Detailed final report

FK17_140346 (formerly FK17_124019): Determination of virulence factors in avian *Mycoplasma* species

I. Background

The economically most important pathogenic *Mycoplasma* species infecting poultry worldwide are *M. gallisepticum* and *M. synoviae.*[1] The infections are leading to respiratory diseases in chickens and turkeys, and *M. synoviae* may also cause infectious synovitis and eggshell-apex abnormalities.[2-5] Breeding flocks are usually free from mycoplasmosis but the infections occur worldwide in commercial broilers, turkeys and layers.[1] Control of *Mycoplasma* infections consists of three general aspects: eradication followed by prevention, vaccination or medication.[1] Where maintaining flocks free of mycoplasmosis is not feasible (e.g. in multi-age commercial layer farm) vaccination can be a viable option.[6] Live, attenuated vaccine strains are commercially available to control *M. gallisepticum* and *M. synoviae* infections. Understanding the mechanisms of attenuation of the *M. gallisepticum* and *M. synoviae* vaccine strains improves control and vigilance of the existing live vaccines, control of success of the vaccination and enables the development of new vaccine candidates. Medication with antimicrobial agents decreases the clinical symptoms of mycoplasmosis and consequently the economic losses, but it does not eradicate mycoplasmosis from the flocks.[1,7] The often prolonged antibiotic treatments of mycoplasmosis likely increase the emergence and spread of resistance to these agents. The alarming tendency of increase of resistance to antibiotics, especially with importance in human medicine, in pathogens infecting food producing animals demands new strategies in the fight against bacteria.[8-9]

II. Achievements of the project

During the five project years of the present grant we successfully performed whole genome sequence analyses and selected genes and genome regions (i) for the genetic characterisations of *M. gallisepticum* and *M. synoviae*, (ii) for the development of PCR based assays discriminating virulent, wild-type strains from live, attenuated vaccine strains, (iii) and which are potentially virulence-associated to submit for targeted inactivation studies. We established, validated and standardized the protocols and methods for the transformation studies and cell adherence and cell invasion tests. We managed to prepare vector plasmids for both examined mycoplasmas, and quality control transformant for *M. gallisepticum*, and executed preliminary examinations. We also initiated another approach for the examination of virulence of *M. synoviae* and for the contribution of vaccine development and control studies by establishing a novel experimental infection model.

The results related to the project were published in five publications at international, peer-reviewed journals, and presented at nine national and international conferences. In addition, 12 articles were published based on studies supported by but not closely related to the project.

1. Analyses of genetic characteristics of *Mycoplasma gallisepticum* and *M. synoviae*

Samples and sequence data

As limited information is available about virulence-associated determinants in avian *Mycoplasma* species, our primary objective was to determine, analyse and compare whole genome sequences of *M. gallisepticum* and *M. synoviae*, in order to identify putative virulence factors and to genetically characterise the isolates in our collection. First we compiled two collections of *M. gallisepticum* and *M. synoviae*, each containing a total of 200 clinical isolates, vaccine re-isolates and DNA samples. We analysed the whole genome sequences of 30 *M. gallisepticum* and 80 *M. synoviae* isolates, either determined previously or in this project. We used the next generation sequencing platforms IonTorrent (New England BioLabs, Hitchin, UK) and Illumina (NextSeq 500/550 High Output Kit, Illumina Inc., San Diego, USA), and mapped the gained short reads to the type strains of each species as reference in Geneious software (Biomatters Ltd., Auckland, New Zealand). Whole genome sequences were aligned and analysed in Geneious software and regions containing mutations (point mutation, insertions, deletions) and tandem repeat units were selected for further analyses.

