Summary of Ph.D. thesis

GENETIC STUDIES ON *MYCOPLASMA GALLISEPTICUM* AND *M. SYNOVIAE* AND DEVELOPMENT OF MOLECULAR ASSAYS APPLICABLE AS DIAGNOSTIC METHODS

dr. Katinka Bekő

Supervisor: Dr. Miklós Gyuranecz

UNIVERSITY OF VETERINARY MEDICINE

Doctoral School of Veterinary Science

Budapest, 2021

Supervisor and consultants:

.....
Dr. Miklós Gyuranecz, Ph.D. Habil.
Institute for Veterinary Medical Research
Centre for Agricultural Research
Supervisor

Dr. Zsuzsa Kreizinger, Ph.D.
Institute for Veterinary Medical Research
Centre for Agricultural Research
Consultant

Dr. Krisztián Bányai, Ph.D.
Institute for Veterinary Medical Research
Centre for Agricultural Research
Consultant

.....
dr. Katinka Bekő

Introduction

Mycoplasma gallisepticum and *M. synoviae* are widespread facultative pathogens of chickens and turkeys. *M. gallisepticum* infection most commonly manifests as respiratory symptoms, while *M. synoviae* usually induces infectious synovitis and it can be related to eggshell apex abnormalities as well (Ley and Yoder, 1997; Feberwee *et al.*, 2009). In Hungary, Bamberger and Csontos reported respiratory disease associated with *M. gallisepticum* infection for the first time in 1953, while the first occurrence of infectious synovitis was in 1958 (Bamberger and Csontos, 1953; Derzsi and Tóth Baranyi, 1960). Thereafter, with the global spread of intensive poultry farming, avian mycoplasmosis caused by *M. gallisepticum* and *M. synoviae* has been reported increasingly, and now, they are the most economically significant *Mycoplasma* species in the chicken and turkey industry worldwide. The control programs are primarily based on eradication, however, there are many difficulties in maintaining flocks free of infection. Vaccination provides an effective measure of long-term disease control, while antibiotic therapy can offer a short-term

solution (Ley and Yoder, 1997; Levisohn and Kleven, 2000; Kleven, 2008).

For phylogenetic studies and epidemiological investigations, it is necessary to reveal genetic relationships between *M. gallisepticum* strains. The most reliable genotyping assay is multi-locus sequence typing (MLST), which classifies strains in sequence types due to the nucleotide sequence of certain housekeeping genes (Ghanem and El-Gazzar, 2019).

Since live vaccines are used in many parts of the world, differentiation of *M. gallisepticum* vaccine strains from wild, virulent isolates is essential in control programs. Mismatch amplification mutation assay (MAMA), which is a PCR-based technique used for single nucleotide polymorphism discrimination, can be suitable for this purpose (Birdsell *et al.*, 2012).

Recently, several studies found high minimal inhibitory concentration (MIC) values of macrolides and lincosamides against *M. synoviae* isolates. Susceptibility to fluoroquinolones also decreased over the last few decades, which is particularly troublesome as their use is critical in the therapy of humans (Kreizinger *et al.*, 2017; Catania *et al.*, 2019). Prudent use of antibiotics can be improved greatly by the determination of antibiotic

susceptibility prior to treatment. Thus, there is an increasing need for rapid susceptibility tests, like detecting resistance-associated mutations by molecular assays (Sulyok *et al.*, 2018). Numerous resistance-related mutations have already been described in the DNA gyrase and topoisomerase IV coding genes (*gyrA*, *gyrB*, *parC*, *parE*) for fluoroquinolones, the 16S rRNA coding genes (*rrsA*, *rrsB*) for aminoglicosides and tetracyclines, and the 23S rRNA and ribosomal protein L3, L4 and L22 coding genes (*rrlA*, *rrlB*, *rplC*, *rplD*, *rplV*) for macrolides, lincosamides, pleuromutilins and phenicols in many bacteria, including mycoplasmas as well (Le Carrou *et al.*, 2006; Lysnyansky *et al.*, 2013; 2015; Sulyok *et al.*, 2017).

