Szent István University
Postgraduate School of Veterinary Science

Comparative pheno- and genotypic analysis of *Bordetella bronchiseptica* strains, with special regard to bacterial virulence factors and host adaptation

Brief Summary of PhD Thesis

Bernadett Khayer

2015

Supervisor:

Tibor Magyar, DSc
Institute for Veterinary Medical Research,
Centre for Agricultural Research,
Hungarian Academy of Sciences

Introduction

Bordetella bronchiseptica is a widespread Gram-negative bacterium with a broad host range. It plays important role in atrophic rhinitis in pigs and canine kennel cough, but *B. bronchiseptica* can cause respiratory diseases of rabbits, cats, guinea pigs, and it has been isolated from several domestic, laboratory and wild mammals, too. The infection of *B. bronchiseptica* usually does not generate severe clinical symptoms or higher rate of lethality, but it can cause huge economic losses for breeders and livestock farmers. Infection of pet animals − kept individually or in small groups −, could be important since the increasing number of human illnesses indicating the zoonotic hazard of *B. bronchiseptica*.

Major virulence factors of *B. bronchiseptica* are adhesins involved in adhesion to and colonisation of the respiratory mucosa of the host, and toxins, which participate the shaping of the typical symptoms of diseases. The actual virulence of *B. bronchiseptica* is controlled by BvgAS, a two component regulatory system, whereby expression of the genes. BvgAS can activate or inhibit the quantity and function of several enzymes, outer membrane proteins and toxins - in response to specific environmental signals, like temperature or sulphate-ion.

Aims of this study were to characterise *B. bronchiseptica* strains isolated from different host species and various geographic regions by pheno- and genotypic methods, and to detect possible differences and phylogenetic relations between strains. We analysed important virulence associated factors like motility, adhesins and toxins. We tried to reveal the host adaptation of these virulence factors, and to examine the variability of these factors in time and space.

Despite the fact that several articles can be found about special examination of *B. bronchiseptica*, only few of them contain research about the comparison of a large numbers of strains from various host species. Investigation of porcine *B. bronchiseptica* strains is traditional in Hungary. However, this is the first effort for characterisation and comparison of the strains from different hosts. Our results may yield new information not only for fundamental research, but they could be important for diagnostics, targeted treatment and also for vaccine development.

Materials and methods

B. bronchiseptica strains

One hundred and sixty-four fresh isolates and strains from the strain-collection of IVMR CAR HAS were used. These *B. bronchiseptica* strains originated from different geographic regions (Hungary and other countries) and various host species (pig, dog, rabbit, guinea pig, horse, koala, turkey and human). The identification of the bacteria was carried out by colony morphology, biochemical (urease, nitrate, indole, glucose, lactose and saccharose test), and molecular (species-specific PCR) assays. Strains were cultured on Columbia agar plates supplemented with 5% sheep blood under aerobic conditions at 37°C for 24 h. Bacteria were stored at -70°C in skim milk suspension.

Phenotypic characterisation of *B. bronchiseptica*

Haemolytic assay

Haemolysis was observed on six differently composed blood agar media (5 or 15% sheep or horse blood and pH 6.8 or 7.2). Strains were incubated at 37°C for 24 h in each case.

Urea-utilisation assays

Since the activity of the genes responsible for ureum-utilisation is increased at low temperature or in the presence of $MgSO_4$, the traditional biochemical test was done at 24ºC. Dependence of the quantity of $MgSO_4$ was examined in different Luria-Bertani (LB) broth, containing 0, 40, 100 and 150 mM $MgSO_4$. A colony of fresh bacteria was suspended in these broths, and after incubation (37°C, 24 h) traditional ureum broth was inoculated with 30 µL of the suspension. The results were evaluated after incubation at 37°Cfor 24 h. The ability of urea-utilisation was tested with Diatabs diagnostic tablets and API 20 NE plate according to the manufacturers' recommendation.

