VetCAST Newsletter April 2020

Usually, the VetCAST Steering Committee presents to stakeholders the status of VetCAST every year at ECCMID. Since this is not possible this year, we have instead prepared this newsletter to provide an overview of past and ongoing activities. A "quick and dirty" overview of our work towards breakpoints can be seen in the table below. We consider this a management tool to be updated regularly – for us and stakeholders to see what data we possess and need for future work.

<table>
<thead>
<tr>
<th>Drug/Species</th>
<th>ECOFF or TECOFF</th>
<th>PK/PD Cut off</th>
<th>Prot. binding</th>
<th>PK/PD index*</th>
<th>Rationale document on EUCAST homepage</th>
<th>CBP on EUCAST homepage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florfenicol / Calves</td>
<td><em>P. multocida</em></td>
<td>yes</td>
<td>yes</td>
<td>Time kill curve</td>
<td>completed</td>
<td>Awaiting validation of MIC testing method</td>
</tr>
<tr>
<td></td>
<td><em>M. haemolytica</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marbofloxacin / Horses</td>
<td><em>E. coli</em></td>
<td>yes</td>
<td>yes</td>
<td>literature</td>
<td>in progress</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Strep. equi</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoxicillin/ clavulanic acid in dogs</td>
<td></td>
<td>PK data generation</td>
<td>data generation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefazolin / dog</td>
<td><em>Staph. pseudintermedius</em></td>
<td>yes</td>
<td>yes</td>
<td>literature</td>
<td>in progress</td>
<td></td>
</tr>
<tr>
<td>Oxytetracycline / cattle</td>
<td></td>
<td>PK data modelling</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*an executive decision was made, both for the choice of the index and its target value, from literature or from another source that is more relevant to veterinary medicine.

1. **ENOVAT: European Network for Optimization of Veterinary Antimicrobial Treatment**

In November 2019, the 4-year COST Action project ENOVAT was launched. The overall aim is to optimize veterinary antimicrobial use with special emphasis on the development of antimicrobial treatment guidelines and refinement of microbiological diagnostic procedures. VetCAST plays an essential role in ENOVAT, as the ultimate task of Working Group (WG) 3 is to develop veterinary clinical breakpoints. The first objective (and ongoing activity) of WG3 is to make a priority list of animal- and infection specific CBPs that are currently lacking for veterinary pathogens. The second objective is to retrieve data for establishing prioritised CBPs. For that purpose, WG2 plays an essential role, as this group will assist in the collection and creation of relevant MIC data and subsequently ECOFFs. ENOVAT is a networking project with participation of more than 30 European countries. COST is funding various networking activities including meetings, trainings schools and exchange of young researchers via "short-term scientific missions". In the absence of funding for research, the strength of
ENOVAT is the very large network and voluntary contributions of data and labour. More information can be found here, and (from May 1st 2020) on www.enovat.eu. For information on upcoming ENOVAT/VetCAST training schools, please see point 9.

2. Status on florfenicol breakpoint

In August 2019, VetCAST published on the EUCAST homepage a rationale document for florfenicol clinical breakpoints proposed for Mannheimia haemolytica (S ≤ 2 mg/L) and Pasteurella multocida (S ≤ 1 mg/L) of bovine origin. The proposed breakpoints are based on ECOFFs and PK data and apply to a single dosage regimen of 40 mg/kg subcutaneously for an expected duration of action of 96h. We would like to include these breakpoints in an official EUCAST breakpoint table, but first we need to adapt the currently used AST methods to EUCAST approved standards, which differ slightly from those of CLSI. We are in dialogue with the EUCAST SC to plan and conduct this work.

3. PK/PD studies in mink

A PhD project conducted in the lab of Peter Damborg aims to establish antimicrobial dosages and clinical breakpoints targeting common bacterial pathogens in mink. In vivo PK studies and time kill studies have been recently completed by PhD student Amir Ronaghinia, who is now preparing three manuscripts on sulfadiazine/trimethoprim, tylosin, and amoxicillin, respectively. A sister project conducted by PhD student Nanett Nikolaisen contributes with MIC data and ECOFFs for the PK/PD modelling.

4. Marbofloxacin in horses

A paper by Alain Bousquet-Melou et al entitled “Determination of the pharmacokinetic-pharmacodynamic cut-off values of marbofloxacin in horses to support the establishment of clinical breakpoint” has been submitted to the Equine Veterinary Journal. In this paper, it was concluded that the PK/PD cutoff of marbofloxacin in horses was 0.125 mg/L for gram-positive pathogens and 0.0625 mg/L for gram-negative pathogens.

5. Doxycycline in pigs

At the Toulouse Veterinary School, the extent of protein binding of doxycycline in pigs has been revisited, and it was found in 32 pigs that the free plasma fraction of doxycycline in pigs is about two to four-folds higher than previously reported i.e. 27.5% vs. 7% (Riond & Riviere, 1990). This result, and our large database of PK data from around 500 pigs, will allow computing a realistic PK/PD cutoff for doxycycline in pigs.

6. Amoxicillin/clavulanic acid in dogs

VetCAST members coordinate a collaborative project on amoxicillin/clavulanic acid (Royal Veterinary College (RVC), the Toulouse Veterinary School and the Faculty of Veterinary Medicine of Ghent). First, we carried out a population PK study in septic dogs treated with a 20 min IV infusion of amoxicillin/clavulanic acid admitted in the intensive care unit of the RVC. Second, we carried out a crossover PK study in healthy dogs (intravenous vs low and high dose oral amoxicillin/clavulanic acid), collecting serial blood and urine for analysis (study was run from public funding). These studies were in their final stages and sample analysis was imminent, but interrupted by the COVID-19 pandemic. Third, we have obtained permission to use plasma concentration-time courses collected from veterinary drug companies and authors of academic studies. These pharmacokinetic studies will help the computation of a clinical breakpoint and support therapeutic dose adjustment.