Aims of the study

The aims of the study were:

1. to develop an MLST assay applicable for genotyping different *M. gallisepticum* strains.

2. to investigate the genetic relatedness of *M. gallisepticum* strains with the developed MLST assay in order to evaluate the discriminatory power of the method and better understand the epidemiology of *M. gallisepticum*.

3. to develop rapid molecular biological assays (MAMA tests) capable of differentiating *M. gallisepticum* vaccine strains from field isolates.

4. to compare the results of the developed MLST assay with data obtained from the designed MAMA tests in order to confirm their reliability and investigate the congruency of these methods.

5. to investigate the genetic background of decreased susceptibility to fourteen antibiotics of eight antimicrobial groups in *M. synoviae* and to identify potentially resistance-related mutations suitable to be targeted with rapid molecular biological assays.

Materials and Methods

Mycoplasma gallisepticum samples

For the assay developments, 19 *M. gallisepticum* whole genome sequences (WGSs) were used *in silico*. DNA of the *M. gallisepticum* type strain ATCC 19610 and the live 6/85, ts-11, F and K vaccine strains were also included in the study. Besides, DNA of 266 *M. gallisepticum* isolates including pure *M. gallisepticum* cultures and clinical samples were used as well. *M. gallisepticum* positivity of the used samples was confirmed by conventional (Garcia *et al.*, 2005) or real-time Taqman (Raviv and Kleven, 2009) PCR. The presence of other, contaminant mycoplasmas was excluded by a universal *Mycoplasma* PCR system (Lauerman *et al.*, 1995), the products were submitted to Sanger sequencing, and sequences were subjected to BLAST search.

Development of MLST assay for genotyping *M. gallisepticum* strains

Several housekeeping genes of 19 *M. gallisepticum* WGSs were analyzed during the target selection, out of them, 15 selected loci were submitted to primer design.

Primers were tested on ten diverse *M. gallisepticum* samples, and PCR products were submitted to Sanger sequencing. An allelic number was assigned to each unique allele variant, and the discriminatory power for each locus was determined by calculating Simpson's index of diversity (SI) (Hunter and Gaston, 1988). Loci showing the highest discriminatory power were selected for the MLST scheme. The developed MLST assay was performed on 131 *M. gallisepticum* samples. The strains were grouped in sequence types (STs) according to their allelic numbers of the loci. The discriminatory power of the method and for each locus was determined by calculating SI (Hunter and Gaston, 1988). Molecular phylogenetic analysis of the concatenated sequences was inferred by using Maximum Likelihood method (Hasegawa *et al.*, 1985). The evolutionary history was inferred with the inclusion of an outgroup, the *M. imitans* ATCC 51306 type strain as well, using Neighbor-Joining method (Nei and Saitou, 1987; Tamura, 1992). The specificity of the assays was tested with 14 avian *Mycoplasma* species. In order to test the sensitivity of the assays, tenfold dilution series of the ATCC 19610 type strain's DNA were used in the range of 10^5-10^0 template copy number/µl.

Development of MAMAs for differentiating *M. gallisepticum* vaccine strains from field isolates

Next-generation sequencing of the vaccine strains was performed on Ion Torrent or Illumina NextSeq 500 platform. Single nucleotide polymorphisms (SNPs) specific for each vaccine strains were detected, and the selected SNPs were used as targets for mismatch amplification mutation assay (MAMA) development. Assays showing the highest discriminating potential were tested with the DNAs of 250-281 *M. gallisepticum* isolates. Samples which appeared to be ts-11 re-isolates by the assays or originated from Australia were further tested according to Ricketts *et al.* (2017). In the stability tests, each genotypes were subjected to serial passage 10 times, resulted in ~33.22 population doubling (Choi *et al.*, 2017). The specificity of the assays was tested using 14 avian *Mycoplasma* species. In order to test the sensitivity of the assays, tenfold dilutions of the DNA of each genotype were used in the range of 10^6-10^0 template copy number/µl. Mixed samples with different template copy numbers of the two genotypes were also tested. To determine the congruency of the assays, (adjusted) Rand co-efficient was calculated (Pinto *et al.*, 2007).

