Summary of Ph.D. thesis

COMPARATIVE ANALYSIS OF *MYCOPLASMA HYORHINIS* ISOLATES AND STUDYING THE PATHOGENESIS OF INFECTION

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Introduction

Mycoplasma hyorhinis is a facultative pathogen bacterium, which was first described by Switzer in 1955. (Switzer, 1955). The main lesions caused by *M. hyorhinis* infection are polyserositis and arthritis (Kobisch and Friis, 1996), but rarely also it is associated with ear infections (Morita *et al.*, 1995), conjunctivitis (Resende *et al.*, 2019) and meningitis (Bünger *et al.*, 2020). In the United States 97% (Pillman *et al.*, 2019), while in Korea 66.1% prevalence was found on herd level in oral fluid samples (Cheong *et al.*, 2017). *M. hyorhinis* is present in the nasal microbiota of healthy pigs (Switzer, 1955). Piglets get infected from sows, afterwards *M. hyorhinis* spreads rapidly in the nursery phase (Clavijo *et al.*, 2017; 2019; Roos *et al.*, 2019).

Clinical signs of infection typically appear in three to ten-week-old weaners. Predisposing factors such as stress caused by weaning or the comingling with other piglets, decay of colostral immunity or presence of viral infections are considered important for the appearance and severity of the clinical signs (Palzer *et al.*, 2015; Chen *et al.*, 2016b; Clavijo *et al.*, 2019). Clinical signs and pathological lesions of *M. hyorhinis* infection are not

pathognomic, therefore detection of the pathogen from the lesions is essential for proper diagnosis. Due to the fastidious nature of mycoplasmas, PCR became the gold standard in diagnostic testing for their detection (Assunção *et al.*, 2005; Resende *et al.*, 2019).

PCR based typing methods are available for *M. hyorhinis* to determine relationships among the isolates (Tocqueville *et al.*, 2014; Dos Santos *et al.*, 2016; Trüeb *et al.*, 2016; Clavijo *et al.*, 2019). However, novel genotyping methods with improved robustness or resolution capability should enable more precise analyses of this species.

There are no commercially available vaccines against *M. hyorhinis* in Europe and maintaining pathogen free herds is also not feasible. Beside maintaining good health management in herds (Palzer *et al.*, 2015; Clavijo *et al.*, 2019), antimicrobial therapy is the only mode to reduce severity of the lesions. *M. hyorhinis* isolates are usually susceptible to tetracyclines, pleuromutilins and fluoroquinolones (Ter Laak *et al.*, 1991; Bekő *et al.*, 2019a; Rosales *et al.*, 2020). Decreased susceptibility against macrolides and lincomycin is common among isolates (Kobayashi *et al.*, 1996b, 2005; Bekő *et al.*, 2019a).

Aims of the study

The aims of the study were:

1. To determine the MICs of ten frequently used antimicrobials against *M. hyorhinis* isolates collected in Europe between 2019 and 2021.
2. To identify resistance markers in the genome of *M. hyorhinis* isolates and to develop a molecular typing method for the detection of the identified markers.
3. To develop genotyping systems for *M. hyorhinis* isolates with different resolution capacities.
4. To establish a *M. hyorhinis* challenge model for future vaccine efficacy studies.

Materials and methods

Sample collection and isolation

Throughout the study *M. hyorhinis* was cultured in *Mycoplasma* Liquid media (Mycoplasma Experience Ltd., Bletchingley, United Kingdom). Isolates were identified by *M. hyorhinis* specific PCRs (Assunção *et al.*, 2005; this study) and the presence of contaminant *Mycoplasma* species in the isolates was also excluded by PCR assays (Lauerman *et al.*, 1995; Assunção *et al.*, 2005; Martinson *et al.*, 2018a; Wu *et al.*, 2019). Whole genome sequences (WGS) of 38 Hungarian isolates were determined on Illumina platform (Illumina Inc., San Diego, California, USA).

