Guidelines for establishing the prevalence of Paratuberculosis

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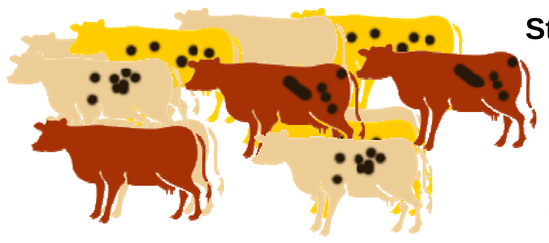
Background & Objective

Reviews of evaluations of diagnostic tests used for detection of *Mycobacterium avium* subsp. *paratuberculosis* (MAP)[1] and prevalences of MAP in farmed animals in Europe[2] have previously been reported from the ParaTBTools project. These reviews revealed that the quality of studies in terms of design, conduction or reporting was generally too poor for further comparisons. Either due to problems with sampling or because of poorly evaluated tests. A set of guidelines assisting researchers in planning prevalence studies was deemed necessary to reduce pitfalls specific to MAP infections.

The objective of this work was to provide an introduction to such guidelines - illustrated by using two different examples.

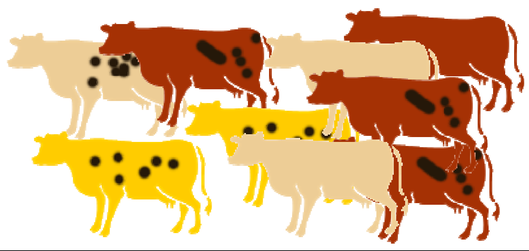
Procedure

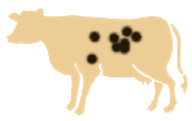


Step 1: Establish the purpose of the study – determine and characterize the target population, e.g. geography, demographics, production types and other relevant traits.

Ex 1: Establish prevalence of ParaTb in Danish dairy cattle, specifically lactating cows > 2yr, in order to decide upon a potential course of actions, i.e. control, eradication, certification, etc.

Ex 2: Establish the prevalence of MAP contaminated carcasses post-evisceration in order to assess the potential risk of transfer to humans





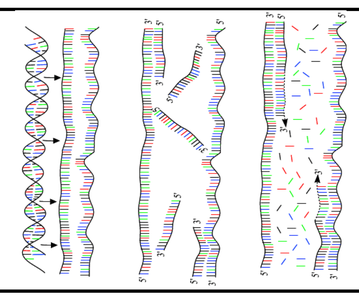
Step 2: Choose the target condition best reflecting the purpose of the study. In [1], three classifications: MAP infected, infectious and affected are used, however also target conditions such as 'Cows excreting MAP in milk' or 'Carcasses contaminated with MAP' could be valid.

Ex 1: MAP infected must be the relevant target, as it encompass both current and potential future sources of infectious animals.

Ex 2: The target condition must be 'Carcass contaminated with MAP', i.e. it is not important if the cow is infected, only if MAP got on the carcass, potentially as transfer.

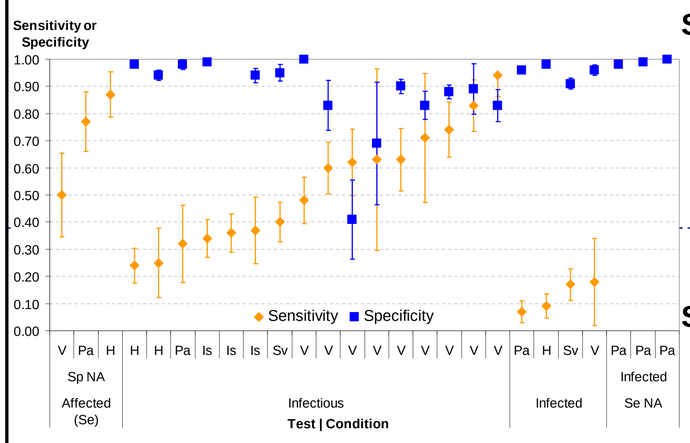
Step 3: Choose a relevant diagnostic test, which reflects the purpose of the study as well as the target condition. Specifically, decide upon:

a) Test type; b) Test make/producer



Ex 1: a) milk-based ELISA b) ID Screen® Paratuberculosis Indirect ELISA kit from ID-Vet (Montpellier, France). The test detects antibodies and may therefore be capable of detecting infected animals, which have not yet started shedding MAP

Ex 2: a) PCR for detection of MAP b) In-house test. The PCR will detect MAP and it is expected to be faster than culture based methods



Step 4.1: Obtain estimates of test sensitivity (Se) and specificity (Sp) for the chosen test and target condition

a) If estimates are available for the chosen target condition and a comparable population, use these (see [1]), if not:

b) Evaluate the test in the population, see [3,4] for guidelines. Warning! The evaluation of diagnostic tests is not simple!

