2.5.1 | Hunter kit^{Step1}

A sampling kit for hunters was developed specifically to collect and send CWD samples (Supplementary Figure S1). The kit includes a pre-stamped envelope, a label^{Step2}, two plastic bags for waste and samples, two short plastic gloves, a spoon designed to collect a brain stem sample (with the obex area) through the foramen magnum, a test tube for the joint brain and lymph node sample^{Step3}, and instructions for all steps in an infographic format (Supplementary Figure S2). The kit was introduced in the 2017 hunting season in selected monitoring areas. From 2018, it was used in all sampled areas for all cervid species.

2.5.2 | Label^{Step2}

The label, printed and made available by the Norwegian Environment Agency, has a unique barcode linking the samples to the correct individual animal (Supplementary Figure S3), and was further developed from a label previously used in cervid population monitoring. Each label has five stickers for labelling the samples, with one intended for the test tube containing the joint brain and lymph node sample. That sticker also contains information about species, reindeer area or municipality for other cervids, name and phone number of the submitter, and whether the sample comes from an animal shot during hunting or an animal found dead/sick/hurted. The remaining stickers or barcode numbers can be used for additional samples (e.g. mandible for ageing) from the same individual. At the minimum, the hunter must indicate location, date, age category, and sex, and is also encouraged to report carcass weight digitally on the web and app (*Hunters self-reporting*^{Step5}).

The label was introduced in 2017 and updated in 2018. It was based on previously used species-specific labels for collecting individual data, mandibles and ovaries from harvested cervids in the Norwegian population monitoring program and labels used by NVI to submit samples from cervids. The motivation to develop a common label was based on challenges identified in 2016 and the first part of 2017 using different labels for different species and several types of labels from different institutions and purposes, some even without a unique identifier.

2.5.3 | Brain and lymph node^{Step3}

The brain sample and RLN were submitted to NVI for testing^{Step6}. The primary test was an ELISA (TeSeE® ELISA SAP, Bio-Rad, until July 2020; thereafter, HerdChek BSE-Scrapie Ag Test IDEXX), routinely performed on a pooled sample of the brain (preferably from the obex) and RLN tissues. The preliminary test's positive or inconclusive results were confirmed by western blotting (TeSeE® Western Blot; Bio-Rad) on individual tissue samples. The analytical tests had high specificity (European Food Safety Authority (EFSA), 2005). The analytical test sensitivity for the Bio-Rad CWD antigen test kit, ELISA, was 92.5% (81.8%–97.9%) for obex and 98.8% (93.5%–99.97%) for RLN in North American cervids (Hibler et al., 2003). However, the sensitivity was not evaluated for the IDEXX ELISA in European cervids. The pooled RLN and brain tissue samples will have slightly lower sensitivity than analysing the two samples separately. However, it enables cost-efficient and simultaneous monitoring of both classical and atypical variants of CWD.

2.5.4 | Ageing using mandibles^{Step4}

Hunters collect mandibles with molars and incisors in selected areas. The mandibles were collected locally and labelled with barcodes identical to the CWD test sample. After the hunting season, samples are submitted to the NINA laboratory for age determination^{Step7}. Usually, the work is completed within a few months after the hunting season. However, the age determination of CWD-positive individuals was completed within a few days after the mandibles/teeth arrived at the laboratory.

2.5.5 | Hunters self-reporting^{Step5}

Each reindeer licence is given to the hunter as a physical licence card. All the information from the label (*Label*^{Step2}) should then be reported digitally using the 'seen-and-shot' app or the website of the Norwegian Cervid Registry ('Hjorteviltregisteret') owned by the Norwegian Environment Agency. The 'seen-and-shot' app was developed prior to the 2017 hunting season and improved in 2018, including the possibility to store information when offline/outside mobile coverage.

2.5.6 | Data flow^{Step8,9,10&11}

Data flow between the NVI Laboratory Information Management System (LIMS)^{Step9} and the Norwegian Cervid Registry (NCR)^{Step8} provides hunters with feedback about CWD test results for an individual deer. The NCR was updated six times a day with the CWD test results, using the barcode number from label^{Step2} as the identifier. The LIMS uses the same barcode number to check for additional data on each individual for their records. The data flow between the NINA laboratory database^{Step11a} and NCR^{Step8} provides feedback on the age of the animals. This is increasingly being done using custom-made web forms^{Step11b} developed for the National population monitoring program that facilitates updating the age and quality checking of other data on individuals already stored in the NCR by hunters or managers.

