ChinaRxiv 中X

AI translation · View original & related papers at
chinarxiv.org/items/chinaxiv-201811.00114

Advances in Detection Techniques for Mycoplasma hyopneumoniae: Postprint

Authors: Xu Zuobo, Li Jiubin, Ding Honglei

Date: 2018-11-25T00:00:00+00:00

Abstract

Porcine enzootic pneumonia (Mycoplasma hyopneumoniae, Mhp) is a disease caused by Mycoplasma hyopneumoniae that is prevalent worldwide, severely impacting the swine industry by significantly impairing feed conversion efficiency and inflicting substantial economic losses. Accurate, sensitive, and rapid Mhp detection methods facilitate understanding of Mhp prevalence in swine herds, thereby enabling the implementation of appropriate preventive, therapeutic, and integrated control measures. This article provides a comprehensive review of domestic and international etiological, molecular biological, and immunological detection methods for Mhp, offering reference materials for scientific researchers to comprehensively understand Mhp detection methods.

Full Text

Research Progress in Detection Technologies for Mycoplasma hyopneumoniae

XU Zuobo[1], LI Jiubin[2], DING Honglei[1],*

[1]Laboratory of Veterinary Infectious Diseases, College of Animal Science and Technology, Southwest University, Chongqing 400715, China
[2]High School Affiliated to Southwest University, Chongqing 400700, China

Abstract: Mycoplasma hyopneumoniae (Mhp) causes mycoplasmal pneumonia of swine (MPS), a disease that poses a severe threat to the global pig industry by significantly reducing feed conversion efficiency and causing substantial economic losses. Accurate, sensitive, and rapid detection methods are essential for understanding Mhp prevalence in swine herds and implementing effective prevention, treatment, and comprehensive control measures. This review systematically summarizes etiological, molecular biological, and immunological detection methods for Mhp reported domestically and internationally, providing a

comprehensive reference for researchers to understand the full spectrum of Mhp detection methodologies.

Keywords: *Mycoplasma hyopneumoniae*; detection; pathogen; molecular biology; ELISA

Mycoplasmal pneumonia of swine is a common infectious disease in the pig industry caused by *Mycoplasma hyopneumoniae* (Mhp). Characterized by high morbidity but low mortality, the disease primarily reduces feed conversion rates, prolongs time to market, and predisposes pigs to secondary infections with pathogens such as porcine circovirus type 2, porcine reproductive and respiratory syndrome virus, *Pasteurella multocida*, and *Streptococcus suis*, leading to porcine respiratory disease complex (PRDC) [1-2]. Epidemiological surveys indicate that over 99% of pig farms in China are infected with Mhp, with conservative estimates suggesting annual direct economic losses exceeding 10 billion yuan [3]. Establishing accurate, sensitive, and rapid detection methods is crucial for understanding Mhp epidemiology and guiding preventive and control strategies. This review provides a comprehensive overview of etiological, molecular biological, and immunological detection methods for Mhp.

1. Etiological Detection

Isolation and identification of Mhp from infected pigs remains the "gold standard" for diagnosing mycoplasmal pneumonia. However, Mhp is among the most difficult mycoplasmas to cultivate due to its stringent nutritional requirements—it needs 20% porcine serum supplementation in cell-free culture media—and its slow growth rate, which is often overshadowed by the faster-growing *Mycoplasma hyorhinis*, a commensal mycoplasma in pigs [4]. The most commonly used culture media include Friis medium, developed by Friis [5-6], and Jiangsu No. II (KM2) medium developed by the Jiangsu Academy of Agricultural Sciences [7]. Adding pyruvate to Friis medium can accelerate Mhp growth [8], and recent modifications to both media have improved cultivation efficiency. Modified KM2 medium increases Mhp color change units (CCU) compared to the original formulation [10], while modified Friis medium supplemented with 2 g/mL kanamycin inhibits *M. hyorhinis* growth without significantly affecting Mhp [9]. Our laboratory has confirmed that Mhp can still grow in KM2 medium with 2 g/mL kanamycin, albeit with slightly reduced growth rate (unpublished data). Overall, Mhp isolation demands highly nutritious media, requires extended culture periods, and suffers from low isolation rates, necessitating the development of rapid and simplified diagnostic methods.