Detecting mutations potentially associated with decreased susceptibility to certain antibiotics in *M. synoviae* strains

In total, 96 *M. synoviae* strains, including the NCTC 10124 type strain, the MS-H and MS1 vaccine strains and 93 field isolates were investigated. *M. synoviae* positivity of the DNA samples was confirmed by conventional (Wang *et al.*, 1997) or real-time Taqman (Raviv and Kleven, 2009) PCR. The presence of other, contaminant mycoplasmas was excluded by a universal *Mycoplasma* PCR system (Lauerman *et al.*, 1995), the products were submitted to Sanger sequencing, and sequences were subjected to BLAST search. Minimal inhibitory concentrations (MICs) were determined by broth microdilution method (Hannan, 2000) for the following antimicrobial agents: enrofloxacin, difloxacin, doxycycline, oxytetracycline, chlortetracycline, spectinomycin, neomycin, tylosin, tilmicosin, tylvalosin, lincomycin, florfenicol, tiamulin, valnemulin. Next-generation sequencing of *M. synoviae* strains was performed on Ion Torrent or Illumina NextSeq 500 platform. Resistance genes were analyzed, and mutations detected in a large number of strains with high MIC values or previously linked to antibiotic resistance in

mycoplasmas were considered as potentially resistance-related mutations. Strains were genotyped by MLST (El-Gazzar *et al.*, 2017) using Maximum Likelihood method (Hasegawa *et al.*, 1985). A conventional PCR followed by a MAMA test was developed for the determination of the nucleotide 2054 in the *rrlA*/*rrlB* genes. In order to investigate the presence of different *tet* genes in strains with high MIC values to tetracyclines, draft genomes were submitted to *in silico* analysis (Bankevich *et al.*, 2012), while *tet*(M) positivity was investigated with a conventional PCR (Shahid *et al.*, 2014b) as well.

Results

Development of MLST assay for genotyping *M. gallisepticum* strains

Among the 15 targeted housekeeping genes, six loci (*atpG*, *dnaA*, *fusA*, *rpoB*, *ruvB*, *uvrA*) were selected for the MLST scheme. Examination of the specificity revealed cross-reactions with other avian *Mycoplasma* species in case of *atpG* and *dnaA*, however, sequence analysis clearly distinguished the cross-reacting species from *M. gallisepticum*. The sensitivity was 10^3 template copy number/µl for all loci. The 131 *M. gallisepticum* samples yielded 17-21 unique alleles for each locus, and 57 unique STs, resulted in a Simpson's index of diversity of 0.958. *DnaA* possessed the highest number of SNPs (36/415), while *rpoB* had the highest diversity (0.913). Samples in ST9-11 were house finch-derived strains (1994-2008), ST29 contained isolates from pheasants, partridges and one chicken (2012-2017), while samples in ST21-22 originated from the Middle East (2006-2016). ST38 contained samples isolated from different Hungarian turkey farms belonging to the same integration. Vaccine strain 6/85 was assigned to ST14 along with 14 other

samples. The closest sequence types for ST14 were ST13 and ST16. Strain ts-11 belonged to ST49 with 12 other samples. The closest sequence types for ST49 were ST48, ST50 and ST45. Beside the vaccine strain F, only one isolate was assigned to ST5, while no samples have been found to share ST57 with the K vaccine strain. Concatenated sequences of *M. imitans* ATCC 51306 type strain had 380/2636 SNPs and 3 bp nucleotide deletion when aligned to the *M. gallisepticum* ATCC 19610 type strain.

