Wild Sheep Foundation Focus Group Questions

1. Have all the PCR swabs collected to date, from wild sheep and/or goats, been processed?
   Those received by USDA-ARS-ADRU from June 2018 onward have all been analyzed. Most of those received prior to this date have also been analyzed, with the exception of some archived (older) samples that were received frozen which will be processed over the next month.
   a. Were these swabs submitted to WADDL, USDA-ARS, or both? **Question for AKFG**
   b. Why or why not? **Question for AKFG**

2. What were the results?
   This information should be obtained from the AKFG.

3. Did the testing on wild and domestic Caprinae follow exactly the same protocol, and did both labs use the same protocols? If so, is there any legitimate reason to doubt the validity of one set of results over the other? If not, what were the differences and what bearing might that have had on the results?
   The nasal swab samples from domestic sheep and goats, received by USDA-ARS-ADRU (and WADDL), were 'dry swabs' (swabs that were contained in a sterile cylinder after collection without culture/broth/agar medium). Most of the nasal/other samples from wildlife were sent in either PBS (early archived samples), and majority of samples were in fresh (unfrozen) or frozen universal transport medium.
   The DNA isolation protocols a bit different between, but USDA hasn't seen indication that one submission type over the other is better for the testing done within the USDA laboratory. USDA-ARS-ADRU has provided all of the information to AKFG for how the samples were tested and the testing method is published in the December 2018 issue of *Emerging Infectious Diseases.*
   There is legitimate reason to doubt the validity of one of results over the other. Testing performed at USDA-ARS-ADRU and the WADDL are different. The WADDL uses a real-time PCR (no sequencing) and has been using a standard universal Mycoplasma PCR which is reported to detected "all" (thus 'universal') mycoplasma species, then they sequence, according to results that have been shared between collaborators (AKFG and USDA). USDA-ARS-ADRU has no information on the details of how the PCR or sequencing is performed. This information would need to be provided by the WADDL for any interpretation of the benefits or limitations of the assay. USDA-ARS-ADRU also uses a universal mycoplasma PCR, likely the same one that WADDL uses since it is a published assay. There are limitations to this method based on what we/USDA have found in samples, as it's not uncommon for animals to carry multiple species of mycoplasmas so the assay may amplify multiple species of mycoplasma or it may preferentially amplify the mycoplasma species that is at a higher amount in the sample, masking, so to speak, mycoplasma bacteria that are at very low levels.
4. Please explain your (or the agency's) interpretation of these results.
   USDA-ARS-ADRU has reported results to AKFG and to the State Veterinarian at DEC. USDA-ARS-ADRU (Highland) is no clear on what this request for interpretation is asking for exactly.
5. There is an oft-stated comment that Dall Sheep are more naïve (i.e. susceptible) to Mycoplasma ovipneumoniae than Bighorns. Is this a valid hypothesis based on scientific studies, or an assumption based on anecdotal examples? If the former, please provide a link to the studies.

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Hypotheses are not facts. There is no reason to think that a Dall's sheep (or herd) is more or less susceptible than a bighorn sheep (or herd). I know of no comparative studies looking specifically at the susceptibility differences between bighorn sheep and Dall's sheep. I can imagine that small enclosure captive studies placing Dall's sheep with domestic sheep and goats (forced commingling in captivity) are on the horizon, by some researcher (no plans of this at USDA-ARS-ADRU), in order to prove (which is not what science really does, it supports, it doesn't "prove") Dall's sheep will get sick or die.
This hypothesis of higher "naivety", to the best of my (Highland) understanding may be due to the thought or hypothesis that Dall's sheep have not ever been exposed to *M. ovipneumoniae* so when exposure happens there is sure to be some sort of massive die-off. Aside from a published report at a zoo in which *M. ovipneumoniae* was associated with pneumonia in barn-penned (as I recall) Dall's sheep, there is no reason to believe that Dall's sheep are more "naïve" or susceptible than are bighorn sheep. If the rationale behind this is that Dall's sheep have never been exposed and do not carry *M. ovipneumoniae*, I would have to disagree since it's been detected in Dall's sheep (the number will have to be obtained from AKFG).
In bighorn sheep, there is a hypothesis that *M. ovipneumoniae* positive bighorn sheep are just as susceptible to a new "strain" of *M. ovipneumoniae* as bighorn sheep that do not carry an "strain" of *M. ovipneumoniae*. So IF this were true, why would an unexposed ("naïve") Dall's sheep be any more or less susceptible than a non-carrier or carrier bighorn sheep?