Antibiotic susceptibility testing

Ten frequently used antibiotics were tested in this study: two tetracyclines (doxycycline and oxytetracycline), a pleuromutilin (tiamulin), a phenicol (florfenicol), a fluoroquinolone (enrofloxacin) four macrolides (tylosin, tilmicosin, tylvalosin and tulathromycin) and a lincosamide (lincomycin). The minimal inhibitory concentration (MIC) of the antibiotics against 76 *M. hyorhinis* isolates (two Belgian, 15 German, 20 Hungarian, 20 Italian and 19

Polish) was defined with the broth micro-dilution method. The MIC was the lowest concentration of the antibiotic where no colour change (no growth) was recorded by the time the growth control changed colour (Hannan, 2000). MIC_{50} and MIC_{90} values were defined as the lowest concentrations that inhibited the growth of 50% and 90% of the tested isolates (Hannan, 2000).

Mismatch amplification mutation assay

Single nucleotide polymorphisms (SNP) associated with high (genotype H) or low (genotype L) MIC values were scouted for in the susceptibility-associated genes (23S rRNA, and 50S ribosomal protein L4 and L22) of the determined 38 WGS using Geneious Prime software version 2019.2.1 (Kearse *et al.*, 2012). To detect and discriminate the SNPs mismatch amplification mutation assays (MAMAs) were developed. The sensitivity of the assays was tested both for genotypes L and H. For the validation of the developed assay a total of 138 samples (123 clinical isolates with MIC data and 15 DNA samples) were used.

Development of genotyping assays

House-keeping genes suitable for MLST were selected from the MLST schemes described in *Mycoplasma* sp. before (Manso-Silván *et al.*, 2012; Tocqueville *et al.*, 2014; Dijkman *et al.*, 2016; Ghanem and El-Gazzar, 2016; Trüeb *et al.*, 2016; Bekő *et al.*, 2019b). Criteria for gene selection is summarised as follows: (1) the selected genes are present in all *M. hyorhinis* genomes, (2) the selected genes have highly diverse internal fragments surrounded by conserved regions, (3) the selected fragments have high Simpson's index of diversity (ID), (4) the amplicon sizes of the selected gene fragments are suitable for Sanger sequencing and (5) the genes are evenly distributed in the genome. A total of 47 samples were used for the validation of the assays (*M. hyorhinis* type strain, six strains available in GenBank and 40 clinical isolates, including three isolates from the same animal). The phylogenetic analysis was performed with the Maximum Likelihood method, using the Hasegawa-Kishino-Yano model in the MegaX software (Kumar *et al.*, 2018).

To develop an MLVA, tandem-repeat regions were identified using the Tandem Repeat Finder program (Benson, 1999). Loci were selected based on period size

(at least 12 bp) and the percent of insertions/deletions (0%). A total of 41 samples (same sample set as MLST except the sequences from GenBank) were characterized by the developed MLVA system. The clustering analysis was performed with the Neighbor-Joining method based on pairwise distances in the MegaX software (Kumar *et al.*, 2018).

Comparison of genotyping methods

The MLST analysis of 45 strains (38 clinical isolate with determined WGS, GenBank sequences and the type strain) was performed according to the previously published MLST systems [previous MLST: MLSTp (Tocqueville *et al.*, 2014; Trüeb *et al.*, 2016) and surface protein MLST: MLSTs (Clavijo *et al.*, 2019)]. Phylogenetic analysis was performed with the Maximum Likelihood method, and Tamura-3 model in the MegaX software (Kumar *et al.*, 2018) for both MLSTp and MLSTs. To compare the three MLST systems, parsimony informative sites and Simpson's diversity of the gene fragments separately and of the concatenated sequences were calculated based on data of the same 45 isolates. The robustness of the constructed phylogenetic trees was compared based on the number of branches with

bootstrap values over 70%. The correlations among epidemiologic information and phylogenetic analysis were examined based on the serial isolates from the same farms.

Challenge model

Sixteen, four-week-old piglets were divided into three groups with similar average weights. The challenge material (MycSu160: a Hungarian *M. hyorhinis* clinical isolate) was prepared freshly for each challenge day. Group IV-IV (n=6) was inoculated by intravenous (IV) route on days 0 and 1 (D0, D1). Group IV-IP (n=6) was challenged IV on D0 and by intraperitoneal (IP) route on D1. The total challenge dose was 1.66×10^7 CCU/pig and 2.86×10^7 CCU/pig in Group IV-IV and IV-IP, respectively. Animals in the Control Group (n=4) received sterile liquid media.

