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Structured approach to design of diagnostic test evaluation studies for chronic progressive infections in animals

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ABSTRACT

Diagnostic test evaluations (DTEs) for chronic infections are challenging because a protracted incubation period has to be considered in the design of the DTE, and the adverse effects of infection may be widespread and progressive over an animal's entire life. Frequently, the specific purpose of the test is not formally considered when a test is evaluated. Therefore, the result is often a DTE where test sensitivity and specificity estimates are biased, either because of problems with establishing the true infection status or because the test detects another aspect of the infection (and analyte) than originally intended.

The objective of this paper is to outline a structured approach to the design and conduct of a DTE for diagnostic tests used for chronic infections in animals, and intended for different purposes. We describe the process from reflections about test purpose and the underlying target condition through considerations of the pathogenesis, and specification of a practical case definition, which can subsequently be used in the DTE for the specific purpose.

The process is illustrated by two examples of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infections in cattle. MAP infections are chronic and can result in different adverse effects at different time points during the incubation period. The description provides input on the process and deductive reasoning which are integral parts to develop a high-quality design of a DTE for chronic infectious diseases.

1. Introduction

Chronic progressive infectious diseases such as brucellosis, tuberculosis, leishmaniasis and paratuberculosis pose special challenges in the design and conduct of diagnostic test evaluations (DTEs). Specifically, the long and to some extent unpredictable disease progression can result in large variation in sensitivity and specificity estimates from DTEs of similar tests (Nielsen and Toft, 2008). Lack of explicit focus on the specific utility of a diagnostic test under evaluation, often termed the index test, is a likely explanation, i.e. the purpose of testing has not been made explicit and therefore test results are interpreted in relation to multiple purposes without really being evaluated specifically in relation to these purposes. The World Organisation for Animal Health (OIE) endorses the use of the "fitness for purpose" criterion, where purposes for example could be to demonstrate freedom from infection in a defined population, to confirm a clinical diagnosis, or to determine the immune status of individuals (OIE, 2009). "Fit for purpose" means that test results must be interpreted to a specific meaning and purpose. This purpose needs to be defined *a priori* by the decision makers, who must specify what they want the test to detect (an objective) before a DTE can be initiated. For chronic infections, the timing of the diagnosis, which

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corresponds to stage of disease, needs to be included in the purpose description.

Early diagnosis can for example reduce the risk of transmission of the causative agent and disease progression. However, some animals at risk of progressing to the stage of "disease" or to the stage of "transmission" may never reach these stages, either because they are genetically resistant to developing disease, are protected by vaccination or because they will not live long enough. Therefore, treatment or culling may be a costly alternative to doing nothing. Late-stage disease diagnosis will often result in a high correlation between test result and clinical signs, and thereby increase the decision makers' confidence in the test-results. However, transmission may already have already occurred without being detected.

Many DTEs of chronic infections are flawed by improper designs and inherent biases both from design issues and from lack of consideration of the close relationship between index test – purpose – target and context of application, exemplified by tuberculosis, HIV and malaria in humans (Fontela et al., 2009), and by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infections in animals (Nielsen and Toft, 2008). We believe that the primary reason for the design flaws stems from not really putting the test and its application into perspective prior to commencing the DTE.

The process and general principles for DTEs are described by TDR Diagnostics Evaluation Expert Panel (2006) and Greiner and Gardner (2000a). However, these generic guidelines do not specifically deal with the challenges of chronic infectious diseases in modern veterinary medicine. Thus, the objective of this paper is to provide readers with a structured approach to address the most important issues involved in designing (and conducting) a DTE for chronic, progressive infections. Specifically, we consider biases imposed by the choice of case definition compared to target condition, biases imposed by the choice of design, and biases occurring from the practical issues in conducting the study.

To illustrate the specific considerations, we use MAP infection in ruminants (paratuberculosis) as our example. MAP infections cause significant economical losses globally and control is most wanted in countries with a major dairy industry (Kennedy and Nielsen, 2007). Diagnosis is pivotal to infectious disease prevention and control. However, a long and partially unpredictable incubation period makes diagnosis of paratuberculosis challenging. Biased test information inevitably arises, when diagnostic tests are evaluated and used. Specifically, we use two examples of a purpose to illustrate how the initial choices influence the remaining study design decisions.

2. Overview of the diagnostic test evaluation (DTE)

A key element in any successful study involving data collection is the planning phase, where the association between the context (including already known features of the problem), the objective of the current study, and the characteristics of the target and study populations are considered. Only when these aspects of the problem and their interrelationships are understood, should planning commence. Thus before considering a study design, many other decisions or choices have to be made. Hence, development of a well-designed DTE includes considerations and understanding of the following elements in the initial planning phase prior to considering a design:

(a) Pathogenesis, including immune responses, predilection sites for the organism, effects of the infection, clinical manifestations and occurrence of any pathognomonic signs;
(b) Analytical principle of the index test, i.e. what analyte does it detect and how is it detected;
(c) Which alternative tests are available, and what are their strengths and limitations, including performance (e.g. sensitivity, specificity and reproducibility) and operational characteristics (e.g. speed of performing the test, ease of sampling, test-costs);
(d) Context in which the index test is to be used, i.e. what are you trying to identify and why.

We believe that the logical flow in planning and designing a DTE should follow Fig. 1. A proper DTE is usually preceded by an initial phase in which an assay is developed and optimised. This phase should not be mixed with the actual DTE. Once the index test has been defined, it should be optimised. The starting point must be consideration of the purpose, i.e. why, how and where will the test be used once it has been evaluated. The optimisation may be closely related to the pathogenesis, which should therefore also be considered in the initial phase. Understanding the purpose and considering the relevant components of the pathogenesis is an iterative process, i.e. if the purpose of the test is to detect animals which may become infectious, then it is important to understand the process leading up to or causing shedding of the infectious agent, whereas a purpose such as confirming clinical disease might make other components of the pathogenesis more important. Only after the purpose has been defined, should the target condition be specified. The target condition is the underlying infection condition that the index test should detect. This condition has to reflect the purpose of testing as much as possible, i.e. if we want to optimise economically-important production measures and control infection, there may be little reason to focus on diseased animals, if infected non-diseased animals can also transmit the agent.

After selection of the target condition, one may then proceed to determine the case definition. The case definition is based on the results of the reference standard, against which the index test should be compared. Again, it is imperative that there is a high degree of association between the underlying target condition and the more pragmatic case definition, which may be considered as the practical description of the target condition. The more discordant they are the more problems in terms of biases will be inferred. As an example, the purpose could be to prevent transmission of an infectious agent. The target condition could then be infectious animals (those that currently transmit the agent), and a case definition might be all animals testing positive in a specific agent-detection test. The challenges incurred might be that a test-positive