PH.D. THESES

Fingerprinting of fowl pathogenic *Pasteurella multocida* and *Riemerella anatipestifer* with molecular epidemiology methods

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Introduction

Analysis of epidemiologically related cases has an increasingly important role in the diagnostics of infectious diseases, thus in case of food-borne zoonoses or nosocomial infections routine diagnostics include not only the establishment of correct etiological diagnosis, but also epidemiology analysis.

Typing methods addressing this aim (serotyping, phage typing) are used since long both in medical and veterinary diagnostics, but these do not always lead to sufficient results (e.g. when the pathogens to be typed belong to a single serotype or when it is serologically homogeneous). For this reason there is a need for modern, highly discriminatory (DNA- based) typing methods, which allow for analysis of epidemiological relations in case of practically all microbes.

Using of molecular typing methods provides means to discover infectious sources, early detection of outbreaks and for reliable tracing of infections, leading to improvement in the efficiency of medical and veterinary therapy, in preventive measures and in some cases considerable expenses may be saved.

These techniques has been established in Hungary regarding the diagnostics of human pathogens, though still confined to some specialized laboratories and has not yet become routine methods available to most diagnostic laboratories. However, domestic veterinary diagnostic facilities have scarcely or not at all applied molecular typing methods for fingerprinting bacteria. The gold standard pulsed-field gel electrophoresis has been practically unavailable for the veterinary and food safety diagnostic facilities. Present work reports the introduction of these methods to the domestic veterinary diagnostics, and demonstrates their applicability through the example of two important fowl-pathogenic bacteria, *Pasteurella multocida* and *Riemerella anatipestifer*.

Aims

1. To compare *Pasteurella multocida* strains isolated from fowl cholera outbreaks using PFGE and repetitive element based-PCR performing retrospective epidemiologic investigation

2. To develop a PCR assay capable of specific identification of *Riemerella anatipestifer* to facilitate correct identification of the species

3. To develop a PFGE protocol for typing *R. anatipestifer* and its application for characterization of Hungarian *R. anatipestifer* strains

Materials and methods

Isolates

We used isolates stored in the *P. multocida* culture collections of the former Veterinary Institute of Debrecen and the Avilab Ltd. All isolates originated from organ samples of moribund or dead animals, culture and identification techniques followed the accepted protocols. Antibiotic susceptibility testing was performed according to the standard disk diffusion method. Isolates were stored lyophilized or frozen at -80 °C until testing. We studies three series of recurrent fowl cholera outbreaks.

The first series occurred in goose stocks of five owners at a settlement in South-Eastern Hungary, between 2001 and 2003. Two owners were companies, while the three others were private individuals. We studies sixteen isolates from at least twelve outbreaks. The majority (eight) of outbreaks occurred in the stock of one of the companies (B), other owners experienced only one (C, D, E) or two (A) outbreaks.

The second outbreak series happened in turkey stocks of a large-scale breeder situated in different Eastern-Hungarian premises situated relatively far (even at cca. tens of kilometres distance) from each other between 1998 and 2003. Birds were always settled from the same hatchery, and animal exchange did not occur. The outbreaks affected six premises (A-F), we examined 27 isolates representing at least 18 outbreaks.

The third series occurred in goose stocks of a large-scale goose breeder situated in Eastern Hungary geographically far from each other in two sub-series. The first outbreaks occurred in 2003, out of this sub-series we had only two isolates from different premises. To prevent recurrences regular inactivated stock-specific vaccine was produced, containing the isolate from one of the outbreaks plus the two *P. multocida* strains most common in fowl cholera in Hungary. Using the vaccine prevented further outbreaks until 2006, when under unchanged vaccine and vaccination protocol severe outbreaks occurred consecutively in several different premises of the company leading to significant losses. Though outbreaks ceased in autumn 2006, sporadic losses continued to the end of the year. We examined nineteen isolates from these outbreaks as well as four epidemiologically unrelated (originating from stocks kept far from the region affected) isolates. We also compared these strains to representative isolates from the former two cases.

The R. anatipestifer isolates tested originated from the culture collections of the former Veterinary Institute of Debrecen and the Avilab Ltd. Isolates were cultured from different

Southern and South-Eastern Hungarian duck and goose stocks, from samples collected on the premises or from organs of animals sent to the former Institute for necropsy. Culture and identification techniques followed the accepted protocols. We studied 38 isolates altogether.

Methods

We applied species- and serogroup-specific PCR assays, PCR-based typing assays and PFGE. Bacterial DNA was extracted using heat treatment or Chelex-100 resin (Bio-Rad). Heat treatment was performed by incubation a loopful of a 24-hours-old pure broth culture (brain-heart infusion broth) in distilled water or TE-buffer (100 mM Tris, 10 mM EDTA) for 15 min at 98 °C. For resin purification we added 6% Chelex-100 to the suspension prepared as above, incubated for 20, 10 and 5 min at 65 °C, 100 °C and –20 °C, respectively. DNA from clinical samples used in testing of the *R. anatipestifer*-specific PCR was also extracted after homogenization using the resin method.