Animals were observed daily, clinical signs and body temperatures were recorded. Body weight measurement, blood sampling and nasal swab sampling were carried out twice a week. The sera were tested by an in-house whole cell ELISA for anti-*M. hyorhinis* antibodies, while the swabs were checked for the presence of *M. hyorhinis* with PCR and isolation. At necropsy, pathological lesions were

scored and samples were collected from the joints, serosal membranes, conjunctiva, lung and brain for *Mycoplasma* sp. culture and PCR, bacteriology and histopathology. The re-isolates collected after the challenge were genotyped by the developed MLST and MLVA. Histopathological changes were also scored based on severity. Statistical analysis of the pathological and histopathological scores, the average daily weight gain (ADWG) and the ELISA titers were accomplished with the R programing language (R Core Team, 2021).

The experiment was approved by the National Scientific Ethical Committee on Animal Experimentation under the reference number: PE/EA/746-7/2021.

Results

Antibiotic susceptibility testing of recent isolates

Regardless of origin, a bimodal MIC distribution for macrolides and lincomycin and a unimodal distribution for the other tested antimicrobials were detected among the 76 *M. hyorhinis* isolates. The tested isolates were inhibited by low concentrations of tiamulin (MIC_{90} 0.312 µg/ml), doxycycline (MIC_{90} 0.078 µg/ml), oxytetracycline (MIC_{90} 0.25 µg/ml), florfenicol (MIC_{90} 0.2 µg/ml) and moderate concentrations of enrofloxacin (MIC_{90} 1.25 µg/ml). High MICs in the range of 32->64 µg/ml except for tylvalosin (5->10 µg/ml) were detected for the tested macrolides and lincomycin in 42-56% of the cases (32-43/76 isolates). Differences between countries were detected when the MIC_{50} values were compared for macrolides and lincomycin. Lower MIC_{50} values were detected in Hungarian and Polish isolates than in the ones from Italy and Germany.

Mismatch amplification mutation assay

Macrolide and lincomycin susceptibility were decreased in 43% of the examined *M. hyorhinis* clinical isolates with MIC data (61/123). To determine resistance related point mutations two 50S ribosomal proteins (L4, L22) and the 23S rRNA gene were analysed. While no SNP was detected on the ribosomal proteins, one SNP at nucleotide position A2066G (A2058G according to *E. coli* numbering) in the 23S rRNA gene was identified. The results of the molecular assays were in line with the ones of the broth micro-dilution method for the majority of the tested macrolides. False reactions were detected in three cases (Ge-13, Po-4 and Po-9), where genotype L was detected in isolates which showed high MIC values with the broth micro-dilution method. In the case of the clinical specimens, the results of the MAMA tests were also in line with the results of the broth micro-dilution tests of the corresponding isolates.

Development of multi-locus sequence typing and multiple-locus variable-number tandem repeat analysis

Six gene fragments (*lepA*, *rpoB*, *rpoC*, *gltX*, *uvrA* and *valS*) were selected for the establishment of the MLST and the novel *M. hyorhinis*-specific MLVA includes the analysis of six alleles (Mhr205, Mhr396, Mhr438, Mhr441, Mhr442 and Mhr444). The developed MLST and MLVA were validated using the same 41 isolates, and while the analysed strains represented 32 sequence types (ST) with the MLST, 38 genotypes were differentiated among them with the MLVA. Isolates from the same animal could not be distinguished with either system. In certain cases, isolates from the same farm collected in distinct years formed one common ST or belonged to closely related STs with the MLST. Nevertheless, isolates with distinct MLST STs were also detected from the same farms. No further correlations were found between the origin (organ, year of isolation) of isolates and ST or genotype.

Comparison of the developed and previous genotyping assays

The two previously published (MLSTp, Tocqueville *et al.*, 2014; Trüeb *et al.*, 2016; and MLSTs, Clavijo *et al.*, 2019) and the here developed MLST (MLSTn) schemes revealed high diversity among the examined 45 isolates (MLSTn: 38 STs; MLSTp: 31 STs and MLSTs: 32 STs).

The rate of parsimony informative sites for the concatenated sequences was the highest for the MLSTs system (8.12%) mainly due to the high variance on the surface protein coding gene *mtlD* (20.30%). Parsimony informative sites of the MLSTn were 1.24%, and 0.65% was detected for the MLSTp. The Simpson's diversity indices of the concatenated sequences were the highest for MLSTn (0.986), while 0.976 and 0.964 were detected for MLSTp and MLSTs respectively. On the phylogenetic tree of MLSTn system, five branches had bootstraps over 70%, while in the case of MLSTp there were none and for MLSTs the number of these branches was 12. The analysis with the here developed MLSTn supported most the epidemiological background of these isolates.