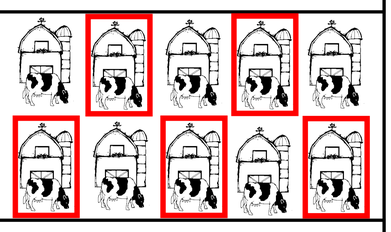
Ex 2: The PCR is evaluated against conventional culturing in a Latent Class Analysis to avoid the assumption of a Gold Standard

OR:

Step 4.2: Choose an approach which does not require estimates of Se and Sp, e.g. use Mixture models for continuous test responses such as OD values from an ELISA [5]

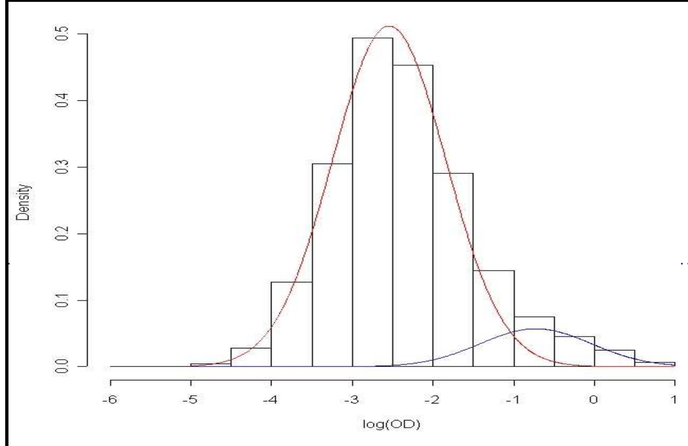
Ex 1: It is chosen to use the OD values from the ELISA in a Mixture model, where other known covariates (e.g. breed, parity and stage of lactation is accounted for)

Step 5: Collect data from target population. Warning: Obey Good Epidemiological Practice in the planning and conduction of the data collection.



Ex 1: A multi-stage sampling is applied to get an estimate of the prevalence at herd-level and animal-level simultaneously. Milk samples collected routinely are randomly selected for further analysis and additional data about samples are obtained from the Danish Cattle registry.

Ex 2: Systematic random sampling (i.e. every 10th carcass) are applied to slaughter lines, selected and visited at random



Step 6.1: Estimate the apparent prevalence (AP) and subsequently the true prevalence (TP) by the Rogan-Gladen estimator:

$$TP = \frac{AP + Sp - 1}{Sp + Se - 1}$$

potentially using some of the software available for a probabilistic or Bayesian approach, e.g. http://www.ausvet.com.au/pprev/ or http://www.epi.ucdavis.edu/diagnostictests/software.html

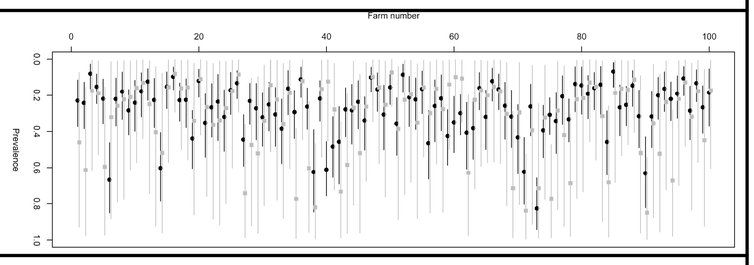
Ex 2: The prevalence is estimated during the evaluation of the PCR test against conventional culturing in a Bayesian analysis.

OR:

Step 6.2: Estimate the true prevalence based on a continuous test response using e.g. an adaptation of the model in [5]

Ex 1: In a Bayesian mixture model, the herd- and animal-level prevalences are estimated, e.g. like in [5]

Step 7: Make the appropriate interpretations based on the collected data and the underlying assumptions!



Ex 1: Some variation was found between herds in terms of the animal-level prevalence. However, the discriminatory power of the test proved to be poor and it was difficult to rank farms and determine any potentially disease-free herds.

Ex 2: A rather low AP was found. However, the Se of the PCR was estimated to be relatively low as well, thus the TP was estimated to be somewhat higher, but with large associated uncertainty.

Step 8: Publish the results!

References

[1]: Nielsen S.S. & Toft, N. (2008). Ante mortem diagnosis of paratuberculosis: A review of accuracies of ELISA, interferon-γ assay and faecal culture techniques. *Vet. Mic. 129, 217-235*

[2]: Nielsen S.S & Toft N. (2009). A review of prevalences of paratuberculosis in farmed animals in Europe. *Prev. Vet. Med. 88, 1-14*

[3]: Greiner M & Gardner, I.A. (2000). Epidemiologic issues in the validation of veterinary diagnostic tests. *Prev. Vet. Med. 45, 3-22*

[4]: Mintiens et *al* .(2009). Guidelines on the use of no-gold standard methods for estimating diagnostic characteristics of microbiological and serological assays. *OIE Sci. Tech. Rev. (submitted)*

[5]: Nielsen, S.S., Toft, N. Jørgensen, E. & Bibby, B.M. (2007). Bayesian mixture models for within-herd prevalence estimates of bovine paratuberculosis based on a continuous ELISA response. *Prev. Vet. Med. 81:290-305*

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