Haemagglutination test

Rocked tile haemagglutination was performed to investigate the adhesion of *B. bronchiseptica* strains using red blood cells from cattle, pig, horse, sheep, dog and chicken as well as from human type A, B and 0 blood. Ten percent red blood cell (RBC) suspension was made with PBS, and 20 µL of these suspensions was mixed gently with a fresh colony of bacteria. The results of the reactions were detected after one minute and evaluated on a five-point scale (0-4). Level 0 meant the lack of haemagglutination, while level 4 indicated the complete haemagglutination. The assay was carried out at room temperature, and repeated at least three times at one occasion.

Motility assay

The motility of the strains was examined on semisolid, 0.4% agar containing Luria-Bertani (LB) and 40 mM $MgSO_4$-enriched LB (LB+$MgSO_4$) agar plates, which were incubated at 24ºC or 37ºC. Motility zones were measured in millimeters after 24 h incubation at each arrangement (LB-37; LB-24; LB+$MgSO_4$-37; LB+$MgSO_4$-24).

Antibiotic susceptibility test

Antibiotic resistance of *B. bronchiseptica* was tested with strains isolated from rabbits (n=40) and pigs (n=15). The antibiotic susceptibility of the *B. bronchiseptica* strains was determined by Kirby-Bauer disk diffusion method. A fresh colony of the bacteria was suspended into 5 mL saline, the density of the suspension was adjusted to 0.5 McFarland, and then the suspension was inoculated onto Müller-Hinton agar plate with sterile swabs. Fourteen different antibiotic disks were applied. The results were evaluated on the basis of the offered zone diameters by Clinical and Laboratory Standards Institute and National Food Chain Safety Office.

Genotypic characterisation of *B. bronchiseptica*

Polymerase chain reaction

Primers for molecular identification (Bb PCR) and amplification of specific genes (urease [*ure*C], dermonecrotic toxin [*dnt*], fimbria [*fim*A], flagellin [*fla*A], adenylate cyclase-haemolysin toxin [*cya*A] and peptide transport protein [*ptp*]) were chosen from the literature or designed by OLIGO 5 Primer Analysis Software. DNA templates were prepared by the boiling method from fresh colonies, the procedure was done with ESCO Swift Mini equipment. The amplification products were analysed in 1.5% (wt/vol) SeaKem agarose with 1×TBE buffer by electrophoresis (9 V/cm). The gel was stained with ethidium-bromide (2 µg/mL) after electrophoresis. For detection and documentation of PCR products we used Kodak Gel Logic 212 Imaging System under UV light.

Restriction fragment length polymorphism

PCR products were cleaved by restriction endonucleases. At *fim*A gene *Hinc*II and *Sal*I enzymes, at digestion of *fla*A *Hinc*II, *Bgl*I and *Msp*I endonucleases, at *cya*A *Nar*I and *Sal*I, and at *ptp* operon *Nar*I and *Bgl*I enzymes were used according to the manufacturers. The resulted fragments were checked by electrophoresis (7 V/cm) in 2.5% MetaPhor agarose gel with 1×TBE buffer. The gel was stained with ethidium-bromide (2 µg/mL) after electrophoresis. Detection and documentation of the products were in UV light with Kodak Gel Logic 212 Imaging System, to determine the length of the fragments Kodak Molecular Imaging Software were used.

Sequence and phylogenetic analysis

Determination of nucleic acid sequence was done at specific region of four genes (*fim*A, *fla*A, *cya*A and *ptp*). The sequencing primers and PCR primers were the same, but the examination of the 2151 bp length *cya*A sequence, we needed an additional, own-designed internal primer.

Purification of PCR products and sequencing reaction were performed by Macrogen Europe Ltd with traditional Sanger dideoxy-nucleotide method. Chromatograms were evaluated with Chromas LITE 2.01, sequences were aligned by SeqMan – Lasergene 7.1.0. (DNASTAR) software. Sequences of our strains were compared with sequences from GenBank by BioEdit 7.1.3.0 and MegAlign – Lasergene 7.1.0. (DNASTAR) software. The similarities of the nucleic acid and deduced amino acid sequences were calculated by the Clustal W algorithm. Following the alignment, phylogenetic dendrogram was constructed using the MEGA 6.06 software, with the Neighbor-Joining method with the Jukes-Cantor correction rate. The resultant tree topologies were evaluated by bootstrap analyses with 1,000 random samplings.