Development of assays for the determination of relationships among the isolates

In order to reveal the relationships among our isolates, we developed novel typing assays based on the whole genome sequences and applied the methods on a wider selection of samples also. In case of *M. synoviae*, using the tandem repeat unit containing regions, we developed a seven-allele based multi-locus variable number of tandem-repeats analysis (MLVA). We also performed the genotyping of 86 strains and compared the results with data gained from sequence typing based on methods available in the literature (multi-locus sequence typing (MLST) and *vlhA* gene targeted sequencing). The study was published in the journal Veterinary Microbiology (Kreizinger et al., 2018). In case of *M. gallisepticum*, we developed a MLST assay using house-keeping genes containing multiple point mutations, and performed the sequence typing of 130 strains and isolates. The results were presented at the XXIst World Veterinary Poultry Association (WVPA) Conference (Bangkok, 2019), the European Mycoplasma Conference (London, 2019) and in the journal Veterinary Microbiology (Bekő et al., 2019).

We also developed a MLVA assay for the genotyping of *M. gallisepticum* examining the tandem repeat units in the whole genome sequences, and presented the method at the annual conference of the Hungarian Academy of Sciences and the University of Veterinary Medicine Budapest (Akadémiai Beszámolók, 2020). The developed assays are suitable for epidemiologic investigations during outbreaks also.

Development of assays for the discrimination of wild-type and vaccine strains

Multiple sequence analysis-based tests have been developed previously for the discrimination of the live vaccine strains from wild, virulent strains. However, due to the highly variable nature of mycoplasmas, these tests require constant improvement (the more samples tested the more exceptions - samples which show ambiguous or discrepant results - are detected), and the development of novel assays covering ever wider selection of samples are therefore justified. The comparison of the whole genome sequences of the live, attenuated vaccine strains and of isolates with known vaccination background in the present project revealed several point mutations in genes associated with virulence and in house-keeping genes also. We successfully used these point mutations as targets for PCR based assays for the differentiation of infected from vaccinated animals (DIVA). We developed assays targeting a single point mutation with competing primers in SYBRgreen system (mismatch-amplification mutation assays, MAMAs) and TaqMan type assays designed with primers and probes containing multiple point mutations. The developed assays are readily usable in diagnostics for the evaluation of effective vaccination. We also presented our assays distinguishing the *M. gallisepticum* live vaccine strains 6/85 (MSD Animal Health Inc.), ts11 (Bioproperties Pty. Ltd.), F (Ceva Santé Animale Inc. and Elanco Inc.) and K (K 5831, Vaxxinova BV.) from wild-type *M. gallisepticum* strains and the *M. synoviae* MS-H (VaxSafe MS-H, Bioproperties Pty. Ltd.) vaccine strain from wild-type *M. synoviae* strains in international journals and at conferences (Sulyok et al., Journal of Clinical Microbiology, 2019; Bekő et al., XXIst WVPA Conference, Thailand, 2019; Kreizinger et al., Poultry Days, Croatia, 2019; Bekő et al., Avian Pathology, 2020; Yvon et al., Akadémiai Beszámolók, Hungary, 2019; Földi et al., Akadémiai Beszámolók, Hungary, 2020).

Comparative genome analyses of MS-H vaccine strain and re-isolates

The comparative analysis of the MS-H vaccine strain and its virulent parent strain was among our objectives, and in parallel the analyses have been published by Zhu and co-workers.[10] The analyses revealed point mutations in predicted coding sequences also, and we collaborated in a study about the examination of stability of these mutations. Whole genome sequences of MS-H vaccine re-isolates (*in vitro* laboratory passaged strains, re-isolates from animal experiments and re-isolates from clinical samples) were determined and compared and the stability of seven point mutations were determined out of the 25 mutations from the original comparison. The results were published at the journal Microbiology Spectrum of the American Society for Microbiology (Klose et al., 2022).