2.5.7 | Feedback to hunters about test results^{Step12}

Feedback on individual CWD test results for hunters was provided through the websites of the NCR. The feedback depends on a successful match between the unique barcode reported by the hunter (*Hunters self-reporting*^{Step5}) and the barcode registered in the LIMS. Express overnight postal tags were provided for each sample kit. The median time between sampling by the hunters and samples arriving at NVI (submission time) was 4 days in 2016–2021. The annual median varied between 2 days in 2016 and 5 days in 2020–2021. Only 4% of the samples with dates registered had a submission time longer than 14 days. For Hardangervidda, the median submission time to NVI varied annually between 5 and 6 days. Usually, the samples are analysed on the same day they arrive at the NVI.

2.5.8 | Information campaigns for hunters, managers and the public

Much effort has been made to provide hunters with general information about CWD. NVI and NINA alone contributed to presentations at about 200 local, regional, and national meetings and conferences in the last half of 2016 and 2017. A decision was made to provide all important information available from national institutions on the website www.hjortevilt.no^{Step14} (a web information portal about cervids and cervid management in Norway), either directly or linking to other web pages. This includes a YouTube video about 'What is CWD' made in 2017, now having received >1.2 million views (January 2022), and instructional videos on how to take tissue samples (Supplementary Table S2). A new website (<http://www.vetinst.no/skrantesjukestatistikk>) was also established by NVI^{Step13}, allowing the public to obtain daily updated statistics on the number of tested animals depending on the year, cervid species, production form (farmed or wild), data source (hunting or other categories) and geographical area. This website also provides aggregated data on the proportion of samples with only brain samples and the proportion of both brain and lymph node samples submitted.

2.6 | Statistical analysis and tools

1. The annual numbers of tested animals from each area during the hunting season were extracted and presented as summary statistics and descriptive figures. When the 'reason for sampling' was not provided, we presumed all samples received in or within a month after the hunting season to originate from ordinary hunting. Information on the sampling date was available for approximately 60% of the samples. For small areas, we set a threshold sample size for inclusion in figures describing of the proportion of hunted animals tested ($n > 15$) and the proportion of samples including RLN ($n > 10$). These thresholds were set a bit arbitrary, but to avoid calculating proportions with very low sample size.
2. We developed statistical tools to estimate CWD prevalence and likelihood for freedom-from-CWD in a given epidemiological unit (Viljugrein et al., 2019). This includes an explicit model of the pathogenesis of CWD accounting for how the specific tissue (brain or RLN) tested affect the likelihood of detecting CWD. The model uses a separate detection function for brain and RLN reflecting their different sensitivity under the course of infection (Supplementary Note 1: Accounting for imperfect CWD detection). We previously estimated the CWD prevalence in the population of Nordfjella zone 1 (Mysterud, Madslie, et al., 2019). Supplementary Note 1 presents a similar analysis for the recent CWD detection in Hardangervidda. A primary objective of this study was to estimate the likelihood of freedom-from-CWD in all other wild reindeer areas of Norway, as detailed in Supplementary Note 1. Samples mainly originate from hunting but include samples from animals found dead/sick/hurted and animals harvested outside the hunting season. The stochastic estimation method is based on a risk-based scenario tree modelling approach (Martin et al., 2007), and the risk of testing positive for CWD infection is assumed to be three times higher in adult males than in adult females and double as high in adult females than in yearlings (Mysterud et al., 2020). We did not separate between further risk groups related to sample category, because there was very few samples registered from high-risk groups such as animals found dead/sick/hurted, including those showing clinical signs.

We assumed a low probability of introduction (0.1%; i.e. 1 introduction per 1000 years) for all years and calculated the probability of freedom for a range of design prevalence (four animals, 0.3%, 0.5% and 1%), that is, the level of infection in a population the surveillance aim to detect. We restricted the lower threshold of the design prevalence to four infected animals for small populations. When information on the age category was lacking, we included the sample in the low-risk category of yearlings. We used an uninformed prior of 50% for the probability of infection before the surveillance started in 2016 (except for Nordfjella zone 2, see Supplementary Note 1).

