modifications may influence trafficking of cytochrome P450 proteins. Localisation of CYP51 protein on acrosome is the first identification of cytocrome P450 on acrosome membrane in mammals. The imporatance of CYP51 protein on acrosome is not yet known, it is possible that CYP51 on acrosome is involved in production of MAS that could be delivered by sperm to the oocyte where it would trigger the conclusion of second meiotic division.

The research work was performed at the Institute for health care of pigs, Veterinary Faculty in Ljubljana and at the Medical centre for molecular biology, Medical Faculty in Ljubljana.

The date of public defense: April 22, 2003.

Mentor: Prof. Dr. Damjana Rozman, co-mentor: Assist. Prof. Gregor Majdič.

Leon Ščuka: ENROFLOXACIN – THE META ANALYSIS OF THE EFFICACY OF DISEASE TREATMENT FOR DOMESTIC ANIMALS

Meta-analysis is the process of using statistical methods to review and combine the results of different independent clinical studies. Glass first used the term meta-analysis in 1976, when he and his coworker Mary Lee Smith have statistically combined the results of 375 studies that evaluated efficacy of psychotherapy. Through meta-analysis, the scientists are able to integrate results and findings from different studies. This analytical method is of particular importance in the assessment of therapeutic efficacy when individual studies do not provide an overview over all studies on a topic. As their samples are too small, individual studies cannot provide a quantitative evaluation of the effect of treatment, nor can they test null hypothesis. Prior to meta-analysis, the traditional method was a narrative discourse on previous findings, which, however, could be misleading and subjective.

In the past few years, meta-analysis has been increasingly used in all fields of science. This is particularly evident for the medical science, where two other methods are used as well –the Systematic Review and Evidence Based Medicine, while especially in medicine, the Decision Analysis and Cost-Effectiveness Analysis have developed. All methods are connected, and the last two are the Upgrade of the first two. Systematic reviews are exact summaries of the best evidences related to exactly specified clinical dilemmas. Special centers, like Cochrane collaboration have been organized in different places around the world, where systematic reviews of scientific literature and their own findings are published in databases collecting data on most appropriate therapies of individual illnesses. These reviews support the synthesis of best evidence for treatment or establishment of best medical practice. In this case, meta-analysis has a broader impact and is not just, as usually, a statistical method for collecting study results. This knowledge in veterinary medicine is only just beginning to develop but centers of this kind have not been established yet.

Our research of literature revealed 21 cases of meta-analysis in veterinary medicine; all of them carried out after 1990. In comparison to more than 1000 meta-analyses carried out only in the year 1997 in human medicine; this is a very small number.

About 16 years ago, fluoroquinolones were introduced to clinical medicine and they promised a new era in the development of antimicrobial agents. Due to its broad spectrum of action, excellent clinically significant pharmacokinetics properties and low toxicity -this being great advantages over other groups of antibiotics -they were classified as almost ideal antimicrobial agents.

Enrofloxacin is a chemotherapeutic from the fluoroquinolone group that was developed exclusively for the use in veterinary medicine. Low concentrations of the substance exert a rapid bactericidal effect against gram-negative and gram-positive bacteria, while the substance also very effective against *Mycoplasma* spp. It acts against bacteria in their reproductive phase and microorganisms in their latent state. It acts in the presence of oxygen and due to this phenomenon does not damage the indigenous anaerobic intestinal micro flora.

We also reviewed literature describing the use of enrofloxacin in domestic animals. In addition to the meta-analysis, we made a systematic review and in the end combined the general findings. We reviewed and evaluated the applicability and efficacy of enrofloxacin in the health care of pigs, poultry and cattle. Individual studies were collected by reviewing databases available on CD ROMs or Online. We also reviewed references in different published studies and data bases on the internet. We first collected many different studies. We obtained 919 articles for the studies for the first

selection, and after review and careful consideration of the studies, we chose for a closer review 110 studies on health care in pigs, 67 in ruminants and 60 in poultry, i.e. a total of 237 studies.