Results

Identification of the strains

All strains were negative in the indole reaction, and did not utilise the tested carbohydrates (glucose, lactose and sucrose) which is typical of *B. bronchiseptica*. On the other hand, only 92% of the strains were nitrate-positive and four urease-negative isolates were also found. During the molecular identification (species-specific PCR), the multiplied 237 bp length DNA portion was detected in every sample, irrespectively of the results of the result of the biochemical tests.

Results of the phenotypic assays

Haemolysis

Various results were noticed in the haemolytic assay, the strains showed diverse haemolytic activity. Nonetheless, it may be declared that fresh isolates showed more pronounced haemolysis than the older ones, presumably as the effect of passages and different maintenance procedures. On blood agar plate with lower pH (6.8 behalf 7.2), stronger β-haemolysis was detected, as well as on horse blood agar plates as compared to sheep blood agar medium. No haemolytic activity was observed with 40 (38 Hungarian and 2 foreign) canine strains.

Haemagglutination

Haemagglutination assay was performed with 79 *B. bronchiseptica* strains from 9 different host species. The examined strains usually agglutinated different types of red blood cells, and the strongest haemagglutination levels appeared with most RBCs. Significant distinction between the reactions with the three different human blood types (A, B and 0) were not found. The most level 4 reaction was observed with canine and porcine RBCs, the lack of the haemagglutination was usual with cattle, horse and chicken erythrocytes. Some strains showed variable haemagglutination with cattle, sheep and horse RBCs. There was no difference between Hungarian and foreign strains in the haemagglutination test, all haemagglutination scores were observed and strains with variable haemagglutination activities were found in both groups.

Antibiotic susceptibility

Strains were resistant to penicillin, ceftiofur, vancomycin and linkomycin, but susceptible against colistin, neomycin and the used quinolones. The resistance to ampicillin and erythromycin was varied widely between strains from both host species. Most of the

strain was susceptible to tetracycline and sulphonamides, but 5% of the rabbit originated and one-third of the porcine *B. bronchiseptica* strains were sulphonamide-resistant and one tetracycline-resistant strain (isolated from pig) was also found.

Urease-negative strains

Four porcine strains were urease-negative in conventional biochemical test after incubation either at 37ºC or 24ºC. The added $MgSO_4$ had no effect on the utilisation of urea. These isolates showed doubtful results in urease test with diagnostic tablets and also in the API 20 NE system, followed incubation at 37ºC for 48 h.

Genotypic examinations showed that all strains possessed a 323 bp gene portion using PCR designed for *ure*C, the major gene of the urease operon. In the further genetic analyses (*dnt*-, *fim*A-, *fla*A- and *cya*A-PCR and RFLP), urease-negative strains yielded the same results with the other porcine strains.

Characterisation of the virulence genes

Dermonecrotoxin

One hundred and fifty-two strains were examined by genotypic methods. 97% of the strains possessed the 224 nucleotide length DNA portion, and only 5 strains were *dnt*-negative. These *dnt*-negative strains were isolated from man (5390, Bb VAL and MBORD 675), pig (PV6) and turkey (MBORD 901).

Fimbria

The 549 bp length part of *fim*A gene was detected from all examined *B. bronchiseptica* strains. In the RFLP analysis – irrespectively of their origin −, all of the strains showed a uniform fragment pattern. Twenty-one strains were chosen for sequence analysis, the 456 bp length part of the *fim*A deposed to the GenBank database with accession number KF211375-KF211395.

The pairwise alignment of 29 own and 8 *fim*A sequences from GenBank revealed 0.0%-3.0% divergence within nucleic acid and deduced amino acid sequences, too. The maximal diversity was detected between two human strains (Bb DEL and 5390). Strains were grouped into 2 major clusters based on phylogenetic analysis. Cluster 2 contained human and atypical animal strains, which differed from the other strains of animal origin.