Development of MAMAs for differentiating *M. gallisepticum* vaccine strains from field isolates

After performing preliminary studies, the number of MAMA assays was narrowed to two for 6/85, three for ts-11, two for F and one for K vaccine strain. The targeted mutations were located in virulence-associated genes (*crmA*, *gapA*, *hlp2*, *lpd*, *plpA, glpK*), a gene (*potC*) coding an ABC transporter protein and a gene (*fruA*) coding fructose-specific enzyme of the phosphotransferase system. The assays clearly distinguished the two genotypes by product size and melting temperature. Negative controls or templates of other avian *Mycoplasma* species were not amplified or generated non-specific

products differing from the profiles of the expected two allelic states. Detection limit of the assays changed between 10^2 and 10^4 template copy number/µl in melt-MAMA tests and 10^3 to 10^5 in agarose-MAMA tests. The assays were able to identify both genotypes in mixed samples (10^6:10^6, 10^6:10^5, 10^5:10^6 template copy number/µl). In the stability test, after the serial passage, strains showed identical genotypes as their parent strains. The MAMA tests showed high congruency (0.920-1.000).

Comparison of the results of the developed *M. gallisepticum* MLST assay and MAMA tests

The MAMA tests showed high congruency with the MLST assay (0.826-1.000). Most incongruent results belonged to isolates closely related to 6/85 or ts-11 vaccine strains. These isolates were identified as vaccine strains (1-2/2636 SNP by MLST), or resulted unambigous genotype calls (7-10/2636 SNP by MLST) with MAMAs. The PCR described by Ricketts *et al.* (2017) revealed poor agreement with the MAMA tests (0.495-0.605) or the MLST assay (0.486-0.505), as five Australian wild-type samples carried, while five vaccine-type samples lacked the ts-11-specific sequences.

Detecting mutations potentially associated with decreased susceptibility to certain antibiotics in *M. synoviae* strains

Due to the lack of *M. synoviae* strains with high MIC values of doxycycline, tiamulin, valnemulin, or low MIC values of neomycin, these antibiotics were omitted from further analysis. In the *gyrA* gene, G28A, A428G, A566G, T1360A, C1361A and G1651A; in the *gyrB* gene, C446T, C1247A and G1250A; in the *parC* gene, A253G, C254T, T256C, G265C/T, G1354A, G1798A and C2442A; while in the *parE* gene, C260T were found to be potentially resistance-realted SNPs. At least one of these mutations were carried by 88.73 % of the M*. synoviae* strains with high MIC values for fluoroquinolones (>1.25 µg/ml). The mutations of a hot spot region (nucleotides 253-265) were found to be of particular importance (85.92 %). In the *rrlA/rrlB* genes, A2054G and A2055G were found to be potentially macrolide and lincomycin resistance-realted SNPs. In total, 14 strains were found to possess the A2054G in both *rrl* genes, while six strains were found to be heterozygous concerning this position. The PCR designed for the determination of this nucleotide revealed that only one sample carried the mutation in the *rrlB* gene, while five strains possessed the SNP in the *rrlA* gene. In

the *rplV* gene, A276C/T was also identified as potentially macrolide resistance-related mutation. All *M. synoviae* strains with high MIC values for these 50S inhibitors (>8 µg/ml for tilmicosin; >1 µg/ml for tylosin; >0.5 µg/ml for tylvalosin; >2 µg/ml for lincomycin) have been found to carry exactly one of the described SNPs. No mutations could be related to the decreased susceptibility to tetracyclines (MIC values of >4 µg/ml), spectinomycin or florfenicol (MIC values of >2 µg/ml), and no *tet* genes could be identified. MLST (El-Gazzar *et al.*, 2017) revealed great genetic diversitiy of the 96 examined *M. synoviae* strains as they were classified into 42 STs. The neighbor-joining tree confirmed that the antibiotic susceptibility profile of the strains can differ within the same ST or even in case of identical origin.