Confirmation of species identification and determination of the capsular type of *P. multocida* isolates was performed using a multiplex PCR assay described in the literature (Townsend és mtsai, J Clin Microbiol. 2001;39(3):924-9). We developed a novel PCR assay for identification of *R. anatipestifer*. We confirmed the identities of five *R. anatipestifer* isolates by means of 16S rDNA sequence determination. As an *in silico* GeneBank search (www.ncbi.nih.gov) did not yield sequences allowing for designing of a specific primer pair, we sequenced (Biomi Ltd., Gödöllő) a cca. 700 bp long common fragment present in enterobacterial repetitive intergenic consensus sequence-based (ERIC)-PCR patterns in case of five isolates representing five different patterns. The resulting 669 bp long consensus sequence was used for primer design. Using the designed primer pair (669AF: 5'-TTACCGACTGATTGCCTTCTAG-3'-669AR: 5'-AGAGGAAGACCGAGGACATC-3') we optimized the PCR assay. To test the reproducibility the assay run in different PCR apparatuses (Perkin-Elmer DNA Thermal Cycler; Bio-Rad GeneCycler; Bio-Rad MyCycler; Bio-Rad ICycler), as well as in another molecular diagnostic laboratory, in the Bacteriology Department of the former Central Veterinary Institute. We tested the specificity on 38 *R. anatipestifer* isolates and some important heterologous pathogens occurring in domestic fowl (*Mycoplasma synoviae*, *M. meleagridis*, *M. gallisepticum*, *M. iowae*, *Ornithobacterium rhinotracheale*, *Bordetella avium*, *Haemophilus paragallinarum*, *Escherichia coli*, *Salmonella* Enteritidis, *S.* Infantis, *S.* Typhimurium, *S.* Virchow, infectious bronchitis virus, *P. multocida*). In case of *P. multocida*, being the most problematic in differentiation from *R.*

anatipestifer, we tested the assay with sixteen reference strains representing the sixteen different Heddleston-serotypes. Though we originally intended to develop an assay for identification of *R. anatipestifer* from pure culture to complement or replace the frequently problematic traditional biochemical tests, we also tested the assay on thirteen clinical samples (lung-trachea, meningeal, nasal sinus samples and one nasal swab sample) from animals suspected to have *Riemerella* infection.

In case of *P. multocida* we used ERIC-PCR, pulsed-field gel electrophoresis (PFGE) and, in case of the first outbreak series, randomly amplified polymorphic DNA (RAPD) metod for fingerprinting of isolates. All methods were adapted using protocols described in the literature (Amonsin és mtsai. J Clin Microbiol. 2002;40(8):3025-31.; Chaslus-Dancla és mtsai. Vet Microbiol. 1996;52(1-2):91-102.; Lainson és mtsai. J Clin Microbiol. 2002;40(2):588-93.). For typing *R. anatipestifer* ERIC-PCR, *Salmonella* Enteritidis repetitive element based (SERE)-PCR and BOX-PCR as well as PFGE were adapted, optimized, tested and applied. In the PFGE analysis of *R. anatipestifer* we used the enzyme SmaI (Promega).

The resulting patterns of repetitive element based PCRs, RAPD and PFGE were analysed by the software Fingerprinting II (Bio-Rad). To quantify similarity we used the Dice coefficient, cluster analysis was performed by means of the UPGMA method. We also performed composite data analysis whenever possible.

Results and discussion

The first fowl cholera outbreak series

All sixteen isolates proved to be *P. multocida* with a capsular type A, and were uniformly susceptible to penicillin, ceftiofur, trimethoprim+sulfamethoxazole, neomycine and fluoroquinolones and resistant to nalidixic acid and tetracyclines. Thus isolates could not be distinguished neither with capsular type determination nor based on resistance patterns. RAPD patterns of isolates from stock of owner B were identical with the exception of isolate number TK2, which pattern was also identical to that of isolate TK3 isolated from the stock of owner C. Patterns of isolates TK1, TK4, TK5 and TK6 originating from stocks of owner A, D and E, respectively were also identical. ERIC-PCR and PFGE yielded highly similar results, though PFGE pattern of isolate TK2 was also identical to that of other isolates of owner B.

Thus, molecular typing grouped the isolates into two clusters. One included all isolates of owner B and the only isolate from the stock of owner C, while the other included the isolates from stocks of the other three owners. Based on thes results we retrospectively hypothesized the following network of epidemiologic relationships. Two different P. multocida strains caused outbreaks in the area, both with capsular type A. The first persisted from 2001 to 2002 in the stock of owner A (causing two outbreaks), then spread to the stocks of owner D and E. The other strain may have spread between stocks of owner B and C, and persisting in the stock of owner B caused several outbreaks. As to our knowledge animals were not transferred between stocks, transmission is likely to have occurred through humans or wild animals.