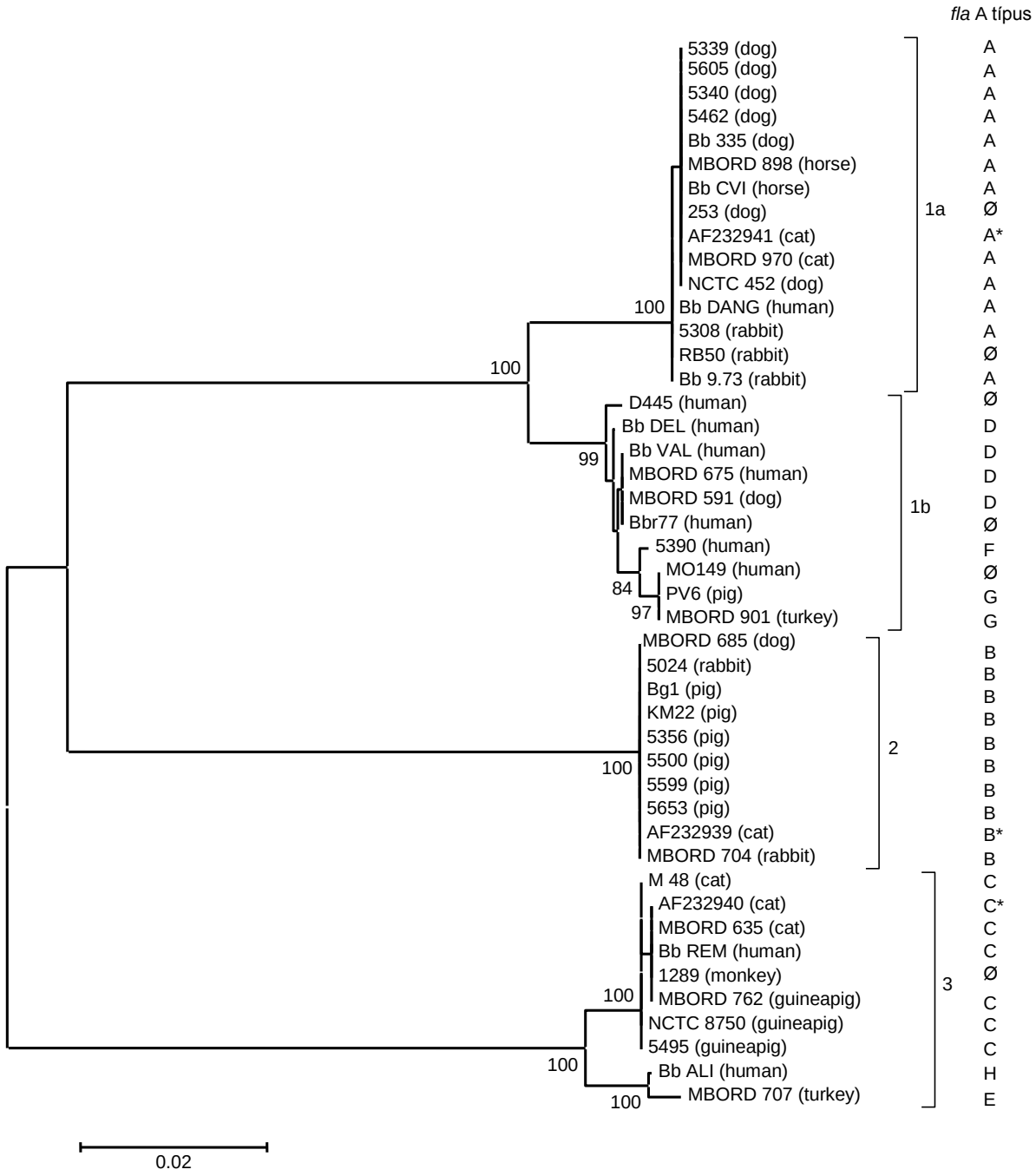

Figure 1: Phylogenetic tree of 1042 bp length *fla*A gene sequence of *B. bronchiseptica*.