Selection of putative virulence-associated genes

In order to identify putative virulent associated genes, favorably shared by both *M. gallisepticum* and *M. synoviae* we searched for predicted coding sequences containing high number of non-synonymous, variant nucleotides, then we used allXall National Center for Biotechnology Information (NCBI) BLASTp searches to encounter these sequences or their homologues in both species. We also developed an in-house statistical method based on the analysis of G+C contents, codon usage, amino acid usage and gene position for the analyses of possible horizontal gene transfer (HGT) events. We analysed the sequence data in correlation with the strains' MLST type and ranked the selected sequences according to the number of variant nucleotides and non-synonymous mutations. As a result, we selected 20 genes (elongation factors, ATP transporter binding proteins, proteins with known function in sugar metabolism, members of the oxidative phosphorylation pathway, hypothetical proteins) for targeted gene inactivation.

2. Transformation studies of *Mycoplasma gallisepticum* and *M. synoviae*

Determination of Minimum Inhibitory Concentration of the used antimicrobials

In order to identify the role of the selected target genes in the pathogenesis of *M. synoviae* and *M. gallisepticum*, the cell adherence and invasion capabilities of transformant strains with disrupted target genes was planned to be examined. We intended to use shuttle vectors (replicative plasmids containing a species specific replication origo (*oriC* gene), the fragment of the target gene and a selective marker) for the targeted gene inactivation of the mycoplasma strains. The tetracycline resistance gene, *tetM* was chosen as selective marker. Therefore, we determined the minimum inhibitory concentration (MIC) of tetracycline against a set of field isolates and the type strains of *M. gallisepticum* and *M. synoviae*. We determined the MICs of the mycoplasma strains to gentamicin also to confirm that the used isolates are susceptible to gentamicin in the cell invasion assays. We used the broth micro-dilution method for the MIC determination, the same method we used in our study (supported by the present project) in which we determined the MIC profile of Asian *M. gallisepticum* and *M. synoviae* strains (Kreizinger et al., 2020).

Establishment and standardization of laboratory conditions, techniques and protocols

We established the laboratory background and techniques for the transformation of the mycoplasma cells based on literature data and with the help of an experienced mycoplasmologist (Rohini Chopra-Dewasthaly) during a study trip at the Institute of Microbiology, University of Veterinary Medicine, Vienna. We received the plasmids pMM-21-7 (containing TetM region) and pGEM®-T Easy Vector (Promega Corporation, Madison, WI) for the construction of the replicative plasmids, and mastered the preparation and handling of plasmid vectors and competent cells. We also established and tested the immortal chicken embryonic fibroblast (CEF) cell line (UMNSAH/DF-1, ATCC CRL 12203, LGC Standards GmbH, Wesel, Germany) and mastered the preparation and handling of primary CEF cell lines. We used the primary CEF cell line in the cell adherence and cell invasion tests. Preliminary examinations of cell adherence of selected *M. synoviae* and *M. gallisepticum* field isolates and type strains were performed on solid mycoplasma medium by incubating mycoplasma colonies overlaid with CEF cells (washed and re-suspended in phosphate-buffered saline). Colonies were examined under light microscope (50x magnification) before and after incubation, and after washing away the unattached cells. Copy number of the mycoplasma strains inoculated on the agar plate, growth phase of mycoplasma colonies, copy number and amount of the CEF cells and washing process were standardized. Preliminary examinations of cell invasion capabilities of the selected strains were performed with primary CEF cells in gentamicin cell invasion assays. We inoculated the mycoplasma cultures into the solution of trypsinized primary CEF cells and after two hours of incubation, we treated the cell solutions with gentamicin or distilled water (used as control) for three hours. We inoculated mycoplasma agar plates with the cell solution at each step of the examination, and checked the plates for growth for 14 days. Intracellular mycoplasma cells could survive incubation with gentamicin only and grow on the solid medium, representing successful cell invasion. Copy number and growth phase of the mycoplasma strains, copy number of the CEF cells and concentration of gentamicin were standardized.