Discussion

Development of MLST assay for genotyping *M. gallisepticum* strains

The large number of STs and high SI indicate good discriminatory power of the assay. The MLST was also shown to be suitable for identifying closely related strains, originating from geographically distinct outbreaks with epidemiologic links (ST38). The occurrence of identical sequence types in distant regions could be explained by extensive international trade. In some cases, closely related strains were detected in a longer period of time (ST9-11, ST21-22, ST29), and infected both game birds and domestic fowl (ST29). Presumably, in the absence of infection control, virulent strains can survive for a longer time in wild or semi-wild birds, followed by transmission of the pathogen from these birds to poultry. Evaluating genetic distances between strains using MLST offers a reliable tool for differentiating wild isolates from vaccine strains. Thus, the designed MLST assay can be used for phylogenetic studies or epidemiological investigations of *M. gallisepticum* strains, and for the differentiation of wild-type isolates from vaccine strains as well.

Development of MAMAs for differentiating *M. gallisepticum* vaccine strains from field isolates

The targeted mutations are located in genes associated directly or indirectly with virulence factors. The SNPs showed genetic stability through the passages, however, it should be noted, that the *in vitro* test may not reflect completely the *in vivo* stability. A limitation of this study is that no (K) or few (F) vaccine strain reisolates were available. Nevertheless, the results of the MAMA tests were in line with the konown vaccination history of the examined animals, flocks, or country of origin. By avoiding isolation, the designed MAMAs represent a convenient, rapid and cost-efficient tool for differentiating F, 6/85, ts-11 and K vaccine strains from field isolates, and are suitable for detecting the simultaneous presence of wild-type and vaccine strains in mixed samples as well.

Comparison of the results of the developed *M. gallisepticum* MLST assay and MAMA tests

Highly congruent results were observed among the developed MAMA and MLST tests, confirming the competence of the assays. However, the MLST system could distinguish closely related field isolates from ts-11 and 6/85 vaccine strains, which were identified as vaccine

strains, or resulted unambigous genotype calls with MAMAs. The MAMA-6/85-gapA and MAMA-ts11-plpA are proved to be the most reliable tests to distinguish vaccine strains. However, in order to achieve the most definite results, the combined use of all MAMAs is recommended, and in ambiguous cases, MLST can be applied as a confirmatory method. The disagreement was remarkable between the results of assays developed in this study and the PCR described by Ricketts *et al.* (2017). Most false positive samples originated from Australia, just like the ts-11 vaccine strain. The negative results are difficult to interpret, because the quality of the DNA and the sensitivity of the PCR systems also influence the results.

Detecting mutations potentially associated with decreased susceptibility to certain antibiotics in *M. synoviae* strains

Numerous molecular markers of fluoroquinolone-resistance could be detected in the examined *M. synoviae* strains. A hot spot region of the *parC* gene, known as resistance-associated region (Le Carrou *et al.*, 2006; Lysnyansky *et al.*, 2013), seems to have a principal role, as mutations located here were possessed by most strains with high MIC values. Mutation C254T could be

related to decreased susceptibility to both fluoroquinolones, while the SNP A253G affecting the same amino acid could be detected for the first time in *M. synoviae* strains resistant against difloxacin but not enrofloxacin. Mutation A428G of the *gyrA* gene also has been mentioned previously as resistance-associated SNP (Le Carrou *et al.*, 2006; Lysnyansky *et al.*, 2013), and mutations C1247A and G1250A of the *gyrB* gene have been reported before in the paper of Lysnyansky *et al.* (2013) as well, however their role was not clarified in that study. Besides, numerous potentially resistance-related mutations could be detected for the first time in the examined genes, mainly occurring simultaneously with the mutations of the hot spot region. However, in two strains with increased MIC values, novel mutations of the *gyrA*, *gyrB* and *parE* genes were detected without any alteration of the hot spot region, suggesting that these mutations may also play a role. Mutations A2054G and A2055G have been previously associated with decreased susceptibility of *M. synoviae* for macrolides and lincomycin (Lysnyansky *et al.*, 2015). Presence of mutation A2054G in the *rrlA* or *rrlB* gene only was found to be enough to increase the MIC values. Non-synonymous mutations revealed in the *rplV* gene