Challenge model

The first clinical signs (swollen tarsal joints) appeared six days after challenge in Group IV-IP and two days later in Group IV-IV. No other clinical signs were detected in the challenge groups and no alterations were detected in the Control Group. The ADWG was 223 g, 170 g and 350 g in Group IV-IV, IV-IP and Control Group. The ADWG in the Control Group was significantly higher than in the challenge groups.

After inoculation PCR positivity was recorded in both challenge groups (on D5 in Group IV-IP and on D8 in Group IV-IV). Regarding the necropsy samples, serosa (pericardium, pleura, peritoneum) and lung swabs were only PCR positive in Group IV-IV. At least one samples from all sampled joints (stifle, elbow, tarsus, carpus) were positive for *M. hyorhinis* by PCR in both challenge groups, however slightly higher positivity were detected in Group IV-IV. Samples from the Control Group were negative for *M. hyorhinis* in the entire study. While all the re-isolates showed the same MLST and MLVA type as the challenge strain, two re-isolates from Group IV-IP differed from the challenge strain on one MLVA allele. No other pathogenic bacteria, or porcine *Mycoplasma* sp. were detected in the necropsy samples.

Arthritis of at least one joint was observed in all pigs in the challenge groups. Arthritis manifested as serous or purulent inflammation and was detected most often in the tarsus (8/12) followed by the elbow (6/12), stifle (5/12) and carpus (4/12) on one or both sides. Diffuse, severe, chronic pericarditis presenting a large amount of connective tissue was detected in two animals in both groups. Additionally, mild or moderate chronic pleuritis and peritonitis presenting filaments of connective tissues were detected in three animals in Group IV-IV. No gross pathological lesions were detected in any examined organs in the Control Group. Pathological scores in both challenge groups differed significantly from the Control Group, but not from each other in both cases.

The main histopathological lesions were detected in the joints and the serosa of parenchymal organs in the thoracic and peritoneal cavities. In the joints inflammation was always associated with the formation of lymphoid follicles around the blood vessels and in some cases with the presence of multinucleated giant cells. Alterations of the serosa in all cases included projections consisting of connective tissue, which combined either with inflammation (in some cases with the presence of multinucleated giant cells) or diffuse thickening.

Multinucleated giant cells in the joints and in the pleura was detected in the same animal from Group IV-IV. Based on the statistical analysis, scores of joints and total scores differed significantly between Group IV-IV and Control. Statistically significant increase in the titre of antibodies against *M. hyorhinis* was detected in both challenge groups compared to the control animals.

Discussion

Antibiotic susceptibility testing

Susceptibility testing of veterinary mycoplasmas is time consuming, requires special expertise and up to day not standardized. Therefore, in most cases the targeted antibiotic treatment is inaccessible and the selection of antibiotics for therapeutic use is often empirical. For this reason, recent and comparable MIC data is essential, however, susceptibility data of recent European isolates are scarce in the literature (Ter Laak *et al.*, 1991; Bekő *et al.*, 2019a; Rosales *et al.*, 2020).

Accordingly, antibiotic susceptibility of 76 European (Belgian, German, Hungarian, Italian and Polish) *M. hyorhinis* isolates from 2019 to 2021 was examined. These isolates were inhibited by low concentrations of tiamulin, tetracyclines (doxycycline and oxytetracycline) and florfenicol, while moderate concentrations of enrofloxacin were also effective against them. On the other hand, in the case of the tested macrolides and lincomycin a bimodal MIC distribution was observed, with 42-56% of the isolates showing decreased susceptibility to these drugs. Slight differences in the MIC_{50} values of macrolides and lincomycin between countries were

detected. These minimal differences can be explained by the hypothesised differences between countries in the route of administration (parenteral or feed/water) and frequency of use. In a study including four European countries (Belgium, France, Germany and Sweden) marked differences in the recommended dosages were found, for example recommended dosages in Germany were lower than in the other countries (Postma *et al.*, 2015).

Evaluating the published MIC values of European *M. hyorhinis* isolates over 35 years, consistently low MIC values were detected only for tiamulin and tetracyclines. The described results indicate the effectiveness of these antimicrobials against *M. hyorhinis* isolates. However, the detected bimodal susceptibility patterns for macrolides and lincomycin and the comparison of the susceptibility profiles of European *M. hyorhinis* isolates over the years emphasize the importance of regular susceptibility monitoring.

