Test and remove for wild sheep: a user guide

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Summary

This user guide is intended to help wildlife biologists and wildlife health specialists design, conduct, and provide feedback on "Test and Remove" management to clear respiratory disease from wild sheep populations chronically infected with Mycoplasma ovipneumoniae (Movi). It provides an overview and background information on test and remove in wild sheep, describes general procedures, highlights considerations for implementation, and addresses frequently asked questions. There are links to more detailed information and references in the Supplement section.

Introduction

Test and remove (also referred to as test and cull) is a tool that has been used for managing infectious diseases in domestic animals when no effective treatment or vaccine is available. It has also been applied or proposed for control of diseases in wildlife such as brucellosis in ibex, bison, and elk; bovine tuberculosis in African buffalo and white-tailed deer; and devil facial tumor disease in Tasmanian devils. Evaluation of test and remove for pneumonia in wild sheep started in 2013 in the Asotin Creek, WA herd of the Hells Canyon metapopulation. Companion crossover commingling experiments were conducted in captive bighorn sheep at South Dakota State University and Washington State University from 2013 – 2019. As of 2020, test and remove has been implemented or is in progress in 15 bighorn sheep populations or metapopulations in 7 states and 1 province (Supplemental table 1). Results from trials in free-ranging and captive sheep have supported the use of test and remove as a management tool to clear Movi and associated pneumonia from bighorn sheep populations (Box 1).

Box 1: Evidence in support of test and remove to clear Movi from wild sheep

Experiments in captivity

- confirm that Movi carriage can be an individual trait that is constant over years
- demonstrate that a single Movi carrier ewe in a nursery group can trigger a pneumonia outbreak in lambs(1)

Health testing and experiments in free-ranging populations

- show that there is individual variation in Movi carriage(2)
- show that removal of chronic carrier ewes can clear Movi and disease in free-ranging bighorn sheep populations(3)

Natural clearance

- instances of natural clearance of disease and infection from bighorn sheep populations have been documented to be associated with the death of Movi carrier ewes (4,5)

When should test and remove be used?

Test and remove is one component of a disease management strategy for wild sheep. The primary objective for this action is to increase recruitment in populations that are chronically infected with Movi and experience recurring fatal pneumonia outbreaks in lambs. It can also be used to clear Movi from chronically infected populations for other reasons, for example to reduce health risks to nearby unexposed populations.

Test and remove is not a management action that can be used to intervene in an acute pneumonia outbreak.

What are the underlying assumptions?

The following assumptions form the foundation of Movi test and remove management in wild sheep:

- Movi is an introduced pathogen in wild sheep. The adapted hosts, domestic sheep and goats, are important reservoirs of infection, and spillover into wild sheep populations can be associated with significant short and long-term negative health impacts (6,7).
- Movi infection predisposes wild sheep to polymicrobial pneumonia and is generally necessary (although not sufficient) for pneumonia epizootics to occur. In other words: no Movi = extremely low likelihood of pneumonia outbreaks affecting all-ages or only lambs in free-ranging wild sheep populations (8,9)
- Most wild sheep that survive initial exposure to Movi clear it and are resistant to reinfection with that strain. They are not carriers.
- There are two Movi carrier classes in bighorn sheep: intermittent (partially resistant) and chronic (Box 2).
- Chronic carriers maintain Movi and respiratory disease in wild sheep populations and are responsible for transmission to intermittent carriers and lambs.

Box 2: Different kinds of Movi carriers

Repeated testing of individuals over multiple years indicates that sheep that test positive on nasal swabs for Movi can be classified into one of several categories of carriers:

Chronic carriers maintain continuous, long-term infections. These animals test PCR-positive on nasal swabs for Movi nearly every time they are sampled, and typically have strong antibody responses to Movi on the blood serum cELISA test.
Chronic carriers should be prioritized for removal.

Intermittent carriers are partially (but not entirely) immune to Movi. They develop shorter-term, low-grade infections. Intermittent carriers test PCR-positive during some sampling events, and negative on others.
Intermittent carriers do not need to be prioritized for removal.

Juveniles (lambs and yearlings) that survive infection are mostly intermittent carriers although the duration of their infections may be longer term than those of adult intermittent carriers. Usually they clear infection and do not need to be removed.
Retest young animals that are positive.

What factors affect Movi carriage and success of test and remove?