Transformation studies with M. synoviae

For the transformation of *M. synoviae* strains, initially we followed the protocol of Shahid et al. (2014).[11] We PCR amplified and ligated the *oriC* region of *M. synoviae* (2146 bp) into pGEM®-T Easy Vector (3015 bp) gaining the plasmid pMS-LoriC. We transformed competent *Escherichia coli* (JM109) cells by heat shock and successfully prepared a stock of the plasmid in the competent cells. We then digested the TetM region (5300 bp) from the plasmid pMM-21-7 and ligated it into the prepared pMS-LoriC plasmid and propagated the gained pMS-LoriC-Tet plasmid in competent cells. We determined the sequence of the TetM region by primer walking method, and prepared a shorter version of the TetM region containing only the tetracycline resistance coding gene (3243 bp, sTet), in order to constitute a smaller, hence more stable plasmid (pMS-LoriC-sTet) for the transformation of *M. synoviae*. We also constructed a modified version of the pMM-21-7 plasmid by replacing the *M. agalactiae* oriC region with the *M. synoviae* oriC region (pMS-mod-pMM). Preliminary trials for the transformation of selected *M. synoviae* strains were performed by electroporation with the prepared plasmids (pMS-LoriC-Tet, pMS-LoriC-sTet and pMS-mod-pMM), in order to check the transformants susceptibility to tetracycline, confirming the transformation of the strains. However, all attempts for the transformation of the selected *M. synoviae* strains failed. Transformation frequency of mycoplasmas by shuttle vectors was reported to be low (10^{-7} – 10^{-8} per colony forming units), therefore the standardization of copy number of target cells and the ratio of the plasmid and the cell culture are crucial. *In vitro* propagation of *M. synoviae* requires special nutrients, the average colour changing unit (CCU) determined in the media used in our laboratory for this species is 10^6 – 10^7 CCU/ml. In order to reach higher copy numbers in the broth cultures, we started optimise and develop distinct culture media.

Transformation studies with M. gallisepticum

For the transformation of *M. gallisepticum* strains, we followed the protocol of Lee et al. (2008).[12] We PCR amplified and ligated the oriC region of *M. gallisepticum* (180 bp) into pGEM®-T Easy Vector (3015 bp) gaining and propagating the plasmid pMG-LoriC. We then ligated the TetM region (5300 bp) from the plasmid pMM-21-7 or only the tetracycline resistance coding gene (3243 bp, sTet) into the prepared pMG-LoriC plasmid and propagated the gained pMG-LoriC-Tet and pMG-LoriC-sTet plasmids in competent cells. Preliminary trials for the transformation of selected *M. gallisepticum* strains were performed by electroporation with the prepared plasmids (pMG-LoriC-Tet and pMG-LoriC-sTet), and the transformants were inoculated on selective agar plates containing tetracycline. Stocks of the transformant *M. gallisepticum* colonies were prepared, and stability of tetracycline resistance was confirmed. The transformants preserved tetracycline resistance after even 20 *in vitro* passages. We successfully tested the newly established cloning technique and transformation process in a preliminary examination by the targeted inactivation of the *gapA* gene (reported to be responsible for cell adherence) of *M. gallisepticum*. We ligated two fragments of the *gapA* gene in the prepared shuttle vector (pMG-LoriC-Tet-gapA-LR), and transformed the selected *M. gallisepticum* field isolate by electroporation. We confirmed the transformation by selective culture and PCR, and propagated the gained transformant. The cell adherence capability of the transformant and the intact *M. gallisepticum* isolate was compared using primary CEF cells. The results were presented at the Akadémiai Beszámolók national conference (Földi et al., 2020) and the constructed shuttle vector

served as quality control in later trials. However, the generation of new *M. gallisepticum* transformants showing difference in cell adhesion or cell invasion capabilities failed. It is suggested, that mycoplasmas elicit their pathogenic effect on the host cells by the combined function of multiple genes. Probably the simultaneous inactivation of multiple genes by shuttle vectors could lead to the expected results, but this approach exceeded the time and costs limits of the project. Therefore, we launched another method for the contribution of control of mycoplasmosis.