Mismatch amplification mutation assay

Macrolide and lincosamide antibiotics are chemically distinct, but share a similar mode of action as their binding sites overlap (also with streptogamins, ketolides and oxazolidinones; Leclercq, 2002). Both inhibit the bacterial protein synthesis by reversely binding to the 50S subunit of the ribosome. Target site modification through mutations in the 23S rRNA gene has been described in several veterinary species and mutations in the L4 and L22 ribosomal proteins in some avian and ruminant mycoplasmas has also been identified (Gautier-Bouchardon, 2018; Bekő *et al.*, 2020, Grózner *et al.*, 2022). A point mutation in *M. hyorhinis* isolates in the 23S rRNA gene V domain A2059G (according to *E. coli* numbering) was described earlier in connection with decreased susceptibility to macrolides (Kobayashi *et al.*, 2005). In this study, a transversion substitution in the same region of the 23S rRNA gene at position A2058G (according to *E. coli* numbering) was identified in isolates with decreased susceptibility to macrolides and lincomycin.

A MAMA was designed to detect the identified SNP and provide a cost effective tool for rapid determination of macrolide and lincomycin susceptibility. Results of the

developed assay were in line with the *in vitro* MIC values. Incogruency between the results was detected in case of three recent isolates. Sequence analysis of the partial 23S rRNA gene sequence in these isolates confirmed the genotypes determined by the MAMA. Based on these findings, presence of other resistance mechanisms can be suspected. Active efflux mechanisms, drug inactivation (Leclercq, 2002) or biofilm formation can also be responsible for elevated MIC values (Tassew *et al.*, 2017).

Considering all the above, the developed assay is reliable to guide antibiotic therapy when the susceptibility data for macrolides and lincomycin are required rapidly.

Genotyping assays

Efficient genotyping tools are the key to better understand and monitor *M. hyorhinis* infections and to conduct epidemiological investigations. A six-gene based MLST was developed before with high discriminatory power, but low reproducibility (MLSTp; Tocqueville *et al.*, 2014; Trüeb *et al.*, 2016). Therefore a modified MLST scheme including surface protein coding genes was applied (MLSTs; Clavijo *et al.*, 2019), however, the use of surface protein genes limits the reliability of the assay as conserved genes are more reliable in indicating

relationships (Estoup *et al.*, 2002; Urwin and Maiden, 2003). Ultimately, a core genome MLST scheme has been established, after the publication of the here developed MLST system, providing the highest resolution of pure isolates (Bünger *et al.*, 2021). Beside the different MLST schemes a two allele based MLVA was also described for the characterization of *M. hyorhinis* isolates (Dos Santos *et al.*, 2015).

In the present study, high resolution MLST (Simpson's ID 0.986) and MLVA (Simpson's ID 0.999) were developed. Typically, isolates from the same farm shared the same or closely related STs with all MLST schemes, as using limited number of house-keeping gene fragments limits the differentiation of closely related isolates (Urwin and Maiden, 2003). Presence of isolates collected over a short period of time (six months to a year) with the detected distinct STs indicates introduction of new strains to the herd. The observation that the same isolate (identical MLST and MLVA type) can be found in different organs indicates that the same strain can cause macroscopic lesions in every affected tissue.

The recently published cgMLST scheme was compared with the MLSTn and MLSTp systems, examining 73 *M. hyorhinis* isolates (from Austria and

Germany; Bünger *et al.*, 2021). The results of the MLSTn to the cgMLST were more congruent (Rand coefficient 0.496) than the MLSTp STs to the cgMLST STs (Rand coefficient 0.179). Moreover, certain distantly related isolates were grouped together by MLSTp, contradicting with the results of the cgMLST (Bünger *et al.*, 2021) and the MLSTn.

The MLSTn and MLVA represent convenient, well reproducible and high resolution molecular tools for the typing of *M. hyorhinis* isolates. To explore relationships of closely related isolates in phylogenetic studies the combined MLSTn and MLVA method is recommended, but the MLVA alone is suitable for epidemiologic studies with basic laboratory equipment and techniques.