So far, two factors seem to have influenced patterns of Movi infection and complicated implementation of test and remove: sinus tumors (10) and new spillovers. Testing should be conducted for these conditions, particularly if Movi carrier prevalence is high (>30%).

Sinus tumors

- In affected populations in captivity and in other populations for which data are available, sinus tumors were rarely detected in sheep that weren't Movi carriers (11).
- Movi and disease may remain after removal of chronic carriers, presumably because of high infection rates. Clearing Movi will take longer and success likely depends on the prevalence of sinus tumors.
- Currently sinus tumors can only be confirmed post-mortem.
- Example populations: Lostine, OR (2), Snowstorms, NV, Poudre River, CO (10)

New Movi spillovers during test and remove

- In affected populations Movi and disease remain after removal of chronic carriers, presumably because of acute infection associated with exposure to new strains (12). Clearing Movi will likely only be possible after the wave of infection from spillover has run its course.
- New invasions can be identified by strain-typing if the invasion strain predominates in the sample. Sheep that switch status from negative to positive are good candidates for strain-typing to detect new spillovers.
- Example populations: Rapid City, SD (3), Pine Ridge, NE (13)

How to test and remove?

The design for test and remove has changed over time and continues to evolve. Wildlife managers have adapted it to fit different situations or perspectives. When carefully thought out and done systematically this helps to improve the technique and increase its utility. Key components of test and remove are covered in Box 3 and described in more detail in the next sections.

Detecting chronic carriers

- At least two positive tests at least one year apart are recommended to distinguish chronic from intermittent carriers.
- Animals that test negative on the first test do not necessarily need to be retested. More tests can help in some cases, for example if a test result is indeterminate.
- A single positive test will identify intermittent as well as chronic carriers and more animals may be removed than necessary. One infected ewe can be enough to maintain Movi and disease in a well-mixed population or subpopulation, so large numbers of removals are usually not necessary to clear disease. The rarity of true chronic infections in the absence of detectable sinus tumors is probably why disease sometimes fades out naturally from infected populations.

- Young animals (lambs and yearlings) are most likely to be positive and are good indicators of active transmission in the population, but those that survive typically clear Movi by their second year even when other sheep in the population are infected, and do not become chronic carriers. Removal of intermittent carriers or young animals is unlikely to clear Movi although it might reduce transmission. The importance of distinguishing carrier types differs on a case by case basis taking into account the proportion of animals that test positive (i.e. the number of intermittent carrier sheep that would be removed on a the basis of a single test vs the number of chronic carriers), the logistics of testing and removals, and other factors.
- Since ewes are responsible for transmission to lambs and because rams seem to play less of a role in maintaining infection, although it can be useful to test rams, the priority is on testing all ewes in the population. Movi has been successfully cleared from populations by removing only carrier ewes. There are some exceptions, for example a case of a possible transmission route from chronic carrier rams to lambs in the summer in a Nevada population with the complicating factor of sinus tumors.

Box 3: Implementing test and remove for Movi

- **Plan for a multi-year project.** While there are different approaches, test and remove in bighorn sheep can take 3 years or more (2 for testing and 1 for follow-up sampling). Focus on testing all ewes in the population or subpopulation that is being treated and individually tag or collar (VHF or GPS) animals so they can be recaptured for retesting or removal.
- **Confirm clearance of Movi through follow-up testing.** Populations can experience periodic years of relatively good lamb recruitment even when infected. A year of higher recruitment doesn't confirm absence of Movi. To verify that Movi has been cleared, sample animals (blood serum and swabs) born after completion of the project. All samples should be negative if the population is free of Movi. This information is crucial for evaluating the outcome of test and remove. No supplementation should be undertaken until Movi is confirmed to be cleared from the population.
- **Removal decisions may not always be obvious or easy.** There can be uncertainty in identifying which animals to remove, even in the presence of repeated testing. Keeping accurate records on individual sex and age, testing, and necropsy results, will contribute to simplifying this process.
- **Test and remove design is still under development. Careful data collection and documentation of what worked and what didn't can advance knowledge and aid in developing approaches for a variety of contexts.**

Sampling and laboratory testing

- Success of test and remove is contingent on the ability to reliably detect most, if not all carriers. Essential samples to collect for detecting chronic carriers and spread of

Movi are nasal swabs for PCR, which detect active infection, and blood for serology, which indicates current or previous exposure. Animal-side tests (for example Biomeme) are currently not sensitive enough to reliably detect carriers but a combination of field and laboratory tests could be used to facilitate a rapid response.

