Update on methods for detecting of pathogenic mycoplasma species

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Introduction

Diagnosis of most (if not all) species of mycoplasmas is often a challenging prospect. A number of factors contribute to this: slow transmission between animals when the infection pressure is low making timing of sample collection difficult, delayed and often impaired immune responses to infection that interfere with antibody production/detection, and a poor appreciation of the extent and impact of field isolate variation, among others. In addition, there are few tools available to perform detailed studies on most mycoplasmas due to the fastidious growth characteristics, unique genetics of this group of organisms that inhibit the study of genes and gene function. Currently, a variety of tests of varying sensitivity and specificity are available for mycoplasma diagnosis that can be used alone or in combination. Understanding the strengths and weaknesses of each assay can help in the development strategies depending on the diagnostic questions being asked.

Swine mycoplasma species

M. hyop causes pneumonia in pigs and is a significant component of the PRDC. *M. hyorhinis* causes arthritis, polyserositis and otitis media in young pigs (usually less than 12 weeks-of-age). *M. hyosynoviae* induces arthritis in older pigs (generally 12-20 weeks-of-age). *M. suis* (formerly *Eperythrozoon suis*) affects all ages of pigs and causes icteroanemia and hypoglycemia. It generally presents with non-specific signs of ill-thrift in piglets, retarded growth and immune suppression in feeder pigs, and impaired fertility and/or poor lactation in sows.

Diagnostic tools

Culture

Typically culture is considered the “gold” standard for diagnosis and confirmation of an organism in the host. Yet as a general rule, Mycoplasmas are difficult to isolate which limits the usefulness of this technique. Some species are relatively more difficult to grow than others, but (nearly) all require specialized medium. For example, routine isolation of *Mycoplasma hyopneumoniae* is impractical, and often impossible, for a number of reasons. *M. hyop* is cultured in a highly enriched growth media that requires high concentration of *M. hyop* antibody-negative serum[1]. In addition, primary isolation commonly requires four to eight weeks to reach measurable levels in culture. Contamination from other bacteria and mycoplasmas that grow more readily in this media frequently interfere with attempts to obtain pure cultures of *M. hyop*. In contrast, isolation of *M. hyorhinis* and *M. hyosynoviae* is relatively more successful than culture of *M. hyop*, but still can over a week to reach measurable levels. *M. suis* is not a cultivable species. It is maintained through serial passage in pigs.

Antigen-based tests

Serology is the most commonly used tool to indirectly determine the presence or absence of many mycoplasmas such as *M. hyop* due to the ease of sample collection, rapid results and relatively low cost. However, interpretation of serological results may

be challenging when organism are slow to spread, or seroconversion after infection is delayed as is the case for *M. hyop*. This complicates the use of serology as a reliable screen for replacement animals.

Current *M. hyop* ELISAs include the Tween-20 ELISA [2,3] and the HerdChek® *M. hyop* Antibody Test Kit (IDEXX Laboratories, Westbrook, ME), which are both indirect ELISAs made from detergent extracts of whole-cell *M. hyop* organisms. The IDEIA™ *M. hyop* EIA KIT (Oxoid, Ely, Cambridgeshire, UK, formerly produced as the DAKO *M. hyop* ELISA) is a competitive-inhibition (blocking) ELISA based on a single protein. Previous studies have found both the competitive-inhibition and indirect ELISAs to be highly specific to *M. hyop,* but to have somewhat low sensitivities [4,5]. These studies identified a number of discrepant results among the assays. While some of these discrepancies were in pigs of known status and can at least partially be explained by differences in sensitivities, others were found in field cases where information was not available on their true status. Antigenic variability between isolates of *M. hyop* has been documented [6,7]and recent studies suggest that the current ELISAs vary in their ability to detect antibodies from pigs experimentally infected with different field isolates of *M. hyop* (unpublished data). Western blot has been proposed as a confirmatory assay to determine the true status of potential false positives,[8] but has not been fully validated; therefore, suspect results must be followed up with serology at subsequent time-points to determine if early stages of infection are being detected, and potentially with PCR in an attempt to confirm the presence of *M. hyop*.

Due to the ubiquitous nature of *M. hyorhinis*, *M. hyosynoviae* and *M. suis,* antibodies to these organisms are wide-spread and often not diagnostic, except in the case of paired sera in conjunction with a specific disease outbreak. No commercial assays are currently available.

Genetic-based tests

A number of PCR assays have been developed that are reported to be both sensitive and specific to many mycoplasmas. As a result, this technique is now among the most widely used for detection. Although for many species there are few genes that are known or presumed to be conserved which limits assay development and has sometimes led to assays producing false negatives due to genetic variability[9]. Real-time assays for *M. hyosynoviae* and *M. hyorhinis* have also been developed for direct testing of clinical samples (unpublished data). *Mycoplasma suis* has historically been diagnosed through staining of blood smears. Recently a real-time assay was described that greatly improves our ability to diagnose this organism[10].

Additionally, several assays have recently been described that differentiate or 'type' field isolates of *M. hyop* based on sequence differences [11-13]. These assays differ in their power to discriminate between isolates and because little information exists on specific genes involved in pathogenesis or protective immunity, these typing assays are currently limited to epidemiological examinations. By themselves, they are not predictive of virulence, or the ability of vaccines to protect against a given isolate. The ever-increasing accessibility of whole-genome sequencing will allow for major improvements in PCR assays to detect mycoplasmas and type strains.

Antibiotic susceptibility tests

Attempts to standardize MIC testing for veterinary mycoplasma species have been made [14], but significant difficulties for antibiotic susceptibility testing still exist due to the

poor growth characteristics of these organisms. Recently a method using flow cytometry to count live mycoplasmas after incubation with antibiotics has been described [15,16] that may be able to replace more traditional methods and also provide more rapid results. Yet the need for pure-culture isolates still remains which is a significant barrier for some species. The ISU-VDL will soon offer MIC testing for *M. hyosynoviae* as a routine test.

Sample collection

Given the difficulties associated with application of many of the diagnostic assays for mycoplasmas, special consideration should be paid to optimizing sample collection. Improved collection systems such as flocked swabs [17] improve the sensitivity of downstream assays. While techniques such as oral fluids [18], sampling of air filters [19], and targeted pooling strategies allow for economic and efficient sampling of large populations. A recent study comparing ante-mortem sampling techniques for the detection of *M. hyop* confirmed previous studies showing lung samples are much more sensitive than nasal swabs, but also described a method for tracheo-bronchial swabbing that was equivalent to, or slightly better than lung lavage in sensitivity[20]. In this same study, oro-pharyngeal swabs were intermediate between nasal swabs and the tracheo-bronchial swabs or lavages. Based on comparisons performed at ISU, this is similar to where oral fluids appear to fall in sensitivity.

Conclusions

In the past, very limited tools were available for the diagnosis of mycoplasma species. Our ability to detect various species of mycoplasma is growing, but certainly has room for significant improvement. Recent advances in genome sequencing, genetic manipulation and protein identification are continuously providing more data that can be applied to improving existing tests and the development of novel assays.

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