3 | RESULTS

3.1 | Data from the surveillance system

We retrieved 14,693 samples from wild reindeer from the annual hunts 2016–2020 (Table 2, Supplementary Table S5). Surveillance was conducted in most reindeer management areas in the form of collection of samples from ordinary harvest. However, there was intensified hunting effort to increase the sample size (i.e. proactive surveillance) in Hardangervidda and Nordfjella zone 2. Samples from hunted wild reindeer constituted 84% of the total samples tested. The remaining samples were outside the hunting season (marksmen culling) and animals found dead/sick/hurted. The total sample was 65.5% out of the 22,123 reindeer aged 1 year or older harvested, with considerable variation among reindeer areas, and markedly lower in most areas in 2016 and 2017 compared to later years (Figure 3a). This was mainly due to the fact that sample collection was first systematically organized in all parts of all wild reindeer areas from 2018. For 29% of samples, the age category or sex of the adults was unknown, and for 4% of samples, the reindeer area was not registered. The proportion of samples with both lymph node and brain tissue averaged 65.0% (Figure 3b). It was highest in the samples from the CWD-affected range of Nordfjella, partly due to extra logistics (field stations with veterinarians collecting samples) and the inclusion of reindeer culled by marksmen. Prior to 2018, only the brain tissue was included in the sampling of several populations, explaining the abrupt increase in lymph node samples.

3.2 | Status of the CWD occurrence

In Nordfjella zone 1, 6 females and 13 males tested positive for CWD until the entire population had been culled in May 2018 (Mysterud, Madslie, et al., 2019). In 2020, one adult male (aged 8.5 years) shot during the regular hunting season tested positive for CWD in Hardangervidda. The estimated prevalence in Hardangervidda is low (~0.1%; 95% credible interval: 0–0.5% or 0.6%, depending on prior distribution, Supplementary Note 1) and lower than the estimated prevalence of 0.6% in adult females and 1.8% in adult males in Nordfjella zone 1.

Despite intense surveillance, a high level of freedom-from-infection probability was only achieved in a few populations for a 1% design prevalence (Figure 4), that is, the lower threshold prevalence that the surveillance is designed to detect. For stricter design prevalence of 0.3% or 0.5%, or set as four individuals, the probability of freedom-from-infection was low for most areas. An exception was Nordfjella zone 2, which had an increased hunting effort and culling by marksmen during the winter of 2019 due to its geographical location between the two areas with CWD detection. With the current harvest rate and composition, it will take several years to reach 95% probability of freedom-from-infection for the stricter design prevalence in most populations (Supplementary Table S6).

TABLE 2 An overview of population sizes, area, harvest and sample sizes from all wild reindeer areas of Norway. Samples of CWD are for harvest data from 2016 to 2021 (<http://apps.vetinst.no/skrantesykestatistikk/NO/#omrade>; updated 22th of Nov. 2021); hence, total sample size (not shown) is slightly larger including animals found dead/sick/hurted and marksmen culling. RLN = retropharyngeal lymph nodes. Harvest of >1 year old reindeer is summed for 2016–2021. Unfit = samples did not have quality to be tested. Complete sample = proportion of harvested being sampled with both RLN and brain