Phylogenetic tree was constructed with a Neighbor-Joining method of MEGA 6.06 software. The percentages of bootstrap test are shown next to the branches. RFLP types of the strains are presented next to the tree. *: feline strains with known RFLP type from GenBank;
Ø: sequences from GenBank

Flagellin

All of the 152 examined *B. bronchiseptica* strains produced an 1165 bp product by *fla*A PCR. After restriction cleavage of PCR products eight different RFLP types (from A to H) were described. The most common profile was type A (44%), followed by type B (41%) and type C (8%), also within strains from Hungary and other countries. On the other hand, *fla*A type D, E and H did not occur among Hungarian strains. Type E, F and H were represented by individual strains and G type was detected at a foreign strain (MBORD 901) from turkey and a Hungarian porcine strain (PV6). Several RFLP types correlated with the host species of *B. bronchiseptica*; canine strains belonged to type A, porcine strains represented type B, and type C were found within strains isolated from cats, guinea pigs and koalas. Type A and B were also detected in rabbit originated strains, however, human strains (n=7) presented diverse RFLP profiles (A, C, D, F, H).

The sequences of the 36 strains selected for sequence analysis of *fla*A region, were submitted into the GenBank database under accession numbers JX673952-JX673981 and KF211396-KF211401. The multiple-sequence alignment showed that the *fla*A sequences had conserved regions in the N-terminal and C-terminal portions, whereas the central region (circa 500 bp) was considerably variable and showed nucleotide substitutions, deletions and insertions indicating non-synonymous changes in amino acid sequences. Hereby the maximum pairwise genetic distance could be 15% among this gene portion of the strains. Correspondence between *fla*A-types and host species of the strains were also demonstrated on the nucleic acid-based phylogenetic tree, because strains with different RFLP profiles (host species) belonged to different clusters of the tree (Figure 1).

Adenylate cyclase-haemolysin

Investigation of *cya*A gene (that codes adenylate cyclase-haemolysin toxin), resulted a specific 2151 bp length PCR product in 73.5% (n=112) of the strains. At the remaining 40 strains, no PCR product was found. Each of these *cya*A-negative strains was isolated from dog, and they were equivalent with the previously detected non-haemolytic strains. Using restriction endonucleases, our strains were sorted into 4 RFLP types (A, B, C and D). Most of the strain (90%) showed type A fragment pattern. The next most frequent profile (6%) was type B, which was typical in human strains (57%), while type C and D were represented by 2-2 foreign strains.

Sequence analysis was performed with 25 strains, our *cya*A sequences are accessible at GenBank (KF220450-KF220474). The pairwise alignment of the 2007 bp length *cya*A sequences and the deduced amino acid sequences was also presented 0.0%-3.8% divergence among the strains. Some strains possessed unique RFLP profile and nucleotide sequence, however, differences between the strains appeared sparsely. Two separate

clusters were found by phylogenetic analysis of *cya*A. Cluster 1 particularly contained animal strains, while human isolates rather got into cluster 2.

Peptide transport protein

For examination of *ptp* operon, the specific PCR product (958 bp) was detected only at 40 strains. All *ptp*-positive strains were identical with the *cya*A-negative strains, and all of them were isolated from dogs. Digestion of *ptp* PCR products by *Nar*I and *Bgl*I endonucleases resulted the same fragment pattern in each strain.

Since the patterns were identical and most of the samples (95%) were originated from Hungarian dogs, 3 Hungarian and 2 foreign strains (Bb 335 and MBORD 843) were chosen for sequence analysis. Five, 909 bp length *ptp* sequences were submitted into GenBank (KF220475–KF220479) after *in silico* analysis. Our examined *ptp* sequences were entirely consistent with each other and with the only available *ptp* sequence from the GenBank database 253 (HE965806), even a single nucleotide difference was not found.

Conclusions

In the background of differences in the antibiotic susceptibility patterns of *B. bronchiseptica* strains might be a plasmid-encoded resistance. On the other hand, diverse bacterial strains could be spread in certain populations mostly due to differences in antibiotic treatment strategies of the farms.