3. Pathogenicity studies of *Mycoplasma synoviae* and establishment of a new infection model

Challenge studies and protocols are essential for vaccine development and for the understanding of the pathogenicity of bacteria. We aimed to find a virulent *M. synoviae* strain which could induce primary infection and generate explicit pathological changes alone, in order to establish a challenge model simulating natural infection, in which clear differences could be examined in the respiratory tract. Applying *M. synoviae* in a single infection model would be favourable in vaccine efficiency trials. Two *M. synoviae* strains containing possibly virulence-associated mutations in multiple genes (selected for targeted inactivation in previous years) were submitted for the examination of their pathogenicity *in vivo*. In previous experiments where pathogenicity of distinct strains of *M. synoviae* were compared additional vaccination of the experimental birds was applied to enhance incidence and severity of lesions caused by the agent. We performed the experimental infection of chickens with the selected two *M. synoviae* strains in the presence of infectious bronchitis virus (IBV) vaccine as a predisposing factor. In the second and third parts of the experiment, we repeated the infection with the strain of higher virulence without the predisposing factor in specific pathogen free (SPF) birds and in commercial layer type chickens (target group) also. We gained permission for the experiment from the Division of Food Chain Safety, Animal Health, Plant Protection and Soil Conservation of the Pest County Government Office (permission number: PE/EA/747-7/2021). The birds were infected by eye drops and individual nebularization of broth cultures of the selected *M. synoviae* strains on days 0 and 7 of the experiment. In the first part of the experiment, the birds were vaccinated with IBV strain intraocullary on day 0 also. Half of the treatment groups were euthanized and submitted to pathological examinations on day 14, and the other half of the treatment groups were euthanized on day 21. As pathological changes were more explicit on day 14, in the second and third parts of the experiment all birds were submitted to pathology on day 14. Colonization capabilities of the strains were compared based on PCR tests and isolation from the respiratory tract, cloaca and joints. Capability to trigger host immune response was examined based on serology tests (ELISA and RPA). Pathological and histopathological lesions were examined and compared especially in the trachea (upper, middle and lower regions), air sacs, lungs and joints. Pathology and histopathology changes were scored in the three examined sections of the trachea and in the air sacs, and thickness of the mucosa of the trachea at three sections (upper, middle and lower regions) were compared in the infected and no-treatment control birds. Significant changes were detected between the challenged groups and the negative control group when the experiment was performed in SPF birds, but not in the commercial layer-type birds. We presented the established infection model for SPF birds at the Akadémiai Beszámolók national conference (Kreizinger et al., 2021).

We continue to improve the established infection model to be suitable for the modelling of natural infection of commercial layer type chickens by adjusting the infectious dose and growth phase of the infectious agent. The standardization of the technique of infection of a whole group instead of individual nebularization is in progress as well. In addition, based on the *in vivo* differences between the pathogenicity of the two strains we can proceed with the design of shuttle vectors focusing on genes carrying virulence-associated mutations in both strains and on genes carrying mutations characteristic for the strain of higher virulence.

III. Differences between the results and primary objectives of the project

The main aim of the present project was to provide potential targets of *M. gallisepticum* and *M. synovie* for the development of new vaccines and antivirulence drugs, hence promoting the control of mycoplasmosis in poultry. The objectives involved the comparative analysis of the whole genomes of *M. synoviae* and *M. gallisepticum* in order to determine putative virulence factors responsible for the attenuation processes and shared by both species; targeted inactivation of the selected genes; and confirming the role of the putative virulence factors in the attenuation mechanisms of the vaccine strains or in the pathogenesis using *M. gallisepticum* and *M. synoviae* wild-type and gene disrupted strains in *in vitro* cell adherence and cell invasion tests. The original four-year long time-frame was extended to five project years due to the restrictions during the COVID-19 epidemic (Government Regulations No. 709/2020. (XII. 30.) and 27/2021. (I. 29.), and LVIII. law of year 2020., modified by CIV. law of year 2020.)