The second fowl cholera outbreak series

The first three isolates and the two isolates from premise F proved to possess capsular type A, while all other isolates were capsular type F. ERIC-PCR and PFGE concordantly revealed three groups of genetically related isolates. The first cluster contained the first three isolates (with capsular type A, all from 1998), the second consisted of the two isolates from the two outbreaks at premise F (with capsular type A, both from 2001), the third included all other isolates (with capsular type F, isolated between 2000 and 2003)

These results show that following the outbreaks in 1998, a new strain with capsular type F had been introduced. This strain spread to five out of the six affected premises and caused several outbreak recurrences, most probably by peristing in an unidentified reservoir (or reservoirs) and reaching the premises from this reservoir(s). The infection may have been transmitted with feed or with the contaminated surfaces in the transportation vehicles. The role of wild animals in transmission is unlikely due to the considerable geographical and temporal distances between outbreaks. On the sixth premise (F) two related but not identical strains (also unrelated to the strain isolates in 1998) caused two consecutive outbreaks. The strain with capsular type F did not reach the latter premise (F).

All isolates were susceptible to penicillin, ceftiofur, trimethoprim+sulfamethoxazole and tetracyclines, resistant to neomycine and nalidixic acid. The first eight isolates (N1-N8) and the two isolates from premise F (N15, N16) were also susceptible to fluoroquinolones, but other isolates (N9-N14 and N17-N27) were resistant. As these isolates were genetically related, we observed the development of acquired fluoroquinolne resistance in the strain.

The lesson to be learned from these two cases is that by discovering the faults in the outbreak prevention practice considerable losses can be avoided. The applied methods are capable of distinguishing introduction of a new strain from the recurrence caused by a persistent strain. In case of persistence the reservoir can be discovered with meticulous and extensive sampling, leading to identification of the point in the prevention process, which being insufficient allows the strain to persist.

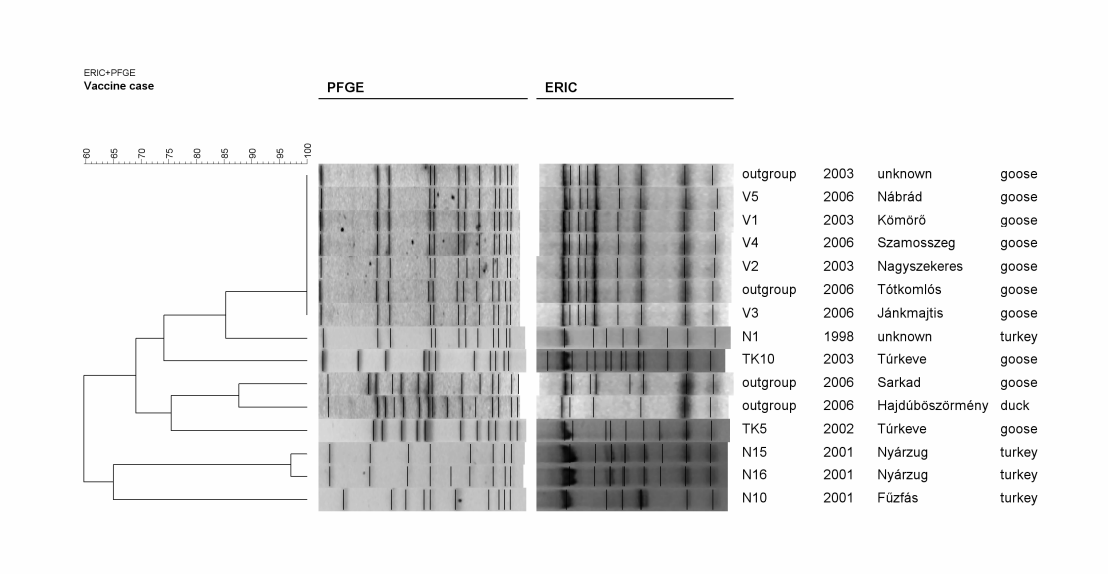
Therefore, if, in spite of the retrospective analysis of the above two cases, fingerprinting of the outbreak isolates had been performed after the first several outbreaks, by demonstrating persistence in the first case five and in the second twelve outbreaks could have been prevented. If we consider the significant losses due to the disease, these investigations could have prevented severe economic losses, thus their application would have been cost-efficient in spite of the relatively high direct costs.

The third fowl cholera outbreak series

All nineteen isolates from the series had capsular type A, and were indistinguishable both by ERIC-PCR and PFGE. Interestingly, two out of the epidemiologically unrelated isolates were also indistinguishable from isolates from the outbreak series, suggesting the existence of a particularly successful strain widely spread in the region. The strains connected

to the first two cases and the other two independent controls, however, were clearly distinct from the outbreak strain.

Dendrogram built using composit data analysis of ERIC-PCR and PFGE patterns of five isolates of the third outbreak series (V1-V5; the other fourteen isolates were also indistinguishable), of the four studied independent isolates (outgroup), and of isolates representing the different strains from series one (TK5, TK10) and two (N1, N10, N15, N16).



