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CONTROL MEASURES ON MYCOPLASMA HYOPNEUMONIAE INFECTIONS IN PIGS - (REVIEW)

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ABSTRACT

Control of swine enzootic pneumonia involves a wide range of measures including immunoprophylaxis, therapy and metaphylaxis. The article is dedicated to research in the field of therapy, control of the disease and prevention of bacterial resistance. An overview of antimicrobial therapy mentions the suitable drugs against *M. hyopneumoniae*, the optimal dosage regimes and routes of application. Types of vaccines, immunization strategies and some unfavorable factors that influence immunity to swine enzootic pneumonia are being described.

Key words: enzootic pneumonia, *Mycoplasma hyopneumoniae*, pigs, control.

INTRODUCTION

Respiratory disease is one of the important health issues leading to economic loss in swine-breeding worldwide. It is one of the main causes of antibiotic treatment and results in 40% mortality rate (Loeffen, 2001). Respiratory symptoms are due to different primary etiological agents: *Porcine Reproductive and Respiratory Syndrome Virus* (*PRRSV*), *Porcine Circovirus type 2* (*PCV-2*), *Actinobacillus pleuropneumoniae* (*App*), *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) as well as secondary infections that complicate the main disease. This led to the formulation of a term in medical literature - Porcine Respiratory Disease Complex (PRDC) (Bochev, 2007; Pepovich, 2015). The main pathogen in PRDC is *M. hyopneumoniae*, which causes swine enzootic pneumonia (Thacker and Minion, 2012; Pepovich, 2015).

Control of swine enzootic pneumonia is a combination of management optimization, hygiene conditions, immune-prophylaxis and/or therapy and metaphylaxis with antimicrobial drugs (Martelli et al., 2006; Maes et al., 2008).

Application of antimicrobial therapy as a control measure of swine enzootic pneumonia.

Vaccination strategies, diminishing stress factors and modern farm strategies are necessary and useful, but even the most careful management cannot protect swine population from secondary bacterial infections. According to Bosch (2004) antibacterial therapy is a main point in infectious disease prevention.

Present day swine-breeding is focused in raising the group, not the separate individual. Therapy and methaphylaxis to diseases are usually conducted by adding medication in fodder or water that make application easier and diminish stress. Giving medication in fodder is useful in

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enzootic and chronic diseases like swine enzootic pneumonia. The use of drugs in water is more suitable in acute respiratory conditions. Diseased swine continue to drink water even if they refuse food (Henry and Apley, 1999).

The emergence of multiple antimicrobial resistances focuses modern human and veterinary medicine to this problem. Application of antibiotics is done only if needed and the concrete drug is chosen carefully. *M. hyopneumoniae* lacks a cellular wall which makes it resistant to all beta-lactamase antibiotics. It owns also inherent resistance to some 14-membered macrolides like erythromycin and oleandomycin (Tanner et al., 1993; Chambaud et al., 2001; Bébéar and Bébéar, 2002; Francoz et al., 2005). Though *M. hyopneumoniae* shows acquired resistance to tetracyclines, 16-membered macrolides (tylosin, tilmicosin), lincosamides (lincomycin) and fluoroquinolones (Stakenborg et al., 2005; Le Carrou et al., 2006; Vicca et al., 2007; Thongkamkoon et al., 2013; Tavio et al., 2014; Maes et al., 2017), these drugs are in use of swine enzootic pneumonia treatment.

The group of potentially active antibiotics against *M. hyopneumoniae* includes tetracyclines, macrolides, lincosamides, pleuromutilins, fluorchinolones, amphenicols and aminoglycosides (Vicca 2005; Pepovich et al., 2016; Pepovich, 2018), but tetracyclines and macrolides are the most often used for treatment and control of respiratory infections (Timmerman et al., 2006). Table 1 shows antimicrobial drugs, its doses, therapy and metaphylaxis regimes in case of enzootic pneumonia.

Table 1. The effect of different antimicrobial regimens for treatment of *M. hyopneumoniae* infections in herds clinically affected by enzootic pneumonia (Vicca, 2005).