Management area	Population size (n)	Area (km ²)	Harvest ≥ 1 year old (n)	N samples harvest	Prop. Sampled (%) ^a	Lack age and/or sex (n)	RLN and brain (n)	Brain (n)	RLN (n)	Unfit (n)	RLN and brain (%)	Complete sample (%)
Setesdal Ryfylke	3500	6154	1557	674	43.3	100	500	167	7	4	73.7	32.1
Setesdal Austhei	2000	2400	437	192	43.9	47	143	49	0	1	74.1	32.7
Skaulen - Etnesfjell	60	486	78	60	76.9	34	35	20	3	3	57.4	44.9
Våmur-Roan	240	406	172	80	46.5	32	59	18	4	0	72.8	34.3
Brattefjell-Vindeggen	500	357	461	287	62.3	135	197	89	4	2	67.5	42.7
Blefjell	140	186	89	53	59.6	10	45	10	0	0	81.8	50.6
Hardangervidda ^b	7000	8136	7350	5212	70.9	1286	3355	1826	50	31	63.8	45.6
Norefjell-Reinsjøfjell	700	314	792	489	61.7	168	225	259	2	4	45.9	28.4
Oksenhalvøya	12	80	0	2		2	0	2	0	0	0	
Fjellheimen	440	1705	175	160	91.4	38	113	47	0	0	70.6	64.6
Nordfjella zone 2 ^c	450	3004	530	593	100	44	606	61	3	0	90.4	100
Lærdal-Årdal	120	488	32	37	100	2	29	10	0	0	74.4	90.6
Vest-Jotunheimen	400	985	93	93	100	8	74	18	1	0	79.6	79.6
Sunnfjord	125	700	35	25	71.4	9	14	13	0	0	51.9	40
Førdefjella	100	700	32	21	65.6	4	8	13	1	0	36.4	25
Svartebotnen	55	99	34	24	70.6	9	16	10	0	0	61.5	47.1
Reinheimen-Breheimen	2900	4551	2824	1712	60.6	569	1210	481	13	15	70.4	42.8
Snøhetta	2700	3345	2846	1549	54.4	237	992	546	12	13	63.5	34.9
Rondane ^d	3500		1677	962	57.4	386	623	334	7	10	64	37.1
Sølnkletten	800	1330	753	300	39.8	116	218	81	3	0	72.2	29
Tolga Østfjell	2000	453	99	68	68.7	1	52	15	0	1	76.5	52.5
Forollhogna	1700	1843	1134	783	69	271	293	490	4	2	37.1	25.8
Knutshø	1500	1776	914	515	56.3	185	284	223	4	4	55.1	31.1
Raudafjell ^e			9	10	100	0	10	0	0	0	100	
Unknown area				600	NA	580	311	267	7	17	51.7	

^aNote: for areas Nordfjella, Lærdal-Årdal, Oksenhalvøya, Raudafjell, the number of samples are higher than what can be achieved by harvest. This is most likely due to inclusion of some animals found dead/sick/hurted, or because some animals may be registered with different age class in the two registries of harvested versus tested animals.

^bSamples from small northern part of Hardangervidda included in Nordfjella zone 2 due to limited movement over road.

^cWe separated Nordfjella zone 1 and zone 2 due to limited movement between zones. CWD samples in 2016 were not separated between zones and includes ~210 samples from Nordfjella zone 1. Harvest from Nordfjella zone 1 was included for 2016.

^dPopulation management separate south (2500 deer) and north (1000 deer) part; but CWD samples were from unknown part.

^eRaudafjell was established as a separate management area from 2020. For epidemiological analyses, CWD samples from Raudafjell are included in Nordfjella zone 2 due to extensive movement between areas.