2. Molecular Biological Detection Methods

2.1 Conventional PCR

PCR technology is widely used for pathogen detection due to its high sensitivity and specificity, operational simplicity, and ability to amplify target genes

rapidly. Since its first application for Mhp diagnosis [11], numerous PCR methods targeting different Mhp genes have been developed for various detection purposes. Early PCR assays primarily targeted the 16S rRNA gene, mainly because limited genomic information was available at the time [12-14]. These methods could detect Mhp in pure cultures, nasal secretions, bronchoalveolar lavage fluid, and lung tissues from pigs with mycoplasmal pneumonia symptoms [13,15-17], with detection limits reaching 4×10^{2} pathogens [16], generally meeting clinical needs. Multiplex PCR based on 16S rRNA sequences was also developed to differentiate Mhp from *M. hyorhinis* and *M. flocculare*, providing a convenient tool for identifying different mycoplasmas in porcine lung tissue [17]. Additionally, PCR methods targeting the conserved P36 and P46 genes showed that P36 primers exhibited higher sensitivity and specificity than P46 primers when detecting Mhp in nasal and lung samples [4].

2.2 Nested PCR

Nested PCR was introduced in the late 1990s for detecting Mhp in nasal secretions, bronchoalveolar lavage fluid, and lung tissues, offering lower detection limits than conventional PCR for clinical samples [18-21]. A nested PCR based on the 16S rRNA gene achieved a detection rate of 61% in nasal swabs, far exceeding the 3.6% rate of conventional PCR and demonstrating 10^{4}-fold higher sensitivity [20]. Stärk developed a nested PCR method capable of detecting Mhp in air samples from farms with acute mycoplasmal pneumonia outbreaks—a feat unattainable with conventional PCR [18]. Kurth et al. [21] established a nested PCR with a detection limit of a single pathogen. While this extremely sensitive method provides efficient detection in both live and dead pigs for herd infection assessment, it requires stringent aseptic technique to avoid false-positive results.

2.3 Real-Time Fluorescent Quantitative PCR

With the widespread adoption of real-time PCR technology, Dubosson et al. [22] established Mhp detection methods targeting repetitive sequences and ABC transporter genes in 2004. Both methods showed 100% specificity without false positives and detection limits of 1 fg of genomic DNA. However, due to polymorphisms in primer sequences, their sensitivities in clinical samples were only 50% and 70%, respectively, with combined use reaching only 85%. Consequently, Strait et al. [23] selected two housekeeping genes, mhp165 and mhp183, to develop more sensitive real-time PCR assays. The mhp165-based method, in particular, could detect Mhp in nasal swabs, bronchial swabs, and bronchoalveolar lavage fluid from infected pigs with extremely high sensitivity. In 2010, a multiplex real-time PCR targeting P46, P97, and P102 genes was developed [24], showing higher sensitivity than the nested PCR method by Calsamiglia et al. [19] with a detection limit of a single Mhp organism. This method currently represents the best available technique for detecting Mhp in clinical samples and is widely used for routine diagnosis [25-26].

2.4 Loop-Mediated Isothermal Amplification (LAMP)

LAMP technology, developed by Notomi et al. [27], enables nucleic acid amplification under isothermal conditions and has been widely applied to detect bacteria, viruses, and parasites due to its simplicity, high sensitivity, and specificity. Li et al. [28] established a LAMP method for Mhp detection that offers short reaction times (30 minutes) and visible results, with a detection limit of 10 Mhp copies. However, its specificity is lower than real-time PCR, often yielding false-positive results. This method is suitable for field detection of clinical samples in resource-limited settings.

2.5 Gene Chip Technology

Gene chip (DNA microarray) technology has experienced explosive development since its introduction by Dr. Fodor at Affymetrix in the early 1990s. For pathogen identification, gene chips offer PCR-level sensitivity and simplicity while simultaneously analyzing multiple genes or gene segments in a single assay, overcoming limitations of PCR non-specificity. In 2016, Bao Yu developed a gene chip method for simultaneous detection of six PRDC pathogens, which showed higher Mhp detection rates in clinical samples than a local standard method [29]. While capable of multi-pathogen detection, the high cost limits its application primarily to inspection and quarantine agencies where cost is less critical.