Antibiotic and dosage	Scheme	Effects	References
Marbofloxacin 3 mg/kg IM	during 3 d	improved ADG and CS	Thomas et al., 2000
Enrofloxacin 50 mg/10 kg b.w. IM	during 3 d	reducing CS, mortality rate and emergency slaughtered	Pepovich et al., 2016
Chlortetracycline 800 ppm in-feed	during 3 w	improvement of CS and oxygen saturation	Ganter, 1995
Chlortetracycline 500 ppm in-feed	1 w medication, 1 w no medication, and again 1 w medication	decreased the prevalence LL and reduced CS	Del Pozo Sacristán et al., 2012a
Doxycycline 11 mg/kg in-feed	during 8 d in the fattening unit	improvement of ADG, incidence of diseased pigs and cure rate	Bousquet et al., 1998
Lincomycin 220 ppm in-feed	during 3 w	no significant improvements	Mateusen et al., 2002
Tiamulin 30 ppm in-feed	during 8 w in pigs from 30 to 70 kg	improvement of ADG, FCR no beneficial effect on MLL	Burch, 1984
Tylosin 4 mg/kg or Lincomycin 5 mg/kg IM	during 3 d after birth or and for 3 d at weaning	Tylosin: improvement of ADG no beneficial effect on FCR and CS for both antibiotics	Kunesh, 1981

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Tilmicosin 300 ppm in-feed	during 9 or 14 d	improvement of ADG, CS and less secondary bacteria	Binder et al., 1993
Tiamulin 200 ppm + Chlortetracycline 600 ppm in-feed	pulse medication: 2 d treatment/2 w during fattening period	lower prevalence on MLL no beneficial effect on severity of MLL	Le Grand and Kobisch, 1996
Tiamulin 40 ppm + Oxytetracycline 300 ppm in-feed	pulse medication: 2 d treatment/w during fattening period	improvement of ADG and mortality rate no beneficial effect on FCR and severity of LL	Jouglar et al., 1993
Tiamulin 100 ppm in feed	during 7 d at weaning and during 7 d at 4 m of age (and during 7 d at 6 m of age)	improved ADG and mortality rate, improvement of FCR and severity of LL	Stipkovits et al., 2003
Florfenicol 40 ppm in feed	during 7 d at fattening	improvement CS, decrease in death, lowering LL and increase ADG	Pepovich, 2018
Florfenicol 30 mg/kg IM	once	reduced CS, improved ADG, mortality rate and LL	Del Pozo Sacristán et al., 2012b
Florfenicol 300 mg/20 kg b.w. IM	twice at a 48-hour interval	reducing CS and mortality rate, normalization in indicators of blood	Pepovich et al., 2016

* Adapted from Vicca (2005); IM: intramuscular; b.w.: bodyweight; d: day(s); w: week(s); m: month(s); ADG: average daily gain; FCR: feed conversion ratio; LL: lung lesions; CS: clinical signs; MLL: macroscopic LL; mLL: microscopic LL;

Application of immunoprophylaxis as a control measure of swine enzootic pneumonia.

Effective application of antibiotics with fodder and water to control the respiratory diseases in swine, including enzootic pneumonia, decreases clinical symptoms and lung lesions and prevents secondary bacterial infections (Thacker, 2006; Pepovich, 2018). However misuse of those drugs increases the risk of antimicrobial resistance and content of antibiotic residues in pork meat (Maes et al., 1999). After the end of antimicrobial treatment there can be new cases of sick animals (Wallgren et al., 2000; Stipkovits et al., 2001). All these factors as a combination make immunoprophylaxis a must in swine enzootic pneumonia measures (Pepovich, 2015). It is a general instrument to control mycoplasmal infection (Simionatto et al., 2013).

Immunopriphylaxis against swine enzootic pneumonia includes the investigation of different vaccines and vaccination schemes. Some authors use inactivated vaccines (Martelli et al., 2014), while others prefer live attenuated vaccines (Feng et al., 2013). The vaccination shedule is chosen according to the herd type, production system and the epidemiological situation. The application of some vaccines against swine enzootic pneumonia is done twice intramuscularly during the first week of life of newborn piglets and then again 3 weeks later (Maes et al., 1998). Vaccine types which are with single use (without need of booster dose) can be injected on third week of age or

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later and lead to a higher average daily growth, better conversion of fodder, lower incidence of pulmonary lesions and decrease in mortality in contrast to unvaccinated individuals (Del Pozo Sacristan et al., 2014; Pepovich et al., 2015b). They are preferred because of lower expenses and lower degree of stress in swine farms (Baccaro et al., 2006). Table 2 shows the most often used vaccines against *M. hyopneumoniae* that can be found on the world market.