(A276C/T) were also found to decrease the susceptibility for tilmicosin. This alteration has been already described previously by Lysnyansky *et al.* (2015), although its role in macrolide resistance was not suggested in that study. No *tet* genes or molecular markers of tetracycline-, spectinomycin- and florfenicol-resistance could be detected, suggesting other resistance mechanisms. The revealed mutations can contribute to the extension of knowledge about the genetic background of antibiotic-resistance in *M. synoviae*. Moreover, the explored potentially resistance-related SNPs can be appropriate targets for molecular biological assays, reducing significantly the detection time of antibiotic susceptibility (Sulyok *et al.*, 2018).

Overwiew of the new scientific results

Ad 1. The designed MLST method is able to differentiate *M. gallisepticum* strains with high discriminatory power and identify closely related strains as well. Relatively high sensitivity of the assay makes it suitable for examining DNAs extracted directly from clinical samples. It can be used in practice for phylogenetic studies and epidemiological investigations as well.

Ad 2. According to the results of the designed MLST assay, the examined *M. gallisepticum* strains can be characterized by high genetic heterogeneity. Our data indicates the high impact of extensive international trade on the worldwide spread of different *M. gallisepticum* strains and also confirms that closely related *M. gallisepticum* strains can infect both domestic fowl and game birds, providing a possible source of infection for chickens and turkeys.

Ad 3. In total, eight MAMA tests were designed and found to be suitable for the differentiation of *M. gallisepticum* vaccine strains from field isolates. The assays reported here represent convenient, rapid and

cost-efficient tools for the monitoring of vaccination and eradication programs against *M. gallisepticum* infections.

Ad 4. The developed MLST and MAMA tests showed high congruency confirming the competence of the designed assays. However, the MLST system could distinguish closely related field strains from vaccines ts-11 and 6/85, which showed the vaccine-type or incongruent results with the MAMA tests. Therefore, the highly reliable MLST assay can be applied as a confirmatory test for the MAMAs.

Ad 5. Previously described molecular markers of high MICs for fluoroquinolones, macrolides and lincosamides were detected within the examined *M. synoviae* strain collection. Besides, genetic alterations presumably associated with decreased susceptibility to fluoroquinolones were identified in the *gyrA*, *gyrB*, *parC* and *parE* genes of *M. synoviae* strains for the first time.

Scientific publications

Publications on the topic of the thesis in peer-reviewed journals

Bekő, K., Gyuranecz, M.: **Baromfiállományok *Mycoplasma gallisepticum* okozta fertőzései - *Mycoplasma gallisepticum* infections in poultry**, Magy. Állatorv. Lapja, 142, 349-364, 2020.

Bekő, K., Gyuranecz, M.: ***Mycoplasma synoviae* okozta baromfi betegségek - Poultry diseases caused by *Mycoplasma synoviae***, Magy. Állatorv. Lapja, 142, 17-28, 2020.

Bekő, K., Kreizinger, Z., Kovács, Á.B., Sulyok, K.M., Marton, S., Bányai, K., Catania, S., Feberwee, A., Wiegel, J., Dijkman, R., ter Veen, C., Lysnyansky, I., Gyuranecz, M.: **Mutations potentially associated with decreased susceptibility to fluoroquinolones, macrolides and lincomycin in *Mycoplasma synoviae***, Vet. Microbiol., 248, 108818, 2020.