We reviewed and evaluated the efficacy of the treatment of various infections with enrofloxacin for individual animal species. A special meta-analysis was carried out and graphically presented for the treatment of each disease. In most cases, we chose the odds ratio to present the size of the effect. By following a systematic way of reviewing, we ensured repeatability of our meta-analyses in case this would be done by other investigators. In heterogeneous meta-analyses, we calculated the total size of the effect according to a random calculation model for total effect size. Additionally the homogeneity of studies was graphically evaluated with funnel plots. In addition to clinical studies, we reviewed and combined data on bacterial in vitro susceptibility to enrofloxacin. These results were also considered in the final opinion about individual meta-analysis of efficacy of enrofloxacin. In a systematic review, we compared efficacy of enrofloxacin and other antimicrobial agents.

We prepared 19 meta-analyses about different uses of enrofloxacin in various diseases in pigs, poultry and domestic ruminants (mainly cattle), while in 7 cases we also calculated the individual effect size (odds ratio) for a specific parameter.

It is evident from the results that enrofloxacin is potently effective in the treatment of respiratory infections in all domestic animals ($P < 0.01$). Enrofloxacin is very effective in the treatment of all coli and salmonella infections in pigs and poultry ($P < 0.001$), while additional studies about colibacillosis and salmonellosis in cattle would be necessary. In cattle, in vitro resistance to enrofloxacin was established in 11.8 % of *E. coli* strains (n = 195), 1.8 % *E. coli* strains, isolated in the udder (n = 1695), and 8.4 % if salmonella strains (n = 1211).

After taking into account all findings (in vivo and in vitro), it was revealed that enrofloxacin is effective in the treatment of mycoplasma infections in poultry and pigs, while additional studies would be necessary in cattle.

A meta-analysis in poultry revealed that administration of enrofloxacin is effective in pasteurellosis in turkeys ($P < 0.001$), and in infectious coryza ($P < 0.001$), staphylococcosis ($P < 0.001$) and *R. anatipestifer* infection in ducks ($P < 0.001$). These results are also confirmed by findings of high in vitro susceptibility to enrofloxacin of the pathogens of these diseases.

In pigs, treatment with enrofloxacin was significantly more effective in the trial group that in the control group for MMA syndrome ($P = 0.002$), urinary tract infections ($P < 0.05$) and streptococcal infections (fewer deaths, $P = 0.045$). These results are also confirmed by findings of high in vitro susceptibility to enrofloxacin of the pathogens of these diseases. For Glässer's disease the difference, in comparison to the control group was not significant ($P = 0.25$), however the pathogen (*H. parasuis*, n = 124) was 100 % susceptible to enrofloxacin. In greasy pig disease, there is a high in vitro susceptibility of S. hyicus to enrofloxacin (98.3 %, n = 744).

To be able to answer the complex questions about mastitis in cattle, one or more additional studies with enrofloxacin would be necessary, as our results indicate that enrofloxacin is not more effective than drugs in control groups (fixed model: odds ratio = 0.3; $P = 0.5$, random model: odds ratio = 1.19; $P = 0.79$). However, the in vitro results on susceptibility of mastitis pathogens to enrofloxacin are good. An additional study would also be necessary for the treatment of endometritis in cattle, since the difference between the trial and the control group was not statistically significant ($P = 0.9$), although the results were in favor of the treatment with enrofloxacin.

In the thesis most of the aims were reached and the majority of tasks necessary for the investigator during the process of meta-analysis were successfully performed: descriptive survey, guidelines for further research, diagnostic survey and transfer of our findings into practice. We reviewed the available studies and could assess sufficiently and insufficiently analysed parameters. Some studies revealed statistically significant results and some not. It occurred in some cases that studies that lacked significant results, due their weight, had a greater impact on the analysis than those with significant results. It was this part of our research that revealed one of the greatest difference between meta-analysis and the narrative comparison of the literature.

Our findings can be considered useful for investigators, doctors of veterinary medicine in practice and for the breeders, as well as for the manufacturers of veterinary medicines and governmental authorities. Our work has a great economic impact too, since it offers an overall survey

of the problem and provides guidelines for further research of the topic.

The research work was performed at the Veterinary Faculty in Ljubljana and Krka d.d., Novo mesto.

The date of public defense: June 27, 2003.

Mentor: Prof. Dr. Jože Drinovec, co-mentor: Prof. Dr. Peter Lazar.