Table 2. Most commonly used commercially available *M. hyopneumoniae* bacterin vaccines. Vaccines available in only one or a few countries are not included (Maes et al., 2017)

Vaccine	Antigen/strain	Adjuvant	Route of administration	Age of administration (days)	Boost needed after . . .weeks
Hyogen (Ceva)	Ceva strain BA 2940-99	Imuvant (W/O J5 LPS)	IM	≥21	—
HYORESP (Merial)	NI[a]	Aluminium hydroxide	IM	≥5	3–4
INGELVAC MYCOFLEX (Boehringer Ingelheim)	J strain isolate B-3745	Impran (water-in-oil adjuvant emulsion)	IM	≥21	—
M+Pac (Intervet Int.)[b]	NI[a]	Mineral oil and Aluminium hydroxide	IM	≥7	3–4
MYPRAVAC SUIS (Hipra Lab)	J strain	Levamisole and carbomer	IM	≥7–10	3
PORCILIS M. HYO (Intervet)	Strain 11	dl-a-tocopherol acetate	IM	≥7	3
Porcilis PCV M. HYO (MSD-Intervet Int)[c]	J Strain	Mineral oil and Aluminium hydroxide	IM	≥21	—
Porcilis MHYO ID Once (MSD-Intervet Int.)	Strain 11	Paraffin oil and dl-a-tocoferylacetaat	ID	≥14	—
STELLAMUNE MYCOPLASMA (Eli Lilly)	NL 1042	Mineral oil and lecithin	IM	≥3	2–4
STELLAMUNE ONE (Eli Lilly)	NL 1042	Amphigen Base, and Drakeol 5 (mineral oil)	IM	≥3	—
SUVAXYN M.HYO[d] (Zoetis)	P-5722-3	Carbopol	IM	≥7	2

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SUVAXYN MH-ONE[e] (Zoetis)	P-5722-3	Carbopol and squalane	IM	≥7	—
SUVAXYN M.HYO—PARASUIS[f] (Zoetis)	P-5722-3	Carbopol and squalane	IM	≥7	2

[a]No information available.
[b]Vaccination scheme when 1 ml is used for each administration. No boost vaccination needed if a 2 ml dose is used the first time.
[c]Combination vaccine with Porcine Circovirus type 2.
[d]Named Suvaxyn RespiFend MH in USA.
[e]Same name is used in the USA, but Amphigen is used as adjuvant in the USA, and vaccine can be administered from day one of age onwards.
[f]Combination vaccine with Haemophilus parasuis - named Suvaxyn RespiFend MH HPS in USA.

The optimal strategy of vaccination should balance between the benefit of a vaccination done later in life and the need of a stable immunity to combat the pathogen (Pepovich et al., 2015a). As long as *M. hyopneumoniae* infection can develops during the first 4 weeks after birth (Vicca et al., 2002; Sibila et al., 2007), vaccination in this age group if often introduced. The effectiveness of this scheme was proved in experimental and field investigation (Jensen et al., 2002; Pepovich et al., 2015b).

The benefit of vaccinating piglets is that animals develop immunity before they encounter infective agents and the number of pathogens that can hinder immune response is lower. Potential negative effects due to vaccination before weaning include higher risk of activating *PCV-2* infection and inhibited effect of maternal antibodies. Investigations done by Opriessnig et al. (2003) show that vaccination against *M. hyopneumoniae* before experimental or natural *PCV-2* infection increases the severity of *PCV-2* induced lesions. This question is still debated because the research of Haruna et al. (2006) concluded that routine vaccination against *M. hyopneumoniae* is not a leading factor in *PCV-2* infection. Influence of maternal antibodies to vaccinal reactions in piglets is still not certain. Animals with high titers may show similar (Martelli et al., 2006) or lower (Hodgins et al., 2004) serological response following vaccination. Surveys of Jayappa et al. (2001) and Pepovich et al. (2013) concluded that high titers of maternal antibodies induced by infection of after vaccination of sows have negative effect on vaccination efficacy in piglets. Infection with *PRRSV* or the application of a modified live virus (PRRSV vaccine) in experimental and field conditions during vaccination against *M. hyopneumoniae* may result in a considerable decrease in the vaccine efficacy (Thacker et al., 2000; Pepovich, 2015).