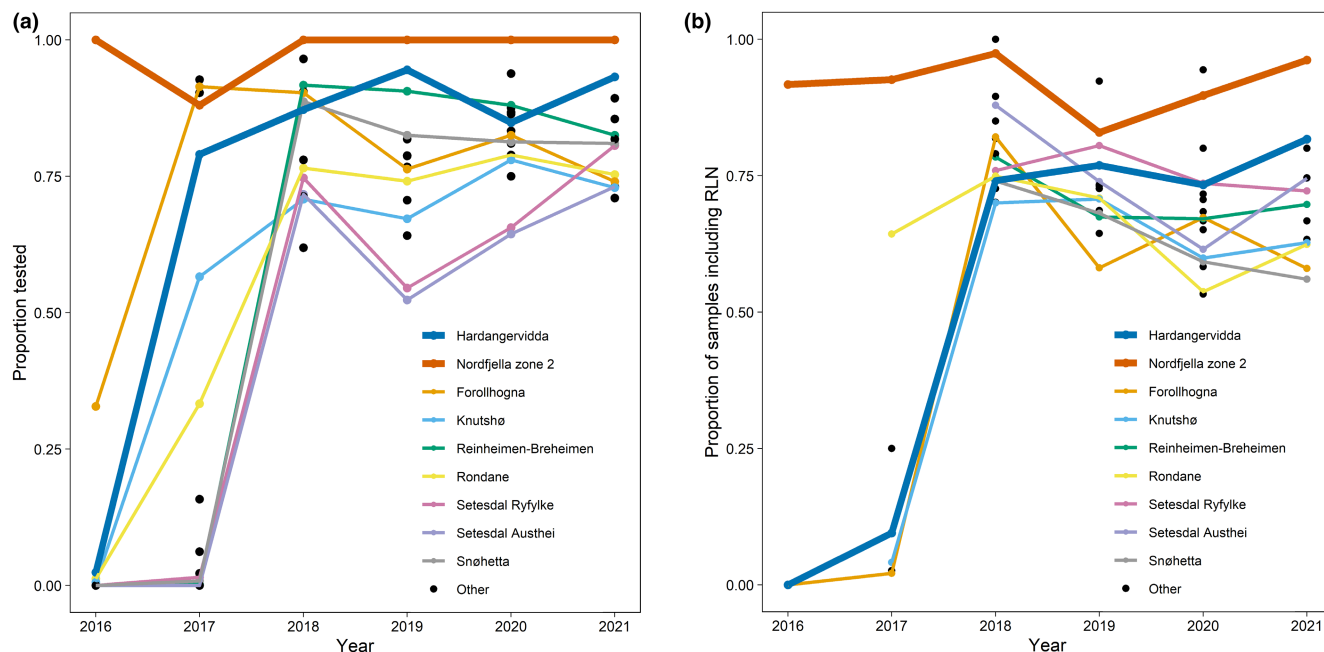


FIGURE 3 The success of data collection of the surveillance system over time for the largest reindeer areas. Smaller reindeer areas are indicated by unlabelled black dots. (a) The proportion of reindeer 1 year or older that was sampled from legal harvests, and (b) the proportion of the samples containing both lymph nodes and brain tissue.

4 | DISCUSSION

Global changes have increased the risk of infectious wildlife disease outbreaks, and establishing robust surveillance systems at broad scales is important and challenging (Lawson et al., 2021). Citizen science has been used for targeted (active), scanning (passive) and syndromic surveillance of wildlife diseases (Lawson et al., 2015). We developed a systematic observation scheme combined with wildlife population monitoring to facilitate evidence-based management of CWD. Surveillance has resulted in CWD detection in a new region, while we are still far from documenting freedom-from-CWD in other reindeer management areas with high certainty (Figure 4).

4.1 | Challenges when using hunters as citizen scientists

The success of surveillance using hunters in the case of CWD involves challenges such as (a) increasing sample size by increased harvest and target demographic groups with a higher probability of infection, (b) proportion of harvest being sampled and reported correctly, (c) proportion of samples containing both targeted tissues (RLN/obex), and (d) data quality. Opportunistic sampling is the most common method for disease surveillance (Lawson et al., 2015). For the populations most at risk (Hardangervidda and Nordfjella zone 2), extra harvesting targeting adult males and culling by marksmen were also performed (Mysterud et al., 2020). This increased harvest was effective in increasing sample sizes, and ~47% of the adult male segment on Hardangervidda was removed in a single year (Mysterud et al., 2021). However, such actions are controversial among hunters.

In all other wild reindeer populations, surveillance is based on ordinary hunting regulations that are usually accepted. The motivation for data collection is critical for citizen science. We obtained data from 65.5% of reindeer harvested at 1 year or older. This percentage would be higher if the sample collection had been initiated from the very beginning in 2016 in all wild reindeer areas (Figure 3a). These are high numbers compared to that from the United States, achieving samples from less than 50% of harvested deer (Belsare et al., 2020a). The reasons for the higher return rates are likely the pre-existing structures of population management and the long tradition of using hunters in citizen science efforts (Cretois et al., 2020).

Relying on citizen science may result in lower data quality (Dickinson et al., 2010). Diagnosis of prion diseases requires post-mortem tissue analysis, and there are logistical constraints to obtaining a sufficient amount of quality tissue during the sampling. In the case of CWD, salivary glands or muscle tissue are occasionally received in lieu of RLN. In addition, the low anatomical integrity of brain tissue samples due to rough extraction or autolysis causes uncertainty as to whether the desired part of the obex is included. There was discussion about which tissue was best suited for CWD analysis when the surveillance system was designed. Initially, only brain stem tissue (obex) was included in testing cervids in Norway for several reasons: (1) diagnostics for TSE in animals in Norway and the EU relies on brain tissue, (2) the brain (obex) is the primary tissue for testing all cervids except genus *Odocoileus* in Canada (Canadian Food Inspection Agency, 2020) and (3) hunters find it more difficult to collect the RLN tissue sample. Furthermore, an atypical/sporadic variant of CWD was detected in moose in the summer of 2016 (Pirisinu et al., 2018), and red deer in 2017 (Vikøren et al., 2019) showed the accumulation of prions in the brain but not in lymph