The lack of urease activity found in some *B. bronchiseptica* strains is a unique and unusual phenotypic property in this species. Furthermore, the presence of the urease-coding *ure*C gene in these strains suggests that mutation(s) and/or deletion(s) in the urease gene cluster might block the activity of the major structural gene or chaperone proteins.

Several pheno- and genoptypic methods were used to examine *B. bronchiseptica* virulence factors, and the heterogeneity of the strains were detected in the majority of the assays. Results of phenotypic assays (haemolysis, haemagglutination, motility) indicated that our strains were in avirulent (Bvg^-) phase. Since modulating signals were not used during cultivation, presumably the phase variation was induced by other extra and/or intracellular conditions. These factors may include number of passages, age of the cells, bacterial density. Moreover, several other yet unknown factors might influence the outcome of these assays.

Inner or outer circumstances had no effect on the results of genotypic methods like PCR, RFLP, sequence and phylogenetic analysis. Genetic assays revealed a diversity of the strains, on the basis of which we could constitute distinct groups. In several cases, this heterogeneity could be regarded as sign of host adaptation, like the deletion of *cya*A gene in strains from dogs. Signs of host adaptation were also observed in distribution of *fla*A RFLP-types. The most canine *B. bronchiseptica* strains belonged to type A while type B was typical for strains from pigs. These properties indicate a clonal population structure of *B. bronchiseptica*.

An unusual porcine strain (PV6) appeared during our genotypic investigations, which was different (*dnt*-negative and *fla*A-type G) from the other *B. bronchiseptica* strains isolated from pigs. The results of PCR-RFLP and sequence analysis of PV6 suggest that this strain is atypical within porcine strains, and it is very probable that the source of the infection was another host. At gene portions where variability was detected within the strains, PV6 showed the biggest similarity with human-adapted *B. bronchiseptica* lineage. So, it cannot be ruled out that man could be the source of infection of susceptible animal species.

New scientific results

1. We first performed a complex, comparative pheno- and genotypic examinations of large numbers of Hungarian (n=107) and foreign (n=45) *B. bronchiseptica* strains from different host species.

2. We first isolated and characterised urease-negative *B. bronchiseptica* strains from the field.

3. We carried out a survey on the antibiotic susceptibility of Hungarian *B. bronchiseptica* strains from rabbits and pigs, and showed out the emergence of tetracycline and/or sulphonamide resistant strains among them.

4. We first performed PCR-RFLP analysis of *fim*A of *B. bronchiseptica*, detecting the uniformity of the strains with this method.

5. During the PCR-RFLP analysis of the *fla*A gene we described 5 new RFLP-types over the 3 well-known RFLP-types. On the basis of the genotypic analysis of the *fla*A we revealed signs of host adaptation, especially at Hungarian strains.

6. We revealed by PCR method that the *cya*A gene is deleted in Hungarian canine originated *B. bronchiseptica* strains, but the presence of *ptp* operon, which replaces *cya*A, were confirmed at each *cya*A-negative strain. Presence of *cya*A gene was detected in all Hungarian *B. bronchiseptica* strains, excluding strains from dogs. Therefore the absence of *cya*A gene in Hungarian canine originated strains might be a host specific property.

7. We found a new allele-type in a Hungarian *B. bronchiseptica* strain of human origin with sequence analysis of *cya*A.

Publications based on the results of the PhD dissertation

Research papers in peer-reviewed journals

Wehmann E., Khayer B., Magyar T.: Heterogeneity of *Bordetella bronchiseptica* adenylate cyclase (*cya*A) RTX domain, Arch. Microbiol., 197. 105-112, 2015.

IF_{2013}: 1,861

Khayer B., Magyar T., Wehmann E.: Flagellin typing of *Bordetella bronchiseptica* strains originating from different host species, Vet. Microbiol., 173. 270-279, 2014.

IF_{2013}: 2,726

Khayer B., Wehmann E., Demeter Z., Rónai Zs., Jánosi Sz., Rusvai M., Magyar T.: Molecular analysis of *Bordetella bronchiseptica* strains of canine origin, Magyar Állatorv. Lapja, 133. 594-600, 2011.