CONCLUSIONS

Mycoplasma hyopneumoniae infection leads to high morbidity rate and economic loss in swine stock. Adequate timely measures to control this disease are necessary. Application of antibiotics in fodder and water and different vaccination schemes may help decrease clinical symptoms, diminish pulmonary lesions, increase mean daily growth and most important – lower mortality.

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REFERENCES

1. Baccaro M., Hirose F., Umehara O., Goncalves L., Doto D., Paixao R., Shinya L., Moreno A. (2006). Comparative efficacy of two single-dose bacterins in the control of Mycoplasma hyopneumoniae in swine raised under commercial conditions in Brazil. Vet. J., 172, 526-531.

2. Bébéar C.M., Bébéar C. (2002). Antimycoplasmal agents. In: Molecular biology and pathogenicity of mycoplasmas. Razin, S., R. Herrmann (Editors). New York: Kluwer Academic/Plenum Publ., 545-566.

3. Bochev I. (2007). Porcine respiratory disease complex (PRDC): A review. I. Etiology, epidemiology, clinical forms and pathoanatomical features. Bulg. J. Vet. Med., 10 (3), 131-146.

4. Bosch G. (2004). Single-dose treatment against respiratory bacteria. Pig international, 34, 30-32.

5. Chambaud I., Heilig R., Ferris S., Barbe V., Samson D., Galisson F., Moszer I., Dybvig K., Wroblewski H., Viari A., Rocha E., Blanchard A. (2001). The complete genome sequence of the murine respiratory pathogen Mycoplasma pulmonis. Nucleic Acids Research, 29, 2145-2153.

6. Del Pozo Sacristan R., Sierens A., Marchioro S., Vangroenweghe F., Jourquin J., Labarque G., Haesebrouck F., Maes D. (2014). Efficacy of early Mycoplasma hyopneumoniae vaccination against mixed respiratory disease in older fattening pigs. Veterinary Record, 174 (8), 197.

7. Feng Zhi-Xin, Yan-Na Wei, Gui-Lan Li, Xiao-Ming Lu, Xiu-Feng Wan, G. Todd Pharr, Zhan-Wei Wang, Meng Kong, Yuan Gan, Fang-Fang Bai, Mao-Jun Liu, Qi-Yan Xiong, Xu-Su Wu, Guo-Qing Shao. (2013). Development and validation of an attenuated Mycoplasma hyopneumoniae aerosol vaccine. Veterinary Microbiology, 167, 417-424.

8. Francoz D., Fortin M., Fecteau G., Messier S. (2005). Determination of Mycoplasma bovis susceptibilities against six antimicrobial agents using the E test method. Vet. Microbiol., 105, 57-64.

9. Haruna J., Hanna P., Hurnik D., Ikede B., Miller L., Yason C. (2006). The role of immunostimulation in the development of postweaning multisystemic wasting syndrome in pigs under field conditions. Can. J. Vet. Res., 70, 269-276.

10. Henry S., Apley M. (1999). Therapeutics. In: Straw, B. E., S. D'Allaire, W. L. Mengeling, D. J. Taylor (Editors). Diseases of Swine. 8th edition. Ames: Iowa State University Press, 1155-1162.

11. Hodgins D., Shewen P., Dewey C. (2004). Influence of age and maternal antibodies on antibody responses of neonatal piglets vaccinated against Mycoplasma hyopneumoniae. J. Swine Hlth. Prod., 12, 10-16.

12. Jayappa H., Davis B., Rapp-Gabrielson V., Wasmoen T., Thacker E. (2001). Evaluation of the efficacy of Mycoplasma hyopneumoniae bacterin follwoing immunization of young pigs in the

presence of varying levels of maternal antibodies. In: Proc. 32nd Annual Meeting Am. Assoc. Swine Vet., Nashville, Tennessee, 237-241.