Janez Posedi: GENETIC MARKERS FOR SHEEP GASTROINTESTINAL STRONGYLIDS SUSCEPTIBLE TO BENZIMIDAZOLE ANTIHELMINTICS

This study defines the genetic markers in rDNA for sheep gastrointestinal strongylids. In the study, parasites of species *Trichostrongylus axei, Trichostrongylus colubriformis, Teladorsagia circumcincta, Cooperia curticei* and *Chabertia ovina* are included.

Previous to molecular analyses, the susceptibility to benzimidazole anthelimintics in investigated population of gastrointestinal strongylids was determined. Egg hatch assay and faecal egg count reduction test for determination of resistance to anthelmintics were performed.

The results of duplicate egg hatch assay showed LD_{50} to be between 0.046 and 0.054 µg/ml thiabendazole. Therefore, the investigated population of gastrointestinal strongylids is susceptible to benzimidazoles, as it has been shown that resistance occurs when the LD_{50} value is equal to or greater than 0.1 µg/ml thiabendazole. Albendazole was used in the faecal egg count reduction test. It was determined that reduction of egg output was 100 %. The faecal egg count reduction test confirmed the result of egg hatch assay and definitely detected the population of gastrointestinal strongylids as susceptible to benzimidazoles.

For all species included in study, the nucleotide sequence for ITS2 and 5.8S region of rDNA was determined. Additionally, for *C. ovina* the nucleotide sequence for ITS1 region was determined. For this purpose, PCR was performed for ITS1, 5.8S and ITS2 region in three individuals of investigated species. Then, PCR products were cloned and nucleotide sequences of the ITS2 region in three plasmids were determined. The length of the 5.8S sequence was 153 bp for all species. The length of the ITS2 sequence ranged from 233 bp to 248 bp.

The length of the ITS2 sequence was 237 bp for *T. axei*. The G+C content was between 31.22 and 31.65 %. Polymorphism was detected at 2, 33, 130 and 189 positions. In our study, intraindividual variation for ITS2 sequence for *T. axei* ranged from 0 to 1.3 %, intraspecific variation was 1.7 %. In all clones obtained from *T. colubriformis*, 100 % identical nucleotide sequences for ITS2 region were determined. The length of the sequence was 238 bp, and the G+C content was 31.09 %. A comparison of all nucleotide sequences for *T. axei* and *T. colubriformis*, showed interspecific variation to be between 3.8 and 4.2 %.

The length of the nucleotide sequence of ITS2 region was 246 bp or 248 bp for *T. circumcincta*. The G+C content was between 33.47 and 34.55 %. The ITS2 sequence in *T. circumcincta* contained 9 polymorphic sites. Intraindividual variation ranged to a high of 2.1 %, and the intraspecific to 2.9 %.

For *C. curticei*, the intraindividual and intraspecific variation ranged to a high of 3.8 %. 13 polymorphic sites were detected, of which 4 had not been discovered before. The length of the ITS2 sequence was 242 bp for *C. curticei*. The G+C content was between 30.17 and 32.23 %.

For *C. ovina*, the length of the ITS2 sequence was 233 bp in five clones and 235 bp in four clones. The G+C content was between 44.26 and 45.06 %. The deletion of two nucleotides at positions 142 and 143 in the sequences of the length 233 bp was determined. Polymorphic positions were also detected at positions 56, 60, 62 and 80. The intraindividual and intraspecific variation of nucleotide sequences for ITS2 region ranged to a high of 2.6 %. The length of the sequence ITS1, 5.8S and ITS2 was 852 bp or 854 bp for *C. ovina*. The length of the ITS1 sequence was 370 bp. Polymorphic sites were detected at positions 258 and 303.

A comparison of the consensus nucleotide sequences for the ITS2 regions for all examined species was performed. It revealed 94.9 % identity between species *T. colubriformis* and *T. axei*. The identity between *T. colubriformis*, and *T. axei* and *T. circumcincta* was 75.5 % and 77.7 %, respectively. The similar percentage of identity was observed between the species of the genus *Trichostrongylus* and *C. curticei*. Thus, the identity between *T. colubriformis* and *C. curticei* was 74.4 %, and between *T. axei* and *C. curticei* 73.8 %. The identity between T. *circumcincta* and *C. curticei* was 71.1 %. The consensus nucleotide sequence of the ITS2 region of *C. ovina* revealed