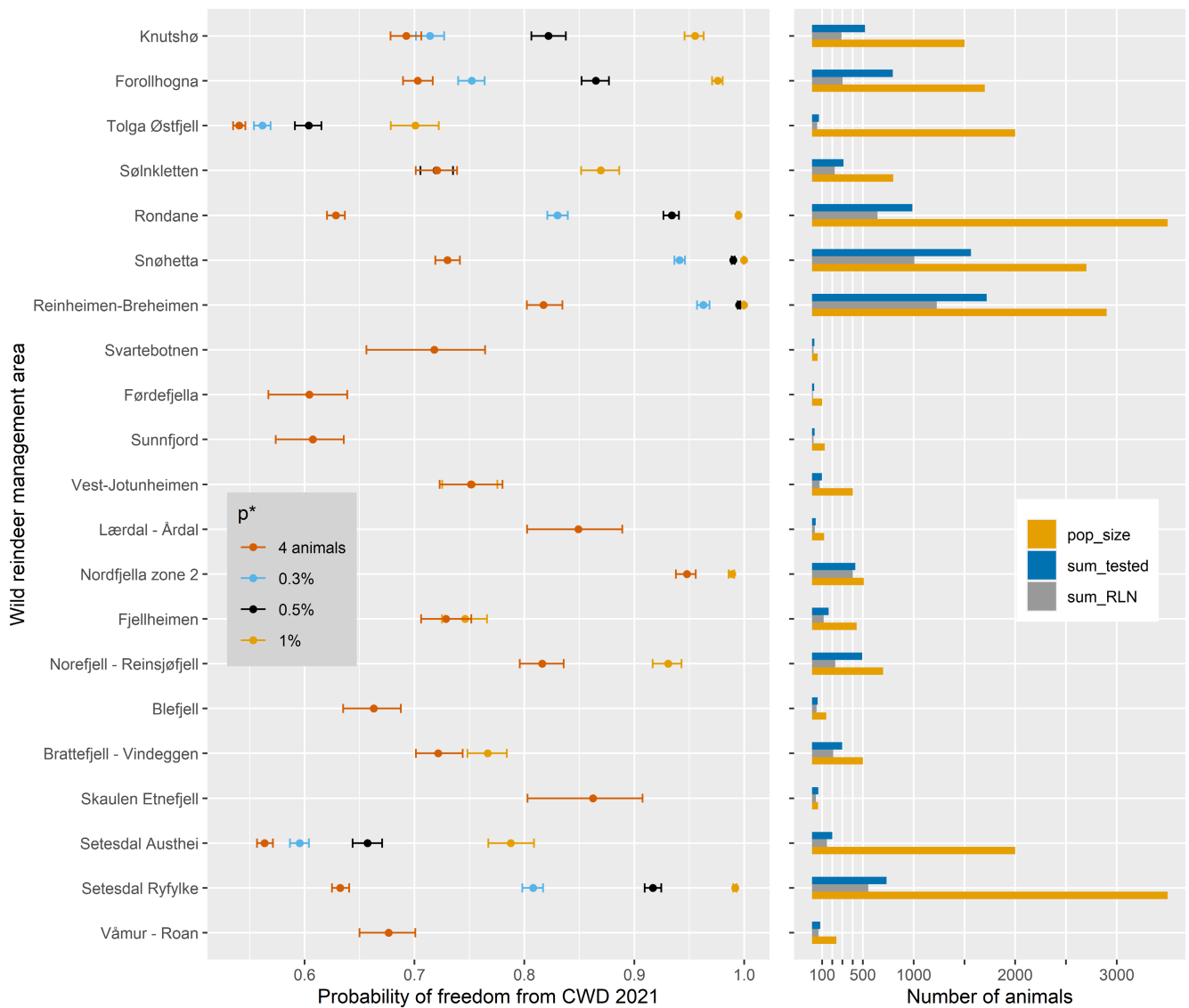


FIGURE 4 The probability of freedom-from-infection in all reindeer populations in Norway without detection of chronic wasting disease (CWD) after 6 years of surveillance (2016–2021). This probability can be calculated for different thresholds of prevalence to be detected in the surveillance system, termed design prevalence. The prior probability of freedom was set to 50% in 2016. Bars to the right are population sizes, overall sample size, and lymph nodes (RLN) sample size.