Bekő, K., Kovács, Á.B., Kreizinger, Z., Marton, S., Bányai, K., Bánáti, L., Catania, S., Bradbury, J., Lysnyansky, I., Olaogun, O.M., Gyuranecz, M.:

Development of mismatch amplification mutation assay (MAMA) for the rapid differentiation of *Mycoplasma gallisepticum* K vaccine strain from field isolates, Avian Pathol., 49, 317-324, 2020.

Bekő, K.*, Sulyok, K.M.*, Kreizinger, Z.*, Forró, B., Marton, S., Bányai, K., Catania, S., Ellis, C., Bradbury, J., Olaogun, O.M., Kovács, Á.B., Cserép, T., Gyuranecz, M.: **Development of molecular methods for rapid differentiation of *Mycoplasma gallisepticum* vaccine strains from field isolates**, J. Clin. Microbiol., 57, e01084, 2019.

Bekő, K., Kreizinger, Z., Sulyok, K.M., Kovács, Á.B., Grózner, D., Catania, S., Bradbury, J., Lysnyansky, I., Olaogun, O.M., Czanik, B., Ellakany, H., Gyuranecz, M.: **Genotyping *Mycoplasma gallisepticum* by multilocus sequence typing**, Vet. Microbiol., 231, 191-196, 2019.

Morrow, C.J., Kreizinger, Z., Achari, R.R., Bekő, K., Yvon, C., Gyuranecz, M.: **Antimicrobial susceptibility of pathogenic mycoplasmas in chickens in Asia**, Vet. Microbiol., 250, 108840, 2020.

Publications on other topics in peer-reviewed journals

Bekő, K., Kreizinger, Z., Yvon, C., Saller, O., Catania, S., Feberwee, A., Gyuranecz, M.: **Development of molecular assays for the rapid and cost-effective determination of fluoroquinolone, macrolide and lincosamide susceptibility of *Mycoplasma synoviae* isolates**, PLoS One, 15, e0241647, 2020.

Bekő, K., Felde, O., Sulyok, K.M., Kreizinger, Z., Hrivnák, V., Kiss, K., Biksi, I., Jerzsele, Á., Gyuranecz, M.: **Antibiotic susceptibility profiles of *Mycoplasma hyorhinis* strains isolated from swine in Hungary**, Vet. Microbiol., 228, 196-201, 2019.

Bekő, K., Koványi, B., Gölöncsér, F., Horváth, G., Dénes, Á., Környei, Z., Botz, B., Helyes, Z., Müller, C.E., Sperlágh, B.: **Contribution of platelet $P2Y_{12}$ receptors to chronic Complete Freund's adjuvant-induced inflammatory pain**, J. Thromb. Haemost., 15, 1223-1235, 2017.

Hornok, S., Szekeres, S., Horváth, G., Takács, N., Bekő, K., Kontschán, J., Gyuranecz, M., Tóth, B., Sándor, A.D., Juhász, A., Beck, R., Farkas, R.: **Diversity of tick species and associated pathogens on peri-**

urban wild boars - first report of the zoonotic *Babesia* cf. *crassa* from Hungary, Ticks Tick Borne Dis., 2012. [submitted]

Kovács, Á.B., Wehmann, E., Sváb, D., Bekő, K., Grózner, D., Mitter, A., Bali, K., Morrow, C.J., Bányai, K., Gyuranecz, M.: **Novel prophage-like sequences in *Mycoplasma anserisalpingitidis***, Infect. Genet. Evol., 2021. [submitted]

Grózner, D., Kovács, Á.B., Wehmann, E., Kreizinger, Z., Bekő, K., Mitter, A., Sawicka, A., Jánosi, S., Tomczyk, G., Morrow, C.J., Bányai, K., Gyuranecz, M.: **Multilocus sequence typing of geese pathogen *Mycoplasma anserisalpingitidis***, Vet. Microbiol., 254, 108972, 2021.