7. Population pharmacokinetic model for oxytetracycline in cattle
Oxytetracycline has been available for decades, and there are many generic products on the market. A population pharmacokinetic model for long-acting injectable formulations of oxytetracycline in cattle is being developed as a first step towards determining PKPD cut-offs for bovine-specific pathogens. Bioequivalence studies conducted to bring the pioneer and generic products to market are a rich source of data for this model. An initial subset of these data is being used for the development phase. The next step will be to refine and validate the model using the full dataset obtained from partnering pharmaceutical companies and academic researchers. The goal is to have a robust model that is flexible enough to accommodate the different study designs and analytical methods used to generate the full pharmacokinetic dataset.

8. MIC data and PK studies for the IZS SA project (Dr. Stefano Lollai)

The ovine species lack official interpretation criteria for antibiotic resistance. VetCAST advised the Istituto Zooprofilattico Sperimentale della Sardegna (Dr. Stefano Lollai) for a study integrating MIC of tetracycline and oxytetracycline against Streptococcus uberis (72 isolates), presence of resistance genes, and pharmacokinetics of long-acting oxytetracycline in blood and milk of 10 healthy sheep and 10 sheep with spontaneous mastitis. A report was submitted in Dec. 2019 to the Italian Ministry of Health.

8. MIC data and ECOFF determination based mainly on the IMPART project

Within the One Health EJP project called IMPART (2018 – 2020) one of the work packages aims for establishing new ECOFFs of antimicrobials for veterinary use by performing susceptibility testing of animal pathogenic bacteria from strain collections of partner institutes. AST of veterinary pathogenic bacteria was performed by broth microdilution with three different antimicrobial panels using commercial plates (Sensititre©). All panels comprised long concentration ranges to determine MICs.

Nine partner institutes (NVI, Norway; BfR, Germany; SVA, Sweden; NVRI, Poland; IZLST, Italy; ANSES, France; APHA, UK; UU, the Netherlands; WBVR, the Netherlands) performed AST on 2,831 bacterial isolates involving nineteen different veterinary pathogenic bacteria including staphylococci (Staphylococcus pseudintermedius, S. hyicus), streptococci (Streptococcus agalactiae, S. dysgalactiae, S. uberis, S. suis, S. canis, S. equi subsp. zooepidemicus, S. equi subsp. equi, S. equisimilis), Pasteurella multocida, Mannheimia haemolytica, Actinobacillus pleuropneumoniae, Bordetella bronchiseptica, Haemophilus parasuis, Pseudomonas aeruginosa, and Klebsiella pneumoniae. This resulted in 1,310 MIC-distributions consisting of 47,640 MIC-values of 34 different antimicrobials. Joined susceptibility testing of a specified collection of animal pathogenic bacteria resulted in a large number of MIC distributions sufficient for setting ECOFFs for several of the bacteria/antimicrobial combinations. This work will be finished before the end of 2020.

9. New VetCAST guideline for veterinary diagnostic labs on the use of interpretive criteria for AST

European veterinary diagnostics labs used a broad variety of different interpretative criteria, e.g. CLSI veterinary clinical breakpoints, EUCAST human clinical breakpoints and/or epidemiological cut-off values (ECOFFs). A guideline on the use of currently available interpretive criteria for AST would be very helpful for veterinary diagnostic labs and support harmonisation of diagnostic AST in veterinary medicine at the European level. Hence, an expert team on behalf of VetCAST was appointed to create and publish a guideline. As a first step, the team compiles currently available interpretative criteria of specific antimicrobial/indication combinations for common pathogens of livestock and companion animals. Next, recommendations of the most appropriate criteria and AST methods will be provided. Finally, general advices for cases, which are not covered by the specific list, will complete the guideline. This guideline will be published on the VetCAST homepage later in 2020.
10. **VetCAST training schools**

A VetCAST workshop on clinical breakpoint determination was held in London in June 2019. A VetCAST session was planned during a conference scheduled for July 2020 in Russia. Unfortunately, this had to be cancelled due to the current COVID-19 pandemic. As for other upcoming training sessions, we anticipate the following VetCAST/ENOVAT training schools in the years to come:

- Early 2021: Basic PK/PD concepts (RVC, London)
- Early 2022: PK/PD modelling (likely in Bulgaria)
- Late 2022: Basic PK/PD concepts (Toulouse)
- Early 2023: Population PK / Monte-Carlo simulation (likely in France or Bulgaria)

These training schools are open to all, although - when co-arranged with ENOVAT to fulfill COST Action rules - a main target audience must be early career investigators and people from less-resourceful countries of Europe. Apart from training schools, VetCAST will contribute with a session/training school at the upcoming EAVPT conference in Lviv, Ukraine, in July 2021. Individual training and exchange of young researchers will also be done in the VetCAST/ENOVAT framework, e.g. one short-term scientific mission with a Serbian veterinary pharmacologist visiting the RVC has been scheduled for late 2020.

11. **Publications**

**2 new papers submitted on behalf of VetCAST to JVPT:**


**2 new papers published in the context of VetCAST:**


NB: Peter Smith, co-author of the latter paper, presented ideas on collaboration for AST of fish pathogens at the recent ENOVAT meeting.

**Any questions or comments on topics in the newsletter?**

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