nodes. The first hunter-harvested reindeer testing positive for CWD shot in 2016 was weakly positive in brain tissue, but the RLN proved to be highly prion positive. RLN is the standard used for CWD testing in deer of the genus *Odocoileus* in most states in the United States (Bloodgood et al., 2020), as RLN accumulates prions at an earlier stage of the disease than the brain. The knowledge gathered from Nordfjella zone 1 confirmed a similar pattern in reindeer, with RLN being the first tissue where prions are detected. In Nordfjella, the RLN was collected from 2016 onwards, whereas only brain samples were collected in other reindeer areas. Based on these new insights, it was decided late in 2016 to increase RLN sampling and use a pooled brain/RLN sample in the primary ELISA test. This explains the significant increase in the RLN from 2018 onwards (Figure 3b).

The return rates of RLN have been highly variable, from 0% in most areas in 2016 and several areas in 2017 up to 97% in Nordfjella zone 2

in 2018 (Figure 3b). To improve the proportion of collected RLNs, field stations were established in the affected Nordfjella region, where trained veterinarians collected the samples from heads delivered by the hunters (Supplementary Note 2). In addition, videos with instructions on how to collect samples were produced (Supplementary Table S2), similar to those developed by for example the Department of Natural Resources in Wisconsin in USA (<https://dnr.wisconsin.gov/topic/WildlifeHabitat/register/sample.html>). Submitting samples in Norway was mandatory in 2017–2019, but non-compliances were not penalized. Current submissions are made voluntarily. However, mandatory testing may again become necessary in cases of demotivation among hunters, or whether some are deliberately resistant and do not want to discover CWD due to potentially very invasive management actions upon disease detection. A possibility is to require submissions to renew the hunting licence the following year.

4.2 | Population demarcation and estimation

Demarcation of wildlife populations, and hence epidemiological and sampling units, is challenging (Joly et al., 2009; Russell et al., 2015). Spatial sampling considerations are important for populations that lack demarcations (Nusser et al., 2008). The demarcation of population boundaries for wild reindeer was due to fragmentation (Figure 1). It is fair to presume close-to-full mixing due to nomadic and grouping behaviour within the most recognized units. For CWD surveillance, incoming samples were adjusted to follow the epidemiological unit rather than the management area for Hardangervidda and Nordfjella zones 1 and 2. However, in at least four formal reindeer areas, further substructures were detected using the GPS-marked reindeer (Figure 1). This may be a problem if the harvest is spatially biased; however, we currently have no solution to this issue. Furthermore, information about connectivity among reindeer populations may be used to adjust certainty for freedom-from-CWD depending on the epidemiological context.

Weighted surveillance for CWD has become a standard in many US states (Jennelle et al., 2018), and we developed a similar system using scenario-tree modelling (Viljugrein et al., 2019). Risk-based disease sampling is an effective way to incorporate samples with different likelihoods of infection. Animals found dead/sick/hurted or animals showing clinical signs form high-risk samples, but unfortunately such cases were rare in these remote areas. We therefore mainly relied on demographic groups as the basis for heterogeneous risk groups for CWD. Estimating the prevalence and the use of risk-based surveillance therefore requires population abundance and demographic composition estimates. With established reindeer population monitoring in 8 of the 24 management areas, pre-existing structures were instrumental. In reindeer populations without information about sex and age composition, we assumed a demographic composition similar to that of the population surveillance areas. Future developments may also include the genetic composition of populations linked to the prion protein gene, *PRNP*, affecting susceptibility to CWD among reindeer (Viljugrein et al., 2021).