Challenge model

Based on available literature data a single dose *M. hyorhinis* challenge through intranasal, intravenous or intraperitoneal inoculation is insufficient to induce all typical lesions (Lin *et al.*, 2006; Gomes Neto *et al.*, 2014; Lee *et al.*, 2018; Martinson *et al.*, 2018a; Fourour *et al.*, 2019; Wei *et al.*, 2020). Therefore, based on previous work of Martinson and co-workers (2018a) a two-dose

intravenous and a two-dose combined intravenous and intraperitoneal challenge were applied in this study.

The double dose IV challenge (which was not mentioned in previous publications) produced equal involvement of joints as the mix of IV-IP route (arthritis of at least one joint was detected in all animals in both groups), which exceeded the rate of animals affected with arthritis in the previous study (single dose IV challenge resulted arthritis in only 1/10 animal; Martinson et al 2018a). The combination of the used infection routes (Group IV-IP) resulted in the earlier appearance of more pronounced clinical signs of arthritis like swollen joints and lameness. On the other hand, in the group which was challenged by the IV route on two consecutive days (Group IV-IV) more organs were affected by serositis than in Group IV-IP. During necropsy, most of the lesions appeared chronic in both groups. Regarding the joints lesions with proliferated synovial membrane and the thickening of the joint capsule were detected and chronic inflammation of the serosa was presented as adhesions with thickening of the membrane, which was detected both in the pericardium and the pleura. The low rate of re-isolation compared to PCR positive samples also indicate the late phase of infection. Therefore, the reduction of the

length of the study is suggested. As clinical signs of *M. hyorhinis* infection gradually alleviate after two weeks (Barden and Decker, 1971; Wang *et al.*, 2022a), the length of study should be determined based on the appearance of the first clinical signs.

Considering the hypothesis of the natural spread of the pathogen via the circulatory or lymphatic system and the more severe pathological lesions in Group IV-IV, a two-dose intravenous challenge is recommended by the authors.

Overview of the new scientific results

Ad. 1.: The performed antibiotic susceptibility tests with 76 *M. hyorhinis* isolates from five European countries provide essential information regarding minimal inhibitory concentration values of ten frequently used antimicrobials in the swine industry. The presented data are useful for current targeted antibiotic treatments and in future analyses of trends in changes of antibiotic susceptibility.

Ad. 2.: A previously described molecular marker of decreased susceptibility to macrolides and lincomycin was identified among the examined *M. hyorhinis* isolates, and a rapid and reliable molecular assay (MAMA) was developed for the detection of this point mutation. The developed assay is a feasible tool to guide targeted antibiotic treatment.

Ad. 3.: Novel, PCR-based, genotyping assays (MLST and MLVA) were developed using the latest technology for the phylogenetic and epidemiologic analyses of *M. hyorhinis* isolates and clinical specimens. The comparison of the developed MLST with the previously published MLST schemes revealed that our MLST scheme has the highest discriminatory power and reflects best the genome-wide relationships among the conventional MLST methods.

Ad. 4.: Genotyping and phylogenetic analyses was performed with the developed MLST and MLVA systems on 41 *M. hyorhinis* isolates. The developed MLST method differentiated *M. hyorhinis* isolates with high discriminatory power. In combination with the developed MLVA, differentiation between closely related isolates could be achieved.

Ad. 5.: Two *M. hyorhinis* challenge models were established in five-week-old piglets. Both the double dose intravenous and the combined intravenous and intraperitoneal challenge could induce typical lesions. The challenge models are suitable to be used in further vaccine efficacy studies.

Scientific publications

Publications in the topic of the dissertation:

Földi D., Klein U., Belecz N., Hrivnák V., Somogyi Z., Gastaldelli M., Merenda M., Catania S., Dors A., Siesenop U., Vyt P., Kreizinger Zs., Depondt W., Gyuranecz M.: **Antimicrobial susceptibility profiles of *Mycoplasma hyorhinis* strains isolated from five European countries between 2019 and 2021**, Plos One, 17, e0272903, 2022.

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Klose SM., Olaogun OM., Disint JF., Shil P., Gyuranecz M., Kreizinger Zs., Földi D., Catania S., Bottinelli M., Dall'Ora A., Feberwee A., van der Most M., Andres DM., Underwood GJ., Morrow CJ., Noormohammadi AH., Marenda MS.: **Genomic diversity of a globally used, live attenuated *Mycoplasma* vaccine**, Microbiology Spectrum, e02845-22, 2022.

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