6. Are there wildlife samples currently waiting to be processed? **Answered in #1, as far as what USDA-ARS-ADRU has received to date.**
    a. Are they going to be processed at WADDL, USDA-ARS, or both?
    b. What is the timeframe for submission of these samples?

7. For Alaska Dall sheep and mountain goats, given Mycoplasma ovipneumoniae PCR positive reports by USDA-ARS, how many of the positive tests have been verified positive by WADDL by species, and does this data allow you to definitively state that Dall sheep and/or mountain goats in Alaska have Mycoplasma ovipneumoniae? Why or why not?
AKFG has this data. To my knowledge, the mountain goats that were PCR/sequence positive were serologically positive by cELISA performed at the WADDL.
To ask if a sample that is tested in USDA-ARS-ADRU (or any USDA or other laboratory) has been "verified" by the WADDL, by definition insinuates that only the WADDL can provide an accurate or true diagnosis. If this were true, that only AAVLD accredited laboratories were "accurate" or must be used to verify test results, I'd guess that most of the published data regarding bacteria in general is not valid/verified.

8. For Alaska non-Caprinae moose and caribou, given Mycoplasma ovipneumoniae PCR positive reports by USDA-ARS, how many positive samples have been verified by WADDL by species?
I (Highland) provide the same answer as in #7

9. Was the testing on all the above moose and caribou positives sufficient to ensure that any positive results were clearly Mycoplasma ovipneumoniae and not some other Mycoplasma species, and does this data allow you to definitively state that moose and/or caribou in Alaska have Mycoplasma ovipneumoniae? Why or why not? **Yes. The "LM40" PCR PLUS sequencing,**

performed at the USDA-ARS-ADRU is a published assay that detects a variable region of the 16S rRNA gene. This detection is then followed by sequence analysis for each sample. The 16S rRNA gene is a well-documented technique to differentiate bacteria (ie. it defines a bacterium to the level of species). Unless the moose and/or caribou samples defy what we know about bacteria and how we define bacteria, then the 16S rRNA PCR plus sequencing should be considered sufficient.

(Ms. Schwanke's version of the question emailed to Highland and Olsen on 12/31/18):
Was the testing on all the above moose and caribou positives sufficient (expanded genomic diagnostics beyond the McAuliffe technique) to ensure that any positive results were clearly M.ovi and not some other Mycoplasma species, and does this data allow you to definitively state that moose and/or caribou in Alaska have M.ovi? Why or why not?
This question implies that McAuliffe technique PLUS sequencing (the technique performed at the USDA-ARS-ADRU Laboratory) is not sufficient to define a species of bacteria. The 3 reviewers of the CDC's *Emerging Infectious Diseases* journal and the editor must support the sufficiency, or they would not have published the data (Highland, *et al.* 2018) regarding moose, caribou, and two species of deer identified to carry *M. ovipneumoniae*. If the McAuliffe PCR (which is followed by sequencing in USDA laboratory) is not sufficient, then the real time PCR assay currently used by WADDL would not be sufficient to identify *M. ovipneumoniae* in any species, as the WADDL qPCR is also based on the same variable region of the 16S rRNA gene. A test used to identify a bacterium should not depend on the host from which a sample is collected, rather it should depend on the bacterium the test is designed to identify.

10. Prior to 2018, have there been any studies on the prevalence of Mycoplasma ovipneumoniae in non-Caprinae species? Is there enough current information to consider these non-Caprinae species as persistent host reservoirs of Mycoplasma ovipneumoniae and capable of passing Mycoplasma ovipneumoniae to wild or domestic Caprinae? Why or why not?
USDA-ARS-ADRU began screening very few (as available) deer species in 2015. Aside from this I (Highland) know of no other screening of non-Caprinae (non-sheep/goat/muskox), as there was published and publicly discussed misconception that only sheep and goats were carries. The research into defining the *M. ovipneumoniae* in non-Caprinae species and analyzing the genomes along with those of *M. ovipneumoniae* in sheep and goats (wild and domestic) is in the early stages and much is yet to be done over the next year. There is no reason to think that if one animal is carrying a bacterium and it comes into close contact with a second animal that is a potential/possible host for that bacterium that the bacterium would not be passed from animal 1 to animal 2.

Ms. Schwanke's version of the question emailed to Highland and Olsen on 12/31/2018:
Is there enough information to consider these non-Caprinae species as persistent host reservoirs of M.ovi and capable of passing M.ovi to wild or domestic Caprinae? Why or why not?
I (Highland) do not see the difference between this and Ms. Olsen's question, aside from the addition of the initial question that Ms. Olsen included about looking for *M. ovipneumoniae* in non-Caprinae species prior to 2018. Therefore I have nothing more to add to my answer.