4.3 | Management facing uncertain and changing disease status

Disease surveillance among wildlife is costly. When to stop surveillance and consider an area free of disease, that is, exit strategies, must consider the sensitivity of any surveillance system relative to international standards (Adkin et al., 2016). For ASF in wild boar, an exit strategy involving a 'screening phase' and a 'confirmation phase' was recommended and also identifying potential pitfalls (lifelong infections) (European Food Safety Authority (EFSA) et al., 2021). Bovine tuberculosis in New Zealand was managed by culling of the primary wildlife host, and optimal stopping rules for active disease management was developed depending on economic costs (Gormley et al., 2018). The economic cost of CWD surveillance in Norway is

also substantial. The environmental contamination of prions in soil increase over time and make eradication more and more difficult (Miller et al., 2004; Zabel & Ortega, 2017). Early detection of CWD is therefore important for mitigation, but massive sampling is required to obtain this (Belsare et al., 2020a). The detection of CWD among reindeer at Hardangervidda after ~3500 negative samples illustrates the challenges of detection, and further surveillance efforts are needed for the southern metapopulation. Hence, despite huge efforts in surveillance, management in Norway will have to make decisions with considerable uncertainty regarding the disease status in different management areas (Figure 4). Active disease management requires extensive harvesting or culling, and such actions are notoriously unpopular in quite large segments of the hunter population and among local and regional stakeholders. Balancing relevant disease mitigation actions to gain public acceptance is a huge challenge facing different emerging wildlife diseases.

AUTHOR CONTRIBUTIONS

Jørn Våge and Christer M. Rolandsen coordinated the CWD monitoring with contributions from Hildegunn Viljugrein, Petter Hopp, Sylvie L. Benestad, Knut Madslie, Torfinn Moldal, Linh Tran and Turid Vikøren. Sylvie L. Benestad led the NVI TSE laboratory. Roy Andersen, Olav Strand and Geir Rune Rauset conducted the population monitoring. Atle Mysterud drafted the paper with parts contributed by Sylvie L. Benestad on CWD testing, Christer M. Rolandsen on CWD sample collection and Hildegunn Viljugrein on statistical methods. Petter Hopp and Hildegunn Viljugrein performed data cleaning and summary. Hildegunn Viljugrein performed the analyses, and Haakon Bakka contributed to the prevalence estimation. Olav Strand created Figure 1, Christer M. Rolandsen created Figure 2 and Hildegunn Viljugrein created Figures 3 and 4. All authors edited the subsequent drafts and approved the final version of the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

All data are publicly available in Zenodo: <http://doi.org/10.5281/zenodo.7410345> (Viljugrein, 2022).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S0. Stochastic distribution of mean diagnostic test sensitivity, defined by mean and standard deviation, was generated by a simulation study (1000 iterations of 30 infected individuals with random time since infection) where test sensitivity is dependent on age category, type of tissue tested and development of infection.

Table S1. A description of the four primary institutions with formal responsibility for CWD management.

Table S2. An overview of tools used to build a CWD surveillance system for reindeer in Norway.

Table S3. An overview of changes to reindeer management areas due to CWD management in Norway, 2016–2021.

Table S4. An overview of ordinary reindeer management and CWD exceptions given in Norway, 2016–2021.

Table S5. An overview of data capture and missing information related to CWD testing of harvested wild reindeer in Norway (updated 22th of Nov. 2021). Harvest of ≥ 1 yr old reindeer is the aim for the monitoring. Age group = calves, yearlings or adults. Complete samples = proportion of harvested that was sampled with both RLN and brain. Unfit = samples did not have quality to be tested.

Table S6. An overview of the approximate number of years it will take to reach 95% (Year_95%) confidence for absence of CWD at design prevalences (p^*) of 0.3%, 0.5%, and 1% prevalence or as 4 individuals. The sample sizes given to reach the 95% (Samples_95%) is given the same harvest rate and composition of harvest (proportion of adult males, $p(\text{adM})$) as for the one of last three years with most samples tested (the year with highest probability to detect disease). Note that this will only be an approximation, and the trajectory required to reach different design prevalences will depend on how harvest and population segments (risk groups) develop over time.

Figure S1. An overview of the kit given to hunters including pre-stamped envelope, tag, 2 plastic bags for waste and samples (“poser

til avfall og prøver, 2 stk), 4 short plastic gloves (“kort hanske 4 stk”), spoon to acquire brain sample (“Skje til hjerneprøve”), and test tube for joint brain and lymph node sample.

Figure S2. Tag to be filled in for each shot animal. Each tag have a unique barcode linked to individual animal.

Figure S3. A two page instruction for how to collect samples and fill in tag.

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