13. Jensen D., Ersboll A., Nielsen J. (2002). A meta-analysis comparing the effect of vaccines against Mycoplasma hyopneumoniae on daily weight gain in pigs. Prev. Vet. Med., 54, 265-278.

14. Le Carrou J., Laurentie M., Kobisch M., Gautier-Bouchardon A. (2006). Persistence of Mycoplasma hyopneumoniae in experimentally infected pigs after marbofloxacin treatment and detection of mutations in the parC gene. Antimicrobial Agents and Chemotherapy, 50, 1959-1966.

15. Loeffen W. (2001). Respiratory diseases, a production balancing act. In: Pig Progress, Elsevier International Business Information, 4-6.

16. Maes D., Deluyker H., Verdonck M., Castryck F., Miry C., Lein A., Vrijens B., de Kruif A. (1998). The effect of vaccination against Mycoplasma hyopneumoniae in pig herds with a continuous production system. Zoonoses and Public Health, 45, 495-505.

17. Maes D., Deluyker H., Verdonck M., Castryck F., Miry C., Vrijens B., Verbeke W., Viaene de K. A. (1999). Effect of vaccination against Mycoplasma hyopneumoniae in pig herds with an all-in/all-out production system. Vaccine, 17, 1024-1034.

18. Maes D., Segales J., Meyns T., Sibila M., Pieters M., Haesebrouck F. (2008). Control of Mycoplasma hyopneumoniae infections in pigs. Vet. Microbiol., 126, 297-309.

19. Maes D., Sibila M., Kuhnert P., Segales J., Haesebrouck F., Pieters M. (2017). Update on Mycoplasma hyopneumoniae infections in pigs: Knowledge gaps for improved disease control. Transbound Emerg Dis. (https://doi.org/10.1111/tbed.12677).

20. Martelli P., Terreni M., Guazzetti S., Cavirani S. (2006). Antibody response to Mycoplasma hyopneumoniae infection in vaccinated pigs with or without maternal antibodies induced by sow vaccination. J. Vet. Med. B Infect. Dis. Vet. Public Health, 53, 229-233.

21. Martelli P., Saleri R., Cavalli V., De Angelis E., Ferrari L., Benetti M., Ferrarini G., Merialdi G., Borghetti P. (2014). Systemic and local immune response in pigs intradermally and intramuscularly injected with inactivated Mycoplasma hyopneumoniae vaccines. Veterinary Microbiology, 168 (2-4), 357-364.

22. Opriessnig T., Yu S., Gallup J., Evans R., Fenaux M., Pallares F., Thacker E., Brockus C., Ackermann M., Thomas P., Meng X., Halbur P. (2003). Effect of vaccination with selective bacterins on conventional pigs infected with type 2 porcine circovirus. Vet. Pathol., 41, 624-640.

23. Pepovich R. (2015). Prevalence, diagnosis and control measures of enzootic pneumonia in industrial swine-breeding. PhD Thesis, Faculty of Veterinary Medicine, University of Forestry, Sofia, Bulgaria.

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24. Pepovich R., Nikolov B., Genova K., Hristov K., Ivanov Y., Ivanova E. (2013). Effect of vaccination against swine enzootic pneumonia on humoral immune response and post-mortem lesions. Tradition and modernity in Veterinary Medicine, 202-212.

25. Pepovich R., Nikolov B., Hristov K., Genova K., Nikolova E. (2015a). Vaccination strategy for control of enzootic pneumonia in swine. Journal of Animal Husbandry, LII (2), 13-16.

26. Pepovich R., Nikolov B., Sirakov I., Genova K., Hristov K., Nikolova E., Hajiolova R., Beltova R. (2015b). Clinical testing of combined vaccine against enzootic pneumonia in industrial pig farming in Bulgaria. Mac. Vet. Rev., 38 (2), 195-201.

27. Pepovich R., Nikolov B., Genova K., Hristov K., Hadjiolova R., Nikolova E., Stoimenov G. (2016). The comparative therapeutic efficacy of antimicrobials in pigs infected with Mycoplasma hyopneumoniae. Scientific Works. Series C. Veterinary Medicine, LXII (2), 76-81.