Comparison of reported PCR-based techniques used to detect *Mycoplasma hyopneumoniae*

For molecular diagnosis of Mhp, nested PCR remains the primary method in most laboratories due to its higher specificity and sensitivity compared to conventional PCR and lower cost than real-time PCR. Laboratories with less cost constraints may adopt real-time PCR for shorter turnaround times, while LAMP technology is suitable for field detection in primitive environments.

3. Serological Methods

Serological diagnosis of Mhp primarily includes complement fixation test (CFT) and enzyme-linked immunosorbent assay (ELISA). Indirect hemagglutination test (IHA) was commonly used in the 1980s but has been largely abandoned due to low sensitivity.

3.1 Complement Fixation Test

CFT detects antigens or antibodies by measuring complement consumption by antigen-antibody complexes. It was the most widely used serological method for Mhp antibody detection in early studies, capable of detecting antibodies two weeks post-infection [30]. However, this method has two major drawbacks: cross-reactivity with antibodies against *M. hyorhinis* and other porcine mycoplasmas

[31-32], and low sensitivity—antibodies become undetectable five months post-infection, whereas ELISA can detect antibodies for up to one year [30]. Additionally, the method is extremely heat-sensitive, with antibody titers decreasing significantly after serum treatment at 60°C for 0.5 hours [31].

3.2 Enzyme-Linked Immunosorbent Assay

ELISA is the most widely used method for clinical Mhp antibody detection. The most common commercial kits are IDEXX' s whole-cell indirect ELISA and Oxoid' s monoclonal antibody-based blocking ELISA. Both kits demonstrate good sensitivity and specificity, detecting antibodies in serum 28 days post-infection. For chronic Mhp infections, both kits perform similarly, but the blocking ELISA shows superior sensitivity in acute infections, detecting antibodies 1-2 weeks earlier than the indirect ELISA [33].

Feng et al. [34] developed three indirect ELISA methods based on P97R1, P46, and P36 proteins. All three could detect Mhp IgG antibodies seven days post-infection, but the P97R1-based ELISA showed higher sensitivity, detecting more positive samples at 14 and 21 days. The P97R1 ELISA could detect secretory IgA (SIgA) at four days post-infection, while P46 and P36 ELISAs detected SIgA at six days. Thus, SIgA detection enables earlier diagnosis than serum IgG and can differentiate between inactivated vaccine-induced and naturally infected SIgA antibodies [35]. However, limitations include difficult nasal swab collection causing pig discomfort and resistance, and non-standardized sampling procedures affecting result interpretation. This kit has obtained national veterinary drug registration certification.

Liu et al. [36] developed a blocking ELISA based on P65 protein monoclonal antibodies for serum IgG detection, achieving 95.7% specificity and 94.8% sensitivity, comparable to IDEXX' s indirect ELISA kit. Okada et al. [32] established a double-antibody sandwich ELISA with extremely high specificity, showing no cross-reactivity with *M. flocculare*, *M. hyorhinis*, or *M. hyosynoviae* sera, though antibodies were only detectable three weeks after experimental infection.

Given the late antibody detection by commercial ELISA kits and the difficulties in standardizing nasal secretion collection for SIgA detection, further research is needed to identify suitable target proteins for early Mhp antibody detection and develop improved early diagnostic kits.

The three categories of Mhp detection methods each have distinct advantages and disadvantages, and selection should be based on specific diagnostic purposes. Pathogen isolation remains the "gold standard" for Mhp detection, commonly used in etiological, epidemiological, and drug resistance monitoring studies. Molecular methods offer high sensitivity for early diagnosis, though results vary by sample type—bronchoalveolar lavage fluid generally yields higher detection rates than nasal swabs and allows earlier detection. Recent studies demonstrate that laryngeal swabs show higher Mhp nucleic acid detection rates than nasal swabs and tracheobronchial lavage fluid, with earlier detection, while oral

fluid samples show the lowest detection rates [37]. Serological methods are simple to perform but detect antibodies later than molecular methods and cannot differentiate between inactivated vaccine-induced and naturally infected antibodies. Developing a simple ELISA that can distinguish vaccine-induced from natural infection represents the most pressing need in current Mhp detection research.