- Strain typing of a minimum of 5 Movi samples is recommended to determine how many strains are in the population, whether a new spillover has occurred, and to identify other reservoirs of Movi that have matching strains and could be epidemiologically connected, either as a source or recipient of infection.
- Necropsies of any animals that die or are removed should follow an established protocol for documenting respiratory disease and sinus tumors (10).
- Collecting other samples can be helpful depending on the situation and project goals.

Carrier removal

Plan ahead for lethal or nonlethal removal to be efficient and to gain the most information possible from this action. Both lethal and nonlethal removals can be time consuming. Animals with multiple rounds of testing can hold a great deal of research value and true chronic carriers are uncommon in most populations and could be useful for captive research. Animals removed lethally can contribute to advancing knowledge if they are removed whole or if good quality samples can be obtained.

How do you know test and remove has been successful?

While increasing recruitment is the objective of test and remove, this is not always a good indication that Movi has been cleared from the population. Sometimes infected populations experience years with good recruitment, then fall back into the vortex of poor lamb survival. No supplementation should be undertaken until Movi is confirmed to be cleared from the population. There is no risk of Movi persistence in the environment.

- Lambs are the most likely age class to be infected, and lambs acquire infection and develop antibodies after birth. If lambs greater than two months of age have antibodies to, or are shedding Movi, then Movi is still circulating in the adults. Follow-up sampling (nasal swabs and blood) of lambs or yearlings that were born after test and remove is completed is the best and most efficient way know whether you are "done" without having to test the entire population. Animals born after a successful test and remove should be negative for infection and exposure (PCR and seronegative).
- Optionally, a subset of adults can be retested as well, to confirm that they are PCR negative and, while they may still be seropositive for several years following exposure, the levels and prevalence of antibody should be declining.

Current and future direction

While test and remove has shown promising results, it is relatively new. Each trial takes several years, so many test and remove experiments are not yet complete. There is also variability in the dynamics of Movi across bighorn sheep populations, and each test and remove is a little different. Every test and remove project is an opportunity to build on what we know so far, even if it doesn't go as planned.

Current research and adaptive management is focused around optimizing implementation. Some of the questions that current and planned test and remove projects hope to address include:

- How can animal handling be minimized? Can particular social or demographic conditions (such as population structure and contact patterns) be exploited to reduce the number of animals that need to be tested?
    - Are there subpopulations that maintain disease in metapopulations through periodic transmission to adjacent populations? Could testing and removal limited to those subpopulations clear Movi from the metapopulation?
    - How much isolation is needed for short term infection status to be independent of status in adjacent subpopulations in order to "divide and conquer" execution of test and remove?
- How do the environment and associated management activities affect transmission? For example, in the desert, overall densities may be low, but there could be frequent contacts at water sources during summer. Populations in northern environments may congregate on small winter ranges or at feeding sites. What is the effect of natural or artificial salt licks?
- How accurate are tests that provide a result in the field?

Additional questions are listed in the supplement

Supplemental information for test and remove in wild sheep

How to collect samples and interpret test results

General information on testing for Movi is available online from WADDL Mycoplasma ovipneumoniae Diagnostics in Domestic and Wild Sheep and Goats (wsu.edu) Also see supplemental table below.

Collecting nasal swabs – The best sample for detecting Movi infection is a deep nasal swab from both nostrils. Swabbing both nostrils is crucial because there are cases of chronic unilateral infection of one nare. Gently rotating the swab in the nare to collect as much bacteria as possible is important for detection of Movi, and for better success in strain-typing. Collecting a second swab as a backup is recommended.