Földi, D., Bekő, K., Kreizinger, Z., Felde, O., Kovács, Á.B., Tóth, F., Bányai, K., Gyuranecz, M.: **Genotyping *Mycoplasma hyorhinis* by multi-locus sequence typing and multiple-locus variable-number tandem-repeat analysis**, Vet. Microbiol., 249, 108836, 2020.

Horváth, G., Otrokocsi, L., Bekő, K., Baranyi, M., Kittel, Á., Fritz-Ruenes, P.A., Sperlágh, B.: **P2X7 receptors drive poly(I:C) induced autism-like behavior in mice**, J. Neurosci., 39, 2542-2561, 2019.

Kreizinger, Z., Sulyok, K.M., Bekő, K., Kovács, Á.B., Grózner, D., Felde, O., Marton, S., Bányai, K., Catania, S., Benčina, D., Gyuranecz, M.: **Genotyping *Mycoplasma synoviae*: Development of a multi-locus variable number of tandem-repeats analysis and comparison with current molecular typing methods**, Vet. Microbiol., 226, 41-49, 2018.

Sulyok, K.M., Bekő, K., Kreizinger, Z., Wehmann, E., Jerzsele, Á., Rónai, Z., Turcsányi, I., Makrai, L., Szeredi, L., Jánosi, S., Nagy, S.Á., Gyuranecz, M.: **Development of molecular methods for the rapid detection of antibiotic susceptibility of *Mycoplasma bovis***, Vet. Microbiol., 213, 47-57, 2018.

Hornok, S., Corduneanu, A., Kontschán, J., Bekő, K., Szőke, K., Görföl, T., Gyuranecz, M., Sándor, A.D.: **Analyses of separate and concatenated cox1 and 18S rRNA gene sequences indicate that the bat piroplasm *Babesia vesperuginis* is phylogenetically close to Cytauxzoon felis and the 'prototheilerid' *Babesia conradae***, Acta Vet. Hung., 66, 107-115, 2018.

Kreizinger, Z., Sulyok, K.M., Grózner, D., Bekő, K., Dán, Á., Szabó, Z., Gyuranecz, M.: **Development of mismatch amplification mutation assays for the differentiation of MS1 vaccine strain from wild-type *Mycoplasma* synoviae and MS-H vaccine strains**, PLoS One, 12, e0175969, 2017.

Beamer, E., Gölöncsér, F., Horváth, G., Bekő, K., Otrokocsi, L., Koványi, B., Sperlágh, B.: **Purinergic mechanisms in neuroinflammation: An update from molecules to behavior**, Neuropharmacology, 104, 94-104, 2016.

Acknowledgements

First of all, I would like to thank my supervisor, Miklós Gyuranecz for all the help and support he gave me and thank for his human attitude through the years.

I am also grateful to my present and previous colleagues, Kinga Görföl-Sulyok, Dénes Grózner, Áron Kovács, Veronika Hrivnák, Orsolya Saller, Cécile Yvon, Orsolya Stammné Felde, Dorottya Földi, Alexa Mitter and Enikő Wehmann for their kindness, helpfulness, and for the cheerful atmosphere. A special thank has to be granted to Zsuzsa Kreizinger for sharing her experiences in research and for her help and useful consultations at all times.

I would like to express my gratitude to my consultant, Krisztián Bányai, for his wise advices during my PhD work and for allowing whole genome sequencing. I would also like to thank Barbara Forró, Szilvia Marton, Eszter Kaszab and Krisztina Bali for their contribution to sequencing.

It is my honour to acknowledge Janet Bradbury, Salvatore Catania, Anneke Feberwee, Inna Lysnyansky, Chris Morrow and Martins Olaogun for their help in the sample collection and data interpretation.

Finally, I would like to thank my family for their love, support and encouragement.

This work was supported by the Lendület (LP2012-22), K_16 (119594), FK_17 (124019) and KKP19 (129751) grants.