# *In vitro* investigation of the effect of tetracycline antibiotics on *Trueperella pyogenes*

Tsvetelina Petkova[1], Nikolina Rusenova[3], Nasko Vasilev[2], Svetla Danova[4], Ivan Fasulkov[2], Manol Karadaev[2], Aneliya Milanova[1]

[1]Department of Pharmacology, Animal Physiology and Physiological Chemistry
[2]Department of Obstetrics, Reproduction and Reproductive Disorders
[3]Department of Microbiology, Infectious and Parasitic Diseases
**Faculty of Veterinary Medicine, Trakia university, 2020**
[4]**The Stephan Angeloff Institute of Microbiology, BAS**

# Introduction

- ***Trueperella pyogenes*** is often associated with purulent clinical endometritis and metritis in cows;
- Antimicrobial therapy is often applied in treatment of these uterine infections;
- Tetracycline antibiotics are among the most often used antibacterial drugs.

# Aim

Current investigation aimed to characterize in vitro effect of oxytetracycline (Oxy) and doxycycline (Doxy) on *Trueperella pyogenes* and its potential for biofilm formation.

# Material and methods

***Drugs***

- Oxytetracycline hydrochloride, Sigma-Aldrich
- Doxycycline hyclate, Sigma-Aldrich

***Bacteria***

- ***Trueperella pyogenes*** has been isolated from cows with clinical metritis. Two strains were further investigated. A number of the isolates shows the number of the cow from which it was isolated
- ***Staphylococcus aureus* O74** kindly provided by Utrecht University, a strain which is a strong biofilm producer, was used as a positive control
- **Tryptic Soy Broth** was used for cultivation of bacteria during experiment for biofilm production

# Material and methods

***MIC determination:***

- According to CLSI (2018) methodology;

***Test for biofilm formation***

- The experiments were run in 96 U-shaped well plates. 100 µl from the bacterial suspension from the isolates of *Trueperella pyogenes* (1x10^6) was added to 100 µl TSB without or with addition of tetracycline antibiotics at the tested concentrations (0.008 – 128 µg/mL). A sterile TSB served as blank. Wells (n=12) with bacterial cell suspension were used as positive control. Plates were incubated at 37°C for 40 h;
- The contents of each well was aspirated and washed 3 times with sterile distilled water to remove all non-adherent bacteria;
- Remaining cells were fixed with 200 µl 95% Metanol for 15 min, emptied and left to dry;
- Each well was stained with 150 µl of 1% Cristal violet for 15 min. Excess stain was rinsed off and plates were left to dry. Dye bound to adherent cells was resolubilized with 70% Ethanol for 30-60 min;
- The optical density was measured at 595 nm wavelength (Synergy LX Multi-Mode Microplate Reader, BioTek, USA);
- All the experiments were performed in triplicate with three independent repetitions.

# Material and methods

***Metabolic assay test for determination of the viability of bacterial cells***

Metabolic assay test for determination of the viability of bacterial cells was used according to manufacturer instructions (Vybrant Cell Metabolic Assay Kit, Molecular Probes, USA) The fluorescence was measured at Exλ=563 nm and Emλ=587 nm (Synergy LX Multi-Mode Microplate Reader, BioTek, USA)

The test was performed in triplicate at a concentrations of Oxy/Doxy at a concentration range between 0.25 – 4 μg/mL. The concentrations were chosen according to the previous results for MIC values. A sterile TSB was used as a negative control. Suspensions from Trueperella pyogenes isolates were used as controls.

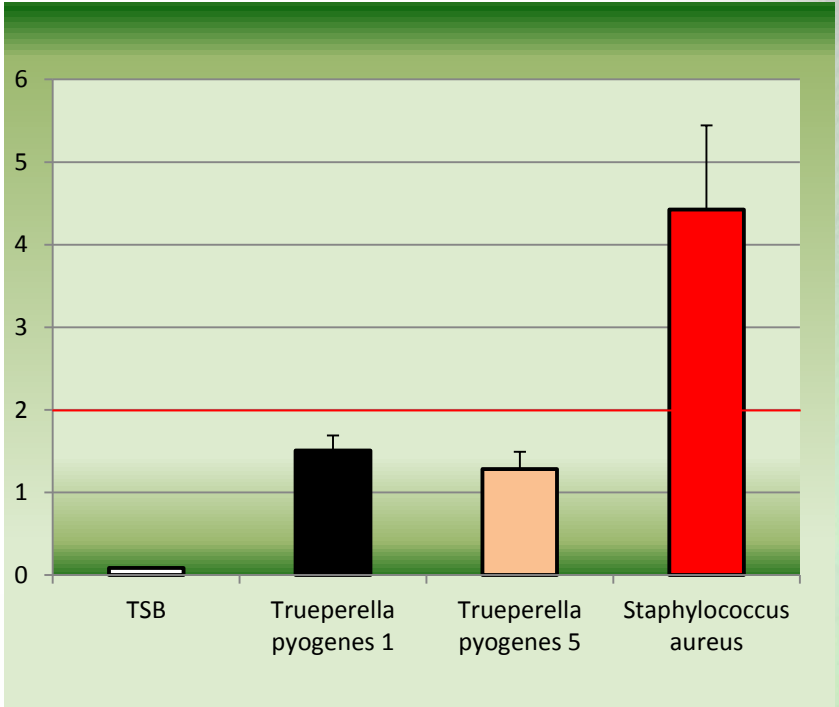
# Results

- MIC values of Oxy and Doxy: 8 µg/mL
- *OD difference compared to control TSB without bacteria:*

***Trueperella pyogenes 1 <2***