References

[1] Maes D, Segales J, Meyns T, et al. Control of Mycoplasma hyopneumoniae, infections in pigs[J]. Vet Microbiol, 2008, 126(4): 297-309.
[2] Maes D, Sibila M, Kuhnert P, et al. Update on Mycoplasma hyopneumoniae infections in pigs: Knowledge gaps for improved disease control[J]. Transbound Emerg Dis, 2018, 1:110-124.
[3] Hua L Z, Shao G Q, Zhou Y Q. Control and eradication of Mycoplasma hyopneumoniae based on vaccine immunization[J]. Chinese Journal of Veterinary Drug, 2012, 46 (7): 50-53.
[4] Caron J, Ouardani M, Dea S. Diagnosis and differentiation of Mycoplasma hyopneumoniae and Mycoplasma hyorhinis infections in pigs by PCR amplification of the p36 and p46 genes[J]. J Clin Microbiol, 2000, 38(4): 1390-1396.
[5] Friis N. A selective medium for Mycoplasma suipneumoniae[J]. Acta Vet Scand, 1971, 12(3): 454-456.
[6] Friis N F. Some recommendations concerning primary isolation of Mycoplasma suipneumoniae and Mycoplasma flocculare a survey[J]. Nord Vet Med, 1975, 27(6): 337-339.
[7] Laboratory of Animal Husbandry and Veterinary Medicine, Jiangsu Institute of Agricultural Sciences. Isolation and culture of Mycoplasma hyopneumoniae, pathogen of porcine enzootic pneumonia[J]. Chinese Veterinary Science, 1978, 1: 17-20.
[8] Kamminga T, Slagman S J, Bijlsma J J E, et al. Metabolic modeling of energy balances in Mycoplasma hyopneumoniae shows that pyruvate addition increases growth rate[J]. Biotechnol Bioeng, 2017, 114(10):
[9] Cook B S, Beddow J G, Manso-Silván L, et al. Selective medium for culture of Mycoplasma hyopneumoniae[J]. Vet Microbiol, 2016, 195:158-164.
[10] Li S, Li J L, Zhou X. Screening of growth medium for Mycoplasma hyopneumoniae [J]. Heilongjiang Animal Science and Veterinary Medicine, 2014, 18: 61-63.
[11] Harasawa R, Koshimizu K, Takeda O, et al. Detection of Mycoplasma hyopneumoniae DNA by the polymerase chain reaction[J]. Mol Cell Probes, 1991, 5(2): 103-109.
[12] Stemke G W, Phan R, Young T F, et al. Differentiation of Mycoplasma hyopneumoniae, M. flocculare, and M. hyorhinis on the basis of amplification of a 16S rRNA gene sequence[J]. Am J Vet Res, 1994, 55(1):
[13] Mattsson J G, Bergström K, Wallgren P, et al. Detection of Mycoplasma hyopneumoniae in nose swabs from pigs by in vitro amplification of the 16S rRNA gene[J]. J Clin Microbiol, 1995, 33(4): 893-897.
[14] Stemke G W. Gene amplification (PCR) to detect and differentiate