IF: 0,201

Khayer B., Rónai Zs., Wehmann E., Magyar T.: Detection of urease-negative *Bordetella bronchiseptica* from the field, Acta Vet. Hung., 59. 289-293, 2011.

IF: 0,673

Conference presentation

Khayer B., Domokos J., Magyar T., Wehmann E.: Antibiotic susceptibility of Hungarian *Bordetella bronchiseptica* strains isolated from pigs. (poster) [*Conference abstract* p. 30.] Congress of the Hungarian Society for Microbiology in 2014. Keszthely, 2014.10.15-17.

Domokos J., Khayer B.: Antibiotic susceptibility testing of *Bordetella bronchiseptica* strains from pigs. (oral) [*Conference abstract* p. 74.] 15th Biologist Day in Cluj-Napoca. Cluj-Napoca, 2014.04.04-06.

Khayer B., Magyar T., Wehmann E.: Comparison of human and animal *Bordetella bronchiseptica* strains by PCR-RFLP and phylogenetic analysis. (poster) [*Conference abstract.*: P1] 10th International Symposium on *Bordetella*. Dublin, 2013.09.08-11.

Khayer B, Sulyok K. M., Wehmann E., Magyar T.: Antibiotic susceptibility of *Bordetella bronchiseptica* strains isolated from rabbits. (poster) [*Conference abstract* p. 184.] EMBO/EMBL Symposium: New approaches and concepts in microbiology. Heidelberg, 2013.10.14-16.

Khayer B, Sulyok K. M., Wehmann E., Magyar T.: Antibiotic susceptibility testing of *Bordetella bronchiseptica* strains from rabbits. (poster) [*Ed.: Janda T., ISBN:978-963-8351-41-8*, pp. 225-228.] 2nd Scientific Day of CAR, Sience which lives with us. Martonvásár, 2013.11.08.

Khayer B., Magyar T., Wehmann E.: Comparative PCR-RFLP and phylogenetic analysis of human and animal originating *Bordetella bronchiseptica.* (oral) [*Ed.: Janda T., ISBN:978-963-8351-41-8,* pp. 50-53.] 2nd Scientific Day of CAR, Science which lives with us. Martonvásár, 2013.11.08.

Magyar T., Khayer B., Wehmann E.: PCR-RFLP analysis of *Bordetella bronchiseptica* isolates from different animal species to detect the possible signs of host-adaptation. (poster) [*Conference abstract* p. 22.] 2nd Prato Conference on the Pathogenesis of Bacterial Diseases of Animals. Prato, 2012.10.09-12.

Khayer B., Magyar T., Wehmann E.: Phylogenetic analysis of *Bordetella bronchiseptica* strains isolated from different host species. (poster) [*Acta Microbiol. Imm. Hung. Supplement* 60:32] Congress of the Hungarian Society for Microbiology in 2012. Keszthely, 2012.10.24-26.

Wehmann E, Magyar T, Khayer B: Virulence factors and host adaptation of *Bordetella bronchiseptica.* [*Ed.: Janda T., ISBN: 978-963-8351-40-1,* p. 69.] 1st I. Scientific Day of CAR, Explorative researches in Centre for Agricultural Research. Martonvásár, 2012.11.14.

Khayer B., Lukács L., Wehmann E., Magyar T.: Characterisation of *Bordetella bronchiseptica* strains isolated from pet animals. (poster) [*Acta Microbiol. Imm. Hung. Supplement* 58:167] 16th International Congress of the Hungarian Society for Microbiology. Budapest, 2011.07.20-22.

Khayer B., Wehmann E., Magyar T.: PCR-RFLP analysis of *Bordetella bronchiseptica* strains originated from different hosts on *fla*A gene. (poster) [*Conference abstract* p. 57.] The Prato Conference on the Pathogenesis of Bacterial Diseases of Animals. Prato, 2010.10.06-09.

Khayer B., Wehmann E., Magyar T.: Identification of host adaptation markers of *Bordetella bronchiseptica* with PCR-RFLP analysis. (oral) [*Acta Microbiol. Imm. Hung. Supplement* 58:51] Congress of the Hungarian Society for Microbiology in 2010. Keszthely, 2010.10.12-15.

Academic reports

Khayer B., Sulyok K. M., Domokos J., Magyar T., Wehmann E.: Antibiotic susceptibility testing of *Bordetella bronchiseptica* strains isolated from rabbits and pigs. Budapest, 2015.01.27.

Khayer B., Magyar T., Wehmann E.: Investigation of virulence factors of *Bordetella bronchiseptica* strains originated from different host species. Budapest, 2013.01.29.

Khayer B., Wehmann E., Magyar T.: Characterisation of flagellin of *Bordetella bronchiseptica* strains isolated from different hosts by traditional and molecular methods. Budapest, 2012.01.17.

Khayer B., Rónai Zs., Wehmann E., Magyar T.: Characterisation of urease-negative *Bordetella bronchiseptica* isolates. Budapest, 2011.01.25.

Khayer B., Wehmann E., Magyar T.: Examination of adenylate cyclase-hemolysin toxin of *Bordetella bronchiseptica.* Budapest, 2011.01.25.

Khayer B., Wehmann E., Magyar T.: Characterisation of flagellin gene of *Bordetella bronchispetica* isolatesby PCR-RFLP analysis. Budapest, 2010.01.26.

Publications not related to the PhD dissertation

Szabó G., Khayer B., Rusznyák A., Tátrai I., Dévai Gy., Márialigeti K., Borsodi A.: Seasonal and spatial variability of sediment bacterial communities in habiting the large shallow Lake Balaton. Hydrobiologia, 663. 217-232, 2011. IF: 1,784

Khayer B., Szabó G., Borsodi A. K., Márialigeti K.: Cultivation based bacterial diversity of the sediment of Lake Balaton. (poster) [*Acta Microbiol. Imm. Hung. Supplement* 54:60] 15th International Congress of the Hungarian Society for Microbiology. Budapest, 2007.07.18-20.

Khayer B., Szabó G., Márialigeti K., Borsodi A. K.: Studies on bacterial polyphosphate accumulation and phosphatase activity in three Hungarian shallow lakes. [*Acta Microbiol. Imm. Hung. Supplement* 53:292] Congress of the Hungarian Society for Microbiology in 2006. Keszthely, 2006.10.18-20.

Acknowledgements

I would like to express my thank to my supervisor, Tibor Magyar, who provided the financial and infrastructural conditions of my work in the Respiratory Bacteriology Group of Institute for Veterinary Medical Research CAR HAS, managed the course of the research, helped me in the interpretation of the results, an correction of the manuscripts prepared for publication. I thank for his patience and confidence and understanding during the years.

I am extremely grateful to Enikő Wehmann, who supported me from the beginning and gave a lot of useful advice. I would like to thank her trust and appreciation as well as her help in acquirement of several methods, management in research, evaluation of results and revision of the publication.

It is my honour to acknowledge Éva Hegedűs and Boglárka Sellyei, just as the other members of the group for their practical and theoretical succour.

I thank Dániel Bartha, bioinformatics of IVMR CAR HAS, who helped in the evaluation of motility data. I am also thankful for every colleague at the IVMR, who gave me professional or technical assistance.

I would like to acknowledgement to the members of NFCSO VDD Bacteriology Laboratory. I thank for Zsuzsanna Rónai for help in examinations with urease-negative *B. bronhiseptica* strains, and for thought-provoking conversation; and I thank for Katalin Szentesi for antibiotic susceptibility standards.

I would also like to thank all veterinarians and my acquaintance, especially my PhD classmates who kindly submitted the samples.

I thank my students (Krisztina Hunyadi, Lilla Lukács, Kinga Mária Sulyok and Judit Domokos) for the part they took in this work. I also thank Andrea Borsodi, Károly Márialigeti, Csaba Bognár and all of my teachers for that I could stand where I am now.

Last, but not least I would like to acknowledge my partner, my family and my friends for their endless favour, confidence and tolerance.

Financial supports were provided mainly by Hungarian Scientific Research Found, OTKA K83332 as well as Postgraduate School of Szent István University.