28. Pepovich R. (2018). Metafillactic efficiency of florfenicol, applied to the fodder of the pigs from the fattening infected with Mycoplasma hyopneumoniae. Trakia Journal of Sciences, 16 (1), 11-16.

29. Sibila M., Nofrarias M., Lopez-Soria S., Segales J., Riera P., Llopart D., Calsamiglia M. (2007). Exploratory field study on Mycoplasma hyopneumoniae infection in suckling pigs. Vet. Microbiol., 121, 352-356.

30. Simionatto S., Marchioro S., Maes D., Dellagostin O. (2013). Mycoplasma hyopneumoniae: from disease to vaccine development (Review). Veterinary Microbiology, 165 (3-4), 234-242.

31. Stakenborg T., Vicca J., Butaye P., Maes D., Minion C., Peeters J., Haesebrouck F. (2005). Characterization of in vivo acquired resistance of Mycoplasma hyopneumoniae to macrolides and lincosamides. Microbial Drug Resistance, 11, 290-294.

32. Stipkovits L., Miller D., Glavits R., Fodor L., Burch D. (2001). Treatment of pigs experimentally infected with Mycoplasma hyopneumoniae, Pasteurella multocida, and Actinobacillus pleuropneumoniae with various antibiotics. Can. J. Vet. Rec., 65, 213-222.

33. Tanner A., Erickson B., Ross R.. (1993). Adaptation of the Sensititre® broth microdilution technique to antimicrobial susceptibility testing of Mycoplasma hyopneumoniae. Veterinary Microbiology, 36, 301-306.

34. Tavío M., Poveda C., Assunção P., Ramírez A., Poveda J. (2014). In vitro activity of tylvalosin against Spanish field strains of Mycoplasma hyopneumoniae. Veterinary Record, 175, 539.

35. Thacker E., Thacker B., Young T., Halbur P.. (2000). Effect of vaccination on the potentiation of porcine reproductive and respiratory syndrome virus (PRRSV) – induced pneumonia by Mycoplasma hyopneumoniae. Vaccine, 18, 1244-1252.

36. Thacker E. (2006). Mycoplasmal diseases. In: Leman, A. D., B. E. Straw, S. D'Allaire, W. L. Mengeling, D. J. Taylor (Ed.). Diseases of Swine, 9th ed. Ames: Iowa St. Un. Press, 701-717.

Online First

Published online: April 18, 2018

37. Thacker E., Minion F. (2012). Mycoplasmosis. In J. J. Zimmerman, A. Ramirez, K. J. Schwartz, & G. W. Stevenson (Eds.), Diseases of Swine, 10th ed. Ames: Wiley-Blackwell Publishing, 779-798.

38. Thongkamkoon P., Narongsak W., Kobayashi H., Pathanasophon P., Kishima M., Yamamoto K. (2013). In vitro susceptibility of Mycoplasma hyopneumoniae field isolates and occurrence of fluoroquinolone, macrolides and lincomycin resistance. Journal of Veterinary Medical Science, 75, 1067-1070.

39. Timmerman T., Dewulf J., Catry B., Feyen B., Opsomer G., de Kruif A., Maes D. (2006). Quantification and evaluation of antimicrobial-drug use in group treatments for fattening pigs in Belgium. Preventive Veterinary Medicine, 74 (4), 251-263.

40. Vicca J. (2005). Virulence and antimicrobial susceptibility of Mycoplasma hyopneumoniae isolates from pigs. PhD Thesis, Gent University, Belgium.

41. Vicca J., Maes D., Thermote L., Peeters J., Hasesbrouck F., Kruif A. (2002). Patterns of Mycoplasma hyopneumoniae infections in Belgian farrow-to-finish pigs herds with diverging-course. J. Vet. Med., 49, 349-353.

42. Vicca J., Mae D., Stakenborg T., Butaye P., Minion C., Peeters J., Haesebrouck F. (2007). Resistance mechanism against fluoroquinolones in Mycoplasma hyopneumoniae field isolates. Microbial Drug Resistance, 13, 166-170.

43. Wallgren P., Vallgarda J., Lindberg M., Eliason-Selling L. (2000). The efficacy of different vaccination strategies against Mycoplasma hyopneumoniae. In Proc. of the 16-th IPVS Congress, Melbourne, Australia, 461.