Despite the initial achievements during the standardization and trial of the freshly established methods, we faced difficulties regarding the production of *M. synoviae* transformants, and the generation of new *M. gallisepticum* transformants showing difference in cell adhesion or cell invasion capabilities. Although we set up

multiple strategies for the solution of the difficulties, we decided to launch another method also for the contribution of control of mycoplasmosis by improving vaccine development and the examination of pathogenicity.

Various experimental infection models have been described before either using *M. synoviae* as a single pathogenic agent, or challenging birds which are vaccinated simultaneously with infectious bronchitis virus (IBV) or Newcastle Disease virus (NDV) in order to enhance the incidence and severity of the lesions.[13-14] Single inoculation of *M. synoviae* intravenously or intra-articularly enabled the examination of joint lesions, while for the examination of both respiratory and articular lesions inoculation of the agent *in ovo*, or into footpad and as eyedrop proved to be suitable.[15-18] Experimental modelling of respiratory tract lesions was successful by direct inoculation of the air sac in turkeys, although failed in turkeys infected via oculonasal route or in chickens infected with *M. synoviae* intranasally or in aerosol.[19-23] Still, a challenge model simulating natural infection, in which clear differences could be examined in the respiratory tract with applying *M. synoviae* as a single infectious agent would be favourable, especially during vaccine licensures. Therefore, we aimed to find a virulent *M. synoviae* strain which could induce primary infection and generate explicit pathological changes alone, in order to establish a challenge model. We managed to find a *M. synoviae* strain containing possibly virulence-associated mutations in multiple genes which caused significantly more severe lesions in SPF birds compared to the negative control group, and we presented the established infection model for SPF birds at the Akadémiai Beszámolók national conference (Kreizinger et al., 2021). Adjustment of the developed model to be suitable for the experimental infection of chickens of all production type is our next goal, in order to provide a universal infection model for the examination of pathogenesis of *M. synoviae*, and for vaccine development and control studies.

IV. Publication list

1. Publications supported by and related to the project

Kreizinger Z, Sulyok KM, Bekő K, Kovács ÁB, 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. 2018;226:41-49.
Quartile ranking: Q1 (IF:2.791)

Bekő K, **Kreizinger Z**, Sulyok KM, Kovács ÁB, Grózner D, Catania S, Bradbury J, Lysnyansky I, Olaogun OM, Czanik B, Ellakany H, Gyuranecz M. Genotyping *Mycoplasma gallisepticum* by multilocus sequence typing. Vet Microbiol. 2019; 231:191-196.
Quartile ranking: Q1 (IF:2.791)

Sulyok KM, **Kreizinger Z**, Bekő K, Forró B, Marton S, Bányai K, Catania S, Ellis C, Bradbury J, Olaogun OM, Kovács ÁB, Cserép T, Gyuranecz M. Development of molecular methods for rapid differentiation of *Mycoplasma gallisepticum* vaccine strains from field isolates. J Clin Microbiol. 2019; 57. pii: e01084-18.
Quartile ranking: Q1 (IF:4.959)

Bekő K, Kovács ÁB, **Kreizinger Z**, Marton Sz, Bányai K, Bánáti L, Catania S, Bradbury JM, Lysnyansky I, Olaogun OM, Gyuranecz M. Development of mismatch amplification mutation assay (MAMA) for the rapid differentiation of *Mycoplasma gallisepticum* K vaccine strain from field isolates. Avian Pathol. 2020;49: 317-324.
Quartile ranking: Q1 (IF:2.338)

Klose SM, Olaogun OM, Disint JF, Shil P, Gyuranecz M, **Kreizinger Z**, Földi D, Catania S, Bottinelli M, Dall'Ora A, Feberwee A, van der Most M, Andrews DM, Underwood GJ, Morrow CJ, Noormohammadi AH, Marenda MS. Genomic diversity of a globally used, live attenuated mycoplasma vaccine. Microbiol. Spectrum. 2022;e02845-22.
Quartile ranking: Q1 (IF:7.171)

2. Conference abstracts supported by and related to the project

Bekő K, Kovács ÁB, **Kreizinger Z**, Grózner D, Gyuranecz M. Development of molecular methods for the rapid differentiation of *Mycoplasma gallisepticum* K 5831 vaccine strain from other live vaccine strains and field isolates. XXIst World Veterinary Poultry Association Conference, Bangkok, Thailand, 16-19 September, 2019.

Kreizinger Z, Bekő K, Sulyok KM, Catania S, Bradbury J, Lysnyansky I, Olaogun OM, Ellakany H, Kovács AB, Grózner D, Forró B, Marton S, Bányai K, Ellis C, Gyuranecz M. Genotyping and vaccine strain discrimination in *Mycoplasma gallisepticum*. European Mycoplasma Conference, London, United Kingdom, 18-19 March, 2019.

Kreizinger Z, Bekő K, Sulyok KM, Gyuranecz M. Development of novel molecular methods to improve the diagnostics and control of avian mycoplasmosis. Poultry Days 2019, Porec, Croatia, 8-11 May, 2019.

Yvon C, **Kreizinger Z**, Wehmann E, Dán Á, Gyuranecz M. Új, molekuláris biológiai módszer fejlesztése a vad-típusú *Mycoplasma synoviae* törzsek és az MS-H vakcina törzs elkülönítésére. Akadémiai Beszámolók, Budapest, 2020. 01. 21., 2019. évi 46. füzet.

Bekő K, Kovács ÁB, **Kreizinger Z**, Marton S, Bányai K, Bánáti L, Gyuranecz M. Molekuláris biológiai módszer fejlesztése a *Mycoplasma gallisepticum* K 5831 vakcinatörzs és vad *M. gallisepticum* törzsek elkülönítésére. Akadémiai Beszámolók, Budapest, 2020. 01. 21., 2019. évi 46. füzet.

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Felde O, Wehmann E, Gyuranecz M, **Kreizinger Z**. Gyors és költséghatékony genotipizáló rendszer fejlesztése Mycoplasma gallisepticum törzsek járványtani vizsgálatára. Akadémiai Beszámolók, Budapest, 2021.01.27., 2020. évi 47. füzet.

Földi D, Wehmann E, Chopra-Dewasthaly R, Kovács ÁB, Yvon C, Gyuranecz M, **Kreizinger Z**. Virulenciához kapcsolódó gének vizsgálata *Mycoplasma synoviae* és *M. gallisepticum* fajokban. Akadémiai Beszámolók, Budapest, 2021.01.27., 2020. évi 47. füzet.

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3. Publications supported by but not closely related to the project

Grózner D, Forró B, Kovács ÁB, Marton S, Bányai K, **Kreizinger Z**, Sulyok KM, Gyuranecz M. Complete genome sequences of three *Mycoplasma anserisalpingitis* (*Mycoplasma* sp. 1220) strains. Microbiol. Resour. Announc., 2019;8: e00985-19.
Quartile ranking: Q4 (IF: 0.383)

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Quartile ranking: Q1 (IF: 3.030)

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Quartile ranking: Q1 (IF: 3.030)

Földi D, Bekő K, Felde O, **Kreizinger Z**, Kovács ÁB, Tóth F, Bányai K, Kiss K, Biksi I, Gyuranecz M. Genotyping *Mycoplasma hyorhinis* by multi-locus sequence typing and multiple-locus variable-number tandem-repeat analysis. Vet. Microbiol. 2020;249: 108836.
Quartile ranking: Q1 (IF: 3.030)

Földi D, **Kreizinger Z**, Gyuranecz M: Sertések *Mycoplasma hyorhinis* okozta megbetegedése. Magyar Állatorvosok L. 2020;142: 515-524.
Quartile ranking: Q4 (IF: 0.107)

Gyuranecz M, Mitter A, Kovács ÁB, Grózner D, **Kreizinger Z**, Bali K, Bányai K, Morrow CJ. Isolation of *Mycoplasma anserisalpingitidis* from swan goose (*Anser cygnoides*) in China. BMC Vet. Res. 2020;16: 178.
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