- **Swab type** - The swab should be made of synthetic material – for example a polyester or polyurethane tip on a plastic handle such as the **BD BBL CultureSwab EZ** here. Natural materials like cotton and wood contain substances that inhibit PCR. A double swab is no better than one single swab because the surface area touching the nostril is not very different. If a duplicate swab is desired, collect two single swabs or split the double swabs up and insert each in the nose separately.
- **Handling the swab after sampling** – Place the swab in a tube, or break off the end into a vial or whirlpak bag, and keep cool. Ship on ice to the lab after sampling or store in a freezer at -20 for future submission. The swab does not need to be placed in media for the PCR test. Avoid repeated thawing and freezing which can degrade DNA.
- **How to interpret PCR test results** – The lab will report three outcomes of the PCR test: Detected, Not detected, and Indeterminate (sometimes referred to as Suspect). Indeterminate means the test is inconclusive. Submit the backup swab or retest the animal. Some animals consistently test indeterminate. They are probably not chronic carriers. For more information on the PCR test at WADDL see Movi PCR Test Details (wsu.edu). PCR testing for Movi is also available at the Kansas State Veterinary Diagnostic Laboratory Movi PCR (k-state.edu), Animal Health Centre, Abbotsford, British Columbia AHC testing, and internally through some state wildlife laboratories.
- **Strain-typing** – Strain-typing is offered at WADDL for animals that tested positive on PCR. There is a high degree of genetic variation in Movi. Multi-locus Sequence Typing (MLST) is a method to genetically fingerprint Movi to determine how many strains are in the population and whether strains match within and across populations and with other potential sources of infection. The current MLST method at WADDL uses sequences from 4 loci as described in (12). The 16S-23S intergenic spacer region (IGS) is the locus that usually provides the best strain discrimination. If the IGS sequences differ, then the strains are different. However, some strains have the same IGS sequences but differ at other loci. Sequences from all 4 loci are recommended to provide a high degree of strain discrimination but it is possible that some biologically different strains will have the same 4 locus sequences and would require sequencing additional loci to be discriminated. If the concentration of DNA in the sample is low, if polymerase inhibitors are present, or if the strain is polymorphic at primer binding sites, not all loci may amplify.

- **Field testing nasal swabs** – Recently developed onsite testing holds promise for providing Movi infection results in the field, prior to releasing animals. Work to date has focused on

using the commercially available Biomeme PCR test although another option is a LAMP (loop-mediated amplification) test developed by the Colorado Parks & Wildlife Health Lab, which is not available commercially. The Biomeme PCR test takes about 60 minutes to run, but early results showed lower sensitivity than the laboratory test. Current research is focused on increasing sensitivity of the Biomeme test. WADDL also offers a same day PCR test for an additional charge.

Box 4: Quick reference for Movi test and remove sampling

Also see Mycoplasma ovipneumoniae Diagnostics in Domestic and Wild Sheep and Goats (wsu.edu)

Sample type	Attribute and characteristic being measured	Materials needed	How to handle and ship samples	Test to request how to interpret result
Deep nasal swab	Movi DNA Identifies current infection status If Movi is detected, strain type can be determined	Synthetic material swab e.g., BD BBL CultureSwab EZ Store in included tube or break off into vial or whirlpak bag No media required	Place in protective tube, vial, or whirlpak bag Short term storage (<1wk) refrigerate or freeze at -20 Ship on ice	PCR - Movi Detected = infected Not detected = not infected Indeterminate = inconclusive Movi strain typing Multi-locus sequence data reported Compare samples using genetic tree-building software (free and web-based)
Blood serum	Antibody to Movi Indicates prior or current exposure	Red- or tiger-top blood tube Storage vial volume 0.5 ml or greater	Centrifuge blood and aliquot 0.5 ml serum into vial Short term storage (<1wk) refrigerate or freeze at -20 Ship on ice	Serology - cELISA for Movi Not detected = unexposed Detected = exposed Indeterminate = inconclusive Herd-level test

Collecting blood serum – WADDL is the only lab currently running a serologic antibody test for Movi. See link at the top of this section. The cELISA test requires about 0.5 ml serum from blood collected into any kind of serum tube (red and gray tiger top is the usual type used) and centrifuged. The serum should be aliquoted into a vial and shipped on ice.

- **How to interpret serology test results** –The report from WADDL will include a "%I" and three outcomes of the cELISA test: Detected, Not detected, and Indeterminate. The %I (inhibition value on the cELISA test) corresponds to the amount of antibody present in the serum.
    - %I < 40% Antibody not detected
    - %I > 50% Antibody detected at levels consistent with previous exposure or current infection with *Mycoplasma ovipneumoniae*
    - % I 40% to 50%: Antibody detection is indeterminate meaning the result is inconclusive

The ELISA test is designed for classifying populations, not individuals. From 30 – 100% of animals usually test positive in exposed populations and <1% of animals will test positive in unexposed populations. It's not possible to tell population exposure status if antibody prevalence is between 1 and 30%. Prevalence of antibody in exposed desert sheep populations is often lower (median 57% range 6% – 100%) than in northern sheep populations (median 76%, range 25 – 100%) (7, Table S2). Antibodies to Movi can be detected in bighorn sheep for several years following exposure, so detections are a good indication that Movi has been circulating in the population, but not always a good indicator of current infection status. This is why it is important to test young animals, born after test and remove has been completed to confirm that Movi has been cleared from the population. No antibody should be detected in these animals.