mycoplasmas in porcine mycoplasmal pneumonia[J]. Lett Appl Microbiol, 1997, 25(5): 327-330.
[15] Blanchard B, Kobisch M, Bové J M, et al. Polymerase chain reaction for Mycoplasma hyopneumoniae detection in tracheobronchiolar washings from pigs[J]. Mol Cell Probes, 1996, 10(1): 15-22.
[16] Baumeister A K, Runge M, Ganter M, et al. Detection of Mycoplasma hyopneumoniae in bronchoalveolar lavage fluids of pigs by PCR[J]. J Clin Microbiol, 1998, 36(7): 1984-1988
[17] Stakenborg T, Vicca J, Butaye P, et al. A multiplex PCR to identify porcine mycoplasmas present in broth cultures [J]. Vet Res Commun, 2006, 30(3): 239-247.
[18] Stärk K D C, Nicolet J, Frey J. Detection of Mycoplasma hyopneumoniae by air sampling with a nested PCR assay[J]. Appl Environ Microbiol, 1998, 64(2): 543-548.
[19] Calsamiglia M, Pijoan C, Trigo A. Application of a nested polymerase chain reaction assay to detect Mycoplasma hyopneumoniae from nasal swabs[J]. J Vet Diagn Invest, 1999, 11(3): 246-251.
[20] Verdin E, Saillard C, Labbé A, et al. A nested PCR assay for the detection of Mycoplasma hyopneumoniae in tracheobronchiolar washings from pigs[J]. Vet Microbiol, 2000, 76(1): 31-40.
[21] Kurth K T, Hsu T, Snook E R, et al. Use of a Mycoplasma hyopneumoniae nested polymerase chain reaction test to determine the optimal sampling sites in swine[J]. J Vet Diagn Invest, 2002, 14(6): 463-469.
[22] Dubosson C R, Conzelmann C, Miserez R, et al. Development of two real-time PCR assays for the detection of Mycoplasma hyopneumoniae in clinical samples[J]. Vet Microbiol, 2004, 102(1-2): 55-65.
[23] Strait E L, Madsen M L, Minion F C, et al. Real-time PCR assays to address genetic diversity among strains of Mycoplasma hyopneumoniae[J]. J Clin Microbiol, 2008, 46(8): 2491-2498.
[24] Marois C, Dory D, Fablet C, et al. Development of a quantitative real-time TaqMan PCR assay for determination of the minimal dose of Mycoplasma hyopneumoniae strain 116 required to induce pneumonia in SPF pigs[J]. J Appl Microbiol, 2010, 108(5): 1523-1533.
[25] Arsenakis I, Panzavolta L, Michiels A, et al. Efficacy of Mycoplasma hyopneumoniae vaccination before and at weaning against experimental challenge infection in pigs[J]. BMC Vet Res, 2016, 12: 63.
[26] Arsenakis I, Michiels A, Del Pozo Sacristán R, et al. Mycoplasma hyopneumoniae vaccination at or shortly before weaning under field conditions: a randomised efficacy trial[J]. Vet Rec, 2017, 181(1): 19.
[27] Notomi T, Okayama H, Masubuchi H, et al. Loop-mediated isothermal amplification of DNA[J]. Nucleic Acids Res, 2000, 28(12):E63.
[28] Li Jia-he, Minion F C, Petersen A C, et al. Loop-mediated isothermal amplification for rapid and convenient detection of Mycoplasma hyopneumoniae[J]. World J Microbiol Biotechnol, 2013, 29(4):
[29] Bao Y. Development and preliminary application of oligonucleotide microarray for simultaneous detection of six pathogens causing porcine respiratory

disease complex[D]. Chongqing: Southwest University, 2016.
[30] Bereiter M, Young T F, Joo H S, et al. Ross evaluation of the ELISA and comparison to the complement fixation test and radial immunodiffusion enzyme assay for detection of antibodies against Mycoplasma hyopneumoniae in swine serum[J]. Vet Microbiol, 1990, 25(2-3):177-192.
[31] Roberts D H, Little T W. Serological studies in pigs with Mycoplasma hyopneumoniae[J]. J Comp Pathol, 1970, 80(2): 211-220.
[32] Okada M, Asai T, Futo S, et al. Serological diagnosis of enzootic pneumonia of swine by a double-sandwich enzyme-linked immunosorbent assay using a monoclonal antibody and recombinant antigen (P46) of Mycoplasma hyopneumoniae[J]. Vet Microbiol, 2005, 105(3-4) 251-259.
[33] Fano E, Pijoan C, Dee S, et al. Longitudinal assessment of two Mycoplasma hyopneumoniae enzyme-linked immunosorbent assays in challenged and contact-exposed pigs[J]. J Vet Diag Invest, 2012, 24(2): 383-387.
[34] Feng Z, Bai Y, Yao J, et al. Use of serological and mucosal immune responses to Mycoplasma hyopneumoniae antigens P97R1, P46 and P36 in the diagnosis of infection[J]. Vet J, 2014, 202(1): 128-133.
[35] Feng Z, Shao G, Liu M, et al. Development and validation of a SIgA-ELISA for the detection of Mycoplasma hyopneumoniae infection[J]. Vet Microbiol, 2010, 143(2-4): 410-416.
[36] Liu M, Du G, Zhang Y, et al. Development of a blocking ELISA for detection of Mycoplasma hyopneumoniae infection based on a monoclonal antibody against protein P65[J]. J Vet Med Sci, 2016, 78(8): 1319-1322.
[37] Pietersa M, Danielsa J, Rovira A. Comparison of sample types and diagnostic methods for in vivo detection of Mycoplasma hyopneumoniae during early stages of infection[J]. Vet Microbiol, 2017, 203:

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv —Machine translation. Verify with original.