

THESES OF PHD DISSERTATION

Molecular characterisation of *Staphylococcus aureus*
strains isolated from rabbits

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1. Introduction and aim of the study

Staphylococcosis is one of the most important diseases in commercial rabbit meat production. *Staphylococcus aureus* is a versatile, opportunistic pathogenic microorganism, which is able to persist and multiply in the animals, humans and environment of farms, and causes a wide spectrum of diseases in both humans and animals.

Scientific studies investigating rabbit staphylococcosis revealed great versatility in both clinical, pathological and microbiological aspects of the disease. International dissemination of virulent variants of the pathogen had been reported, and the only feasible solution for outbreaks caused by such agents was complete depopulation of contaminated units. Present study was originated from the request of Hungarian rabbit breeders and meat producers, because production losses, and ineffective treatment programmes clearly demonstrated, that veterinary practice needed substantial improvement in this regard.

The aim of this study was to gain information on the clinical situation on rabbit farms, collect isolates, and characterise them in order to facilitate more effective treatment and preventive measures in commercial production.

2. New scientific results

2.1 Molecular characterization of clinical *Staphylococcus* isolates originating from Hungary

With hundreds of farm visits and thousands of necropsies I examined the situation about rabbit staphylococcosis on the majority of Hungarian rabbit farms. The diagnostic procedures resulted an archive of more than 500 strains of the pathogen. I implemented a multiplex PCR method in standard diagnostics, which led to the first identification of virulent variants of the pathogen in Hungary. This information facilitated the decision to eradicate the contaminated populations, after this the most dangerous ST121/t645 clone was never detected in following diagnostic procedures. The most abundant genotype on Hungarian farms is an atypical highly virulent variant, similar genotype was only detected in one outbreak in Belgium, in 1996.

2.2 Statistical analysis on data about genotype and organ of origin of the isolates

The statistical analysis of genotype and organotropism of the strains revealed that different genotypes have significantly different organotropism. HV strains are highly correlated to septicaemia. LV and non-aureus *Staphylococcus* strains are much more likely to only cause local lesions on the skin.

2.3 Publication of 52 drafts of the whole genome of rabbit-originated *Staphylococcus aureus* isolates

Whole genome drafts were sequenced with high (more than 100x) on Illumina next generation sequencing platform, the data was publicized in NCBI Genbank and the European Nucleotide Archive. The data is a substantial addition for the practical and scientific rabbit medicine.

2.4 Comparative analysis of the whole genome of a typical and an atypical highly virulent strain

I compared the two draft genomes in two different approaches. Pairwise alignment of the two drafts revealed that the two virulent variants are in fact two very different, far related variants of *Staphylococcus aureus*. The two genomes had several major insertions/deletions, including an approximately 39 kbp phage insertion site in the HV strain and a different 42 kbp phage insertion in another locus in the aHV genome. The two phage insertion sites were somewhat similarly structured regarding major phage component genes, but pairwise nucleotide identity was only 51.8% between these regions. Numerous insertion/deletion sites with length up to 15 kbps are scattered through the two genomes.

Comparing 63 virulence-related genes we concluded that EC enzymes of the two genotypes are very

similar, but the whole enterotoxin gene cluster is absent from the atypical highly virulent strain. This kind of virulence factor gives a remarkable advantage to a microorganism and must have a fundamental role in the pathogenicity of HV strains. Surface components and adhesion factors were proven to be notably different between HV and aHV strains. This result details the illustration of the evolutionary distance between the two variants and can explain the differences in organotropism concluded earlier.

2.5 Epidemiologic analysis of 64 rabbit-originated *Staphylococcus* isolates with whole-genome multilocus sequence typing

I analysed 64 rabbit-originated *Staphylococcus* isolates representing 4 European countries with whole-genome MLST. All typical highly virulent strains formed a closely related cluster and Hungarian strains were almost identical, thus the infections caused by strains labelled as HV in rabbit-focused diagnostics belong to an epidemic spreading worldwide.

My results clarified that the atypical highly virulent isolates, until now only identified by amplification of a few selected genetic elements, constitute a group of closely related, most probably clonal specimens of *Staphylococcus aureus*. The aHV group has a novel MLST pattern (ST5993).

The whole genome of the low virulence strains also provided interesting new discoveries. The majority of

the strains formed a cluster of very closely related strains, despite that both MLST and spa sequence typing methods resulted in great diversity. The 7 genes of MLST and the single spa sequence would have distributed these 10 strains into 7 different clusters, while analysis of 3897 loci revealed that within the main cluster only an average of 1,3% of the genetic features was different. Based on the wgMLST, the clonal origin of this cluster (LV-ST2855) also seems very probable.

This analysis revealed, that three fundamentally different clonal complexes of *Staphylococcus aureus* can be present simultaneously on rabbit farms. This result highlights that application of molecular methods in diagnostics of rabbit staphylococcosis is mandatory to provide the necessary information for decisions resulting effective therapy and prevention.

3. Publication list

Német, Zoltán ; Szenci, Ottó ; Biksi, Imre

A házi nyúl staphylococcosisa : Irodalmi áttekintés és hazai tapasztalatok
Staphylococcosis in domestic rabbit. Literature review and domestic experiences

MAGYAR ÁLLATORVOSOK LAPJA 133 pp. 720-726. , 7 p. (2011)

Német, Zoltán ; Albert, Ervin ; Nagy, Krisztina ; Csuka, Edit ; Dán, Ádám ; Szenci, Ottó ; Hermans, Katleen ; Balka, Gyula ; Biksi, Imre

Virulence type and tissue tropism of *Staphylococcus* strains originating from Hungarian rabbit farms

VETERINARY MICROBIOLOGY 193 pp. 1-6. , 6 p. (2016)

Nemet, Zoltan; Albert, Ervin; Nagy, Tibor ; Olasz, Ferenc ; Barta, Endre ; Kiss, János ; Dan, Adam ; Banyai, Krisztian ; Hermans, Katleen ; Biksi, Imre

Draft Genome Sequence of an Atypical Highly Virulent Rabbit *Staphylococcus aureus* Strain.

GENOME ANNOUNCEMENTS 5 : 42 Paper: e01049-17 (2017)

Nemet, Zoltan; Albert, Ervin ; Nagy, Tibor ; Olasz, Ferenc ; Barta, Endre ; Kiss, Janos ; Dan, Adam ; Banyai, Krisztian ; Hermans, Katleen ; Biksi, Imre

Draft Genome Sequence of a Highly Virulent Rabbit *Staphylococcus aureus* Strain.

GENOME ANNOUNCEMENTS 3 : 4 Paper: e00461-15 , 2 p. (2015)