Sampling considerations

Starting test and remove following an outbreak - Test and remove is simplest when Movi prevalence is low. One to two years after adult mortality rates have dropped following spillover may be a good time to implement test and remove, especially if lamb survival seems to be improving. If testing is started too early clearance will still be in progress and prevalence will be higher because some non-carriers will still test positive from the initial exposure.

Test and remove in interconnected populations, subpopulations, or ewe bands – Strategies for working on interconnected populations have been to start at one end and work through populations consecutively or to do some initial surveillance testing across the metapopulation and start with the population with the highest prevalence and/or the most interconnected.

Marking animals – Unless animals are individually recognizable and accessible, all animals tested should be radio-collared and individually marked so they can be located and recaptured if necessary.

Minimum number of animals to test to detect infection (or clearance) - The minimum number of samples needed to detect infection depends on the prevalence of Movi, the sensitivity of the Movi test, the level of confidence desired, and the population size. A binomial test can be used to estimate the number of tests needed to detect a positive. There are many online sample size estimators available. An Excel spread sheet calculator can be downloaded from USDA APHIS here.

Case histories

Table 1. Summary of Movi test and remove management actions conducted or in progress in free-ranging bighorn sheep populations, 2013 – 2020.

	Population	ST/PR	Month/Year of outbreak(s)	Pop. est[1]	Mo/Yr Start testing	No. sheep removed	Mo/Yr completed[2]
1	Asotin	WA	12/2011	70	9/2013	3	2/2017
2	Custer State Pk	SD	2004	25	8/2014	2	2/2017
3	Black Butte	WA	11/1995, 11/2013	30	8/2014	8[3]	2/2017
4	Other Hells Can.	WA,ID,OR	11/1995, 12/2011	650[4]	2/2017[4]	1	1/2020
5	Lostine	OR	2005	80	1/2012[5]	13	NA
6	Rapid City	SD	2009	60	2014	5	NA
7	Snowstorms	NV	2011	50	2012[6]	18[7]	NA
8	Bison Range	MT	6/2016	33	2/2018	0[8]	12/2020
9	Pine Ridge	NE	2004 - 2014	30(?)	2/2017	10	NA
10	Chasm	BC	2012	35	2018	5	NA
11	Fraser River	BC	1990's	50	2018	11	NA
12	Lower Salmon	ID	1989	425	11/2020	NA	NA
13	Lookout Mt.	OR	2/2020	150	12/2020	NA	NA
14	Yakima Canyon	WA	2009	100	2/2021	7	NA
15	Leppy Hills	NV/UT	2010	40	8/2020	2	NA

[1] Estimated population size at start of test and remove.

[2] When population was confirmed Movi-free. NA = in progress

[3] None of the sheep removed were carriers

[4] Metapopulation – 12 populations range 5 – 110 sheep. Also connected to Asotin, Black Butte, and Lostine

[5] First removals in 12/2014

[6] Comprehensive testing and nonselective removals started in 12/2015

[7] Not all removals were carriers

[8] One carrier ewe died naturally

Additional Management and Research Questions

Some of these larger questions are best answered by replicating testing and/or removals in different contexts.

- What cofactors or individual characteristics are associated with chronic carriage?
    - Are there physical characteristics that can be used to identify chronic carriers without testing?
- In what situations are rams important in maintaining Movi in populations or are rams chiefly transmitting Movi between subpopulations?
- What is the minimum time interval between tests for correctly classifying chronic carriers?
- How might the capacity and options for carrying out removals (lethal or transfer to captivity) affect implementation?
    - What are the tradeoffs between removing all animals that test positive vs only chronic carriers?
- How does infection prevalence influence the success of test and remove?
    - Since surviving lambs are usually infected, does low lamb recruitment facilitate clearance of Movi?
- Are certain strains of Movi easier to clear than others?
- Do populations recover more quickly after high virulence outbreaks?
- Are there any management actions that could facilitate natural clearance without or with minimal testing and removal?

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