The outbreak series, similarly to the former two cases, was caused by the persistence of the outbreak strain for years. In the third series this persistence was not prevented by the regular use of stock specific vaccine (produced from one of the 2003 isolates). Though we demonstrated persistence, based on our data the direct cause of the recurrence of the outbreaks cannot clearly be decided. A possible explanation can be a novel, unaccustomed stress to the animals, but this is contradicted by more premises being affected and by the sporadic occurrences following the outbreaks for months. It is conceivable that the vaccine applied in 2006 was inappropriate (e.g. due to faults in storage), or errors in the vaccination protocol may also led to the vaccine failure.

The lesson conveyed by these investigations is twofold. On one hand, review of the vaccination protocol and control of the vaccine stock used are indispensable, since the case is clearly a vaccine failure. On the other hand, perhaps it is even more important to discover the site of persistence (asymptomatic animals, fomites in the batteries, wild animals in the premises) and the factors aiding pasteurellae to persist. Similarly to the second series, it would be important to find how the pathogen spread between premises. A probable transmission route could be the plucker team, whose role is suggested by the fact that the sequence of outbeaks followed their route between premises.

R. anatipestifer-specific PCR

Sequences of all five isolates undergone 16S rDNA sequencing showed 99% similarity to *R. anatipestifer* 16S rDNA sequences deposited in the GeneBank. Thus we confirmed that our biochemical tests capable of correctly identifying *R. anatipestifer*.

The primer pair designed (669AF-669AR, see above) performed optimally in the following PCR mix; in 50 µl end volume in 1x PCR buffer containing 2,5 mM $MgCl_2$, 2mM dNTP mix, 0,1 µg/µl BSA, 0,5-0,5 µM primers and 1U Taq polimerase (Fermentas). Optimal PCR program was as follows; 94 °C 4 min initial denaturation, 35 cycles of 95°C 1 min, 55°C 1 min, 72 °C 1 min and 72 °C 7 min final extension. This mix and program worked well in all PCR systems applied.

All tested *R. anatipestifer* isolates yielded the expected 546 bp long product, while the heterologous pathogens including all sixteen *P. multocida* somatic serotypes wee negative. PCR results were fully concordant with culture results in case of organ samples, suggesting the applicability of the assay directly on samples in order to demonstrate *R. anatipestifer*, but this issue clearly needs confirmation.

As identification of the pathogen with traditional tests may be problematic and sometimes unequivocal, the assay described providing more precise and faster means to identify *R. anatipestifer*, will expectedly aid in the diagnosis of the disease.

Typing of *Riemerella anatipestifer* isolates

Out of the three repetitive element based PCRs (ERIC-PCR, SERE-PCR and BOX-PCR) ERIC-PCR yielded well evaluable fingerprints, while the other two did not, therefore we stopped the optimization of the latter two PCRs.

Out of the 38 isolates examined ERIC-PCR demonstrated six related groups, a pair of related isolates and five isolates independent of groups, while PFGE grouped 23 isolates into five different groups, pointed out three related pairs and found eight independent isolates. Groups were not homogeneous regarding either the site or the year of isolation, indicating the simultaneous presence of several unrelated strains, which can spread between premises to relatively long distances. These endemic clones are probably carried and transmitted by wild birds.

Summary of results

1. We proved that all three fowl cholera outbreak series originated in persistence of pasteurellae, in the third case we also examined the possible causes of vaccine failure. Real-time (non-retrospective) application of the methods would have provided key information to prevent a significant number of outbreaks, thus the work demonstrates their potential application in veterinary diagnostics.

2. We demonstrated that the strain involved in the second outbreak series acquired secondary fluoroquinolone resistance.

3. Using a common ERIC-PCR fragment as a starting point, we developed, optimized and tested a *R. anatipestifer*-specific PCR assay. This assay is capable of correctly identify the bacterium, moreover, according to preliminary results it may be applicable for direct demonstration of the pathogen from different samples.

4. We optimized an ERIC-PCR and a PFGE protocol for the typing of *R. anatipestifer* based on the methods successfully applied to *P. multocida*. Using these methods we performed a survey on isolates from Southeastern Hungary, and concluded that groups were not homogeneous regarding either the site or the year of isolation, indicating an endemic presence of the pathogen.

5. The work demonstrates an application of molecular typing methods in veterinary diagnostics, and may serve as a model for further application fields in connection with veterinary medicine.

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Publications

Publications used in the dissertation

1. **G. Kardos**, I. Turcsányi, A. Bistyák, J. Nagy, I. Kiss: Application of molecular epidemiology methods to study breakthrough outbreaks occurring in vaccine-protected poultry stocks. Submitted for publication.

2. I. Kiss, **G. Kardos**, J. Nagy, M. Tenk and E. lvanics: DNA-fingerprinting of Riemerella anatipestifer isolates. Vet. Rec. 2007. 160: 26-28.
   IF(2005): 1,017 times cited: 0

3. **G. Kardos**, J. Nagy, M. Antal, A. Bistyák, M. Tenk, I. Kiss: Development of a novel diagnostic PCR assay specific for *Riemerella anatipestifer*. Lett. Appl. Microbiol. 2007. 44: 145-148.
   IF(2005): 1,440 times cited: 0

4. **G. Kardos,** I. Kiss: Molecular epidemiology investigation of outbreaks of fowl cholera in geographically related poultry flocks. J Clin Microbiol. 2005, 43(6): 2959-61.
   IF(2005): 3,537 times cited: 2

Other publications

1. **G. Kardos**, T. Farkas, M. Antal, N. Nógrády, I. Kiss: Novel PCR assay for identification of Salmonella enterica serovar Infantis. Lett Appl Microbiol. Accepted for publication.

2. G. Sóczó, **G. Kardos**, P. M. McNicholas, E. Falusi, L. Gergely, L. Majoros: Posaconazole susceptibility testing against Candida species: Comparison of broth microdilution and Etest methods. Mycoses. 2007. 50 (3):178-182
   IF(2005): 0.765 times cited: 0

3. A. Bistyák, S. Kecskeméti, R. Glávits, I. Tischler, S. T. Nagy, **G. Kardos**, I. Kiss: Pacheco's disease in a hungarian zoo bird population: a case report. Acta Vet. Hung. 2007. 55 (2): 213-218
   IF(2005): 0,530 times cited: 0

4. T. Farkas, M. Antal, L. Sámi, P. Germán, S. Kecskeméti, **G. Kardos**, S. Belák, I. Kiss: Rapid and simultaneous detection of avian influenza and Newcastle disease viruses by duplex polymerase chain reaction assay. Zoonoses Public Health. 2007 54: 38–43
   IF(2005): 1,505 times cited: 0

5. L. Majoros, I. Szegedi, **G. Kardos**, Cs. Erdész, J. Kónya, Cs. Kiss: Slow response of invasive *Candida krusei* infection to amphotericin B in a clinical time-kill study. Eur. J. Clin. Microbiol. Infect. Dis. 2006. 25 (12): 803-806
   IF(2005): 2,061 times cited: 0

6. I. Kiss, P. Germán, L. Sámi, M. Antal, T. Farkas, **G. Kardos**, S. Kecskeméti, Á. Dán, S. Belák: Application of real-time RT-PCR utilising lux (light upon extension) fluorogenic primer for the rapid detection of avian influenza viruses. Acta Vet. Hung. 2006. 54 (4): 525-533
   IF(2005): 0,530 times cited: 0

7. A. Csagola, S. Kecskemeti, **G. Kardos**, I. Kiss and T. Tuboly: Genetic characterization of type 2 porcine circoviruses detected in Hungarian wild boars. Arch. Virol. 2006, 151(3): 495-507
   IF(2005): 1,819 times cited: 0

8. L. Majoros, **G. Kardos**, B. Szabó, and M. Sipiczki: Caspofungin Susceptibility Testing of Candida inconspicua: Correlation of Different Methods with the Minimal Fungicidal Concentration. Antimicrob. Agents Chemother. 2005, 49(8): 3486-8.
   IF(2005): 4,379 times cited: 1

9. Majoros, L., **G. Kardos**, P. Feiszt, B. Szabó: Efficacy of amphotericin B and flucytosine against fluconazole-resistant Candida inconspicua clinical isolates. J. Antimicrob. Chemother. 2005, 56(1): 253-4.
   IF(2005): 3,886 times cited: 1

10. Szládek, G., A. Juhasz., **G. Kardos**, K. Szőke, T. Major, I. Sziklai, I. Tar, I. Márton, J. Kónya, L. Gergely, K. Szarka: High co-prevalence of genogroup 1 TT virus and human papillomavirus is associated with poor clinical outcome of laryngeal carcinoma. J Clin Pathol. 2005, 58(4):402-5
    IF(2005): 2,170 times cited: 3

11. Majoros, L., **G. Kardos**, B. Szabó, M. Kovács, A. Maráz: Fluconazole susceptibility testing of Candida inconspicua clinical isolates: comparison of four methods. J Antimicrob Chemother. 2005, 55(2):275-6
    IF(2005): 3,886 times cited: 2

12. Kiss, I., **G. Kardos**, L.H. Farkas: DNA fingerprinting techniques in bacterial strain typing. Hungarian Veterinary Journal 2004, 126(3):161-6 (in Hungarian)
    IF(2004): 0,158 times cited: 1

13. Majoros, L., **G. Kardos**, Á. Belák, A. Maráz, L. Asztalos, E. Csánky, Z. Barta, B. Szabó: Restriction enzyme analysis of ribosomal DNA shows that Candida inconspicua clinical isolates can be misidentified as Candida norvegensis with traditional diagnostic procedures. J Clin Microbiol. 2003, 41(11):5250-3.
    IF(2003): 3,489 times cited: 4

14. Majoros L, **Kardos G**, Pócsi I, Szabó B.: Distribution and susceptibility of Candida species isolated in the Medical University of Debrecen. Acta Microbiol Immunol Hung. 2002, 49(2-3):351-61
    IF: - times cited: 2

Referenced abstracts

1. (Abstr.) **Kardos G.**, Sóczó G., McNicholas P., Falusi E., Hegedűs J., Majoros L.: Time-kill studies show that posaconazole is fungicidal against some Candida species. The 16th Congress of the International Society for Human and Animal Mycology, Paris, France, 2006. Poster No P-0089.
   IF: 1.46 times cited: 0

2. (Abstr) Tóth B., Falusi E., Miszti C., Borbély Á., **Kardos G.**, Majoros L. Comparison of MICRONAUT-Candida, a new yeast identification system to API ID32C. The 16th Congress of the International Society for Human and Animal Mycology, Paris, France, 2006. Poster No P-0428.
   IF: 1.46 times cited: 0

3. (Abstr) Sóczó G., McNicholas P., **Kardos G.**, Falusi E., Majoros L. Further studies on fungicidal activities of posaconazole 46th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington D.C., Abstr. A-2151. 2006 (poster)
   IF: 4,379 times cited: 0

4. (Abstr) Majoros L., **Kardos G.**, McNicholas P.: Determination of minimal inhibitory and minimal fungicidal concentrations of posaconazole against four human pathogenic Candida Species. Abstr. M-1611. 45th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington D.C., 2005 (poszter)
   IF: 4,216 times cited: 0

5. (Abstr) **G. Kardos** and L. Majoros: Comparison of colonizing ability of Candida albicans and Candida dubliniensis in murine model. 2002. Abstr. Mycoses, 45, Suppl. 2., p. 30
   IF(2002): 0,545 times cited: 0

Oral and poster presentations

1. L. Majoros, C. Miszti, **G. Kardos**, I. Andirkó, B. Szabó: Repeated positive Candida cultures from male urine samples, 1st Joint meeting of the Slovenian Society for Microbiology and the Hungarian Society for Microbiology, Keszthely, 2000. (előadás)

2. L. Majoros, C. Miszti, **G. Kardos**, I. Andirkó, B. Szabó: Fungal sepsis in newborns and childhood. 1st Joint meeting of the Slovenian Society for Microbiology and the Hungarian Society for Microbiology, Keszthely, 2000. (előadás)

3. **Kardos G.**, Majoros L., Erdész Cs.: Egy ritka patogén, Candida dubliniensis izolálása és azonosítása. Ph.D.konferencia, Debrecen, 2001. (előadás)

4. Erdész Cs., **Kardos G.**, Miszti C., Majoros L., Szikszay E., Szegedi I., Kiss Cs.: Szisztémás gombafertőzések neutropéniás betegekben – korszerű diagnosztika és kezelés. Magyar Gyermekaneszteziológiai és Intenzív Terápiás Társaság Országos Tudományos Ülése, Debrecen, 2001. (előadás)

5. Majoros L., **Kardos G.**, Miszti C., Szabó B.: Vesetranszplantált betegek vizeletéből kitenyésztett kórokozók species szerinti megoszlása és rezisztenciája. Magyar Kemoterápiás Társaság Közgyűlése, Hajdúszoboszló, 2001. (előadás)

6. Majoros L., Miszti C., **Kardos G.**, Szabó B.: A cefepime, az imipenem, a meropenem és a piperacillin/tazobactam in vitro érzékenységének összehasonlító vizsgálata vegyes klinikai anyagból gram-negatív baktériumok esetén Magyar Kemoterápiás Társaság Közgyűlése, Hajdúszoboszló, 2001. (előadás)

7. Majoros L., **Kardos G.**, Miszti C., Szabó B.: Klinikai anyagból izolált sarjadzó gombák species szintű eloszlása és antimikotikumok iránti érzékenysége. Magyar Mikrobiológiai Társaság Jubileumi Nagygyűlése, Balatonfüred, 2001. (előadás)

8. **Kardos G.**, Majoros L., Erdész Cs., Miszti C., Szabó B.: Egy ritka patogén, Candida dubliniensis izolálása és azonosítása. Magyar Mikrobiológiai Társaság Jubileumi Nagygyűlése, Balatonfüred, 2001. (előadás)

9. **Kardos G.**, Majoros L.: Candida albicans és Candida dubliniensis kolonizációs képességének összehasonlítása egér modellben. Ph.D. konferencia, Debrecen, 2002. (előadás)

10. **Kardos G.**, Majoros L.: Candida albicans és Candida dubliniensis kolonizációs képességének összehasonlítása egér modellben. II. Magyar Mikológiai Konferencia, Szeged, 2002. (poszter)

11. Majoros L., **Kardos G.**, Pócsi I., Szabó B., Miszti C. (2002) Sarjadzó gombák kóroki szerepe az egyes testtájak fertőzései esetén. Ritkábban izolálható Candida speciesek klinikai jelentősége. II. Magyar Mikológiai konferencia, Szeged, 2002. (előadás).

12. **G. Kardos** and L. Majoros: Comparison of colonizing ability of Candida albicans and Candida dubliniensis in murine model. 8th Congress of the European Confederation of Medical Mycology, Budapest, 2002. (poszter)

13. L. Majoros, **G. Kardos**, C. Miszti, J. Szabó, B. Szabó: Changes in species distribution of yeasts isolated in the University of Debrecen during a six year period. (előadás) 23rd International Specialised Symposium on Yeasts, Budapest, 2003

14. **G. Kardos**, A. Maráz, Á. Belák, M. Sápy, G. Reményi, L. Majoros and B. Szabó: Comparison of different methods for fluconazole susceptibility testing of *Candida inconspicua* (poszter). 23rd International Specialised Symposium on Yeasts, Budapest, 2003.

15. M. Kovács, Á. Belák, L. Majoros, **G. Kardos**, A. Maráz: Molecular characterization of Candida inconspicua clinical isolates (poszter) 23rd International Specialised Symposium on Yeasts, Budapest, 2003.

16. G. Szládek, A. Juhász, **G. Kardos**, T. Major, I. Tar, L. Gergely, K. Szarka: Co-infection with TTV may increase the oncogenic effects of HPV infection in laryngeal carcinomas. 2nd Joint meeting of the Slovenian Society for Microbiology and the Hungarian Society for Microbiology, Balatonfüred, 2003. (előadás)

17. Majoros L., **Kardos G.**, Miszti C., Szabó J., and Szabó., B. (2003) In vivo investigation of fluconazole resistance in case of Candida inconspicua. In program and Abstracts of the

14rd International Congress of the Hungarian Society for Microbiology, Budapest, 2003 Abstract M-13.

18. **G. Kardos**, L. Majoros: Comparison of in vitro development of fluconazole resistance in Candida albicans and Candida dubliniensis. 2nd Joint meeting of the Slovenian Society for Microbiology and the Hungarian Society for Microbiology, Balatonfüred, 2003. (előadás)

19. **G. Kardos**, I. Kiss: Molecular fingerprinting of avian Pasteurella multocida isolates from Hungary. 2nd Joint meeting of the Slovenian Society for Microbiology and the Hungarian Society for Microbiology, Balatonfüred, 2003. (előadás)

20. **Kardos G.**, Kiss I.: Baromfikolera járványok molekuláris epidemiológiai vizsgálata. MTA Állatorvosi Osztályának Gyűlése, Budapest, 2004. (előadás)

21. **Kardos G.**, Kiss I.: Különböző eredetű Salmonella Enteritidis törzsek DNS-ujjlenyomat vizsgálata. Magyar Mikrobiológiai Társaság Nagygyűlése, Keszthely, 2004. (előadás)

22. **Kardos G.**, Majoros L.: Különböző testtájakról 2000-2003 között izolált Candida törzsek amphotericin B és flukonazol iránti érzékenységének összehasonlító vizsgálata Magyar Mikrobiológiai Társaság Nagygyűlése, Keszthely, 2004. (előadás)

23. **Kardos G**., Pappné Falusi E., Bódi T., Szabó B., Majoros L.: Amphotericin B, vorikonazol és 5-fluorocitozin in vitro aktivitása *Candida inconspicua* ellen. III. Magyar Mikológiai Konferencia, Mátraháza, 2005. (poszter)

24. Majoros L., **Kardos G**., Pappné Falusi E., Hegedűs J., Szabó B., Sipiczki M.: Candida inconspicua klinikai izolátumok érzékenysége caspofungin iránt. III. Magyar Mikológiai Konferencia, Mátraháza, 2005. (előadás)

25. **G. Kardos**, L. Majoros: Genetic diversity of *Candida albicans* strains isolated from oral, urine and blood samples. 1st Central European Forum for Microbiology, Keszthely, Hungary, 2005. (előadás)

26. **G. Kardos**, M. Antal, I. Tóth, I. Kiss, B. Nagy: Genetically related clusters among hungarian *Escherichia coli* O157 EHEC and EPEC strains. 1st Central European Forum for Microbiology, Keszthely, Hungary, 2005. (előadás)

27. L. Majoros, **G. Kardos**, P. McNicholas: Determination of Minimal Inhibitory and Minimal Fungicidal Concentrations of Posaconazole against Four Human Pathogenic Candida Species. Abstr. M-1611. 45th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington D.C., 2005 (poszter)

28. **Kardos G.**, Farkas T., Kiss I.: Ételfertőzést okozó baktériumok előfordulásának vizsgálata csirkeállományokban. MTA Állatorvosi Osztályának Gyűlése, Budapest, 2006. (előadás)

29. **G. Kardos**, M. Antal, I. Tóth, I. Kiss, B. Nagy: Discrimination between E. coli O157 isolates using PCR-mediated fingerprinting based on SER element. 16th European Congress of Clinical Microbiology and Infectious Diseases, Nice, France, 2006. (poszter)

30. **Kardos G.**, Antal M., Bistyák A., Turcsányi I., Kiss I.: Baktériumok nyomon követése DNS-ujjlenyomat technikákkal: felmérő vizsgálatok és esettanulmányok. 14. Derzsy Napok, Eger, 2006. (előadás)

31. Tóth B., Falusi E., Miszti C., Borbély Á., **Kardos G.**, Majoros L. Sarjadzó gombák azonosításának összehasonlító vizsgálata Micronaut-Candida és API ID 32C panelek segítségével. Magyar Mikrobiológiai Társaság Nagygyűlése, Keszthely, 2006. (előadás)

32. Sóczó G., **Kardos G.**, Falusi E., Miszti C., McNicholas P., Majoros L. Posaconazol fungicid hatásának a vizsgálata az idő-ölés görbék segítségével különböző Candida fajok ellen. A Magyar Mikrobiológiai Társaság Nagygyűlése, Keszthely, 2006. (előadás)

33. **G. Kardos**, T. Farkas T., Nógrády N., Kiss I.2006 MMT (Keszthely, okt17-20): Development and optimization of a PCR assay specific to Salmonella enterica serovar Infantis. Magyar Mikrobiológiai Társaság Nagygyűlése, Keszthely, 2006. (előadás)

34. **G. Kardos**, L. Fodor, I. Kiss: A pulsed-field gel electrophoresis protocol for typing of Hungarian Histophilus somni isolates. A Magyar Mikrobiológiai Társaság Nagygyűlése, Keszthely, 2006. (előadás)

35. **G. Kardos**, T. Farkas, A. Bistyák, I. Turcsányi, I. Kiss: Isolation of Arcobacter cryaerophilus and A. skirrowi from Hungarian broiler samples. A Magyar Mikrobiológiai Társaság Nagygyűlése, Keszthely, 2006. (előadás)

36. T. Farkas, **G. Kardos**, A. Bistyák, I. Turcsányi, I. Kiss: Detection of potential food-borne pathogens from stool samples of broiler chickens. A Magyar Mikrobiológiai Társaság Nagygyűlése, Keszthely, 2006. (előadás)

37. Ungvári A., **Kardos G**., Nógrády N., Turcsányi I., Pászti J., Spinu M., Kiss I.: Csirke eredetű Salmonella enterica serovar Infantis törzsek molekuláris epidemiológiai vizsgálata. MTA Állatorvosi Osztályának Gyűlése, Budapest, 2006. (előadás)

38. **Kardos G**., Kiss I.: Csatlakozás a Pulsenet-Europe nemzetközi molekuláris járványtani figyelőhálózathoz. MTA Állatorvosi Osztályának Gyűlése, Budapest, 2006. (előadás)

39. **Kardos G.**, Nagy J., Bistyák A., Kiss I.: Telepspecifikus vakcinával védett lúdállományokban ismétlődő áttöréses baromfikolera járványok molekuláris járványtani vizsgálata. MTA Állatorvosi Osztályának Gyűlése, Budapest, 2006. (előadás)

40. **Kardos G.**, Mészáros J., Galántai Zs., Turcsányi I., Bistyák A., Farkas T., Juhász Á., Damjanova I., Pászti J., Kiss I.: Termotoleráns Campylobacter és Arcobacter fajok előfordulásának vizsgálata csirke eredetű mintákban és csirke eredetű élelmiszerben. MTA Állatorvosi Osztályának Gyűlése, Budapest, 2006. (előadás)

41. **Kardos G.**, Sóczó G., McNicholas P., Falusi E., Hegedűs J., Majoros L.: Time-kill studies show that posaconazole is fungicidal against some Candida species The 16th Congress of the International Society for Human and Animal Mycology, Paris, France, 2006 (poszter)

42. Sóczó G., **Kardos G.**, Gesztelyi R., Majoros L. In vitro and in vivo studies with C. tropicalis isolates, exhibiting paradoxical growth in the presence high concentration of caspofungin 8th Europen Congress of Chemotherapy and Infection, Budapest, Hungary, 2006 (poszter)

43. **Kardos G.**, Sóczó G., Gesztelyi R., Majoros L. Killing activity of amphotericin B against Candida krusei cannot be predicted by standard susceptibility testing: An in vitro comparison of methods 8th Europen Congress of Chemotherapy and Infection, Budapest, Hungary, 2006. (poszter)

44. **Kardos G.**, Mészáros J., Galántai Zs., Turcsányi I., Bistyák A., Juhász Á., Damjanova I., Pászti J., Kiss I. Prevalence of thermotolerant Campylobacters in broilers, eggs, chicken abattoir and human samples in a Hungarian county. 17th European Congress of Clinical Microbiology and Infectious Diseases, Muenchen, Germany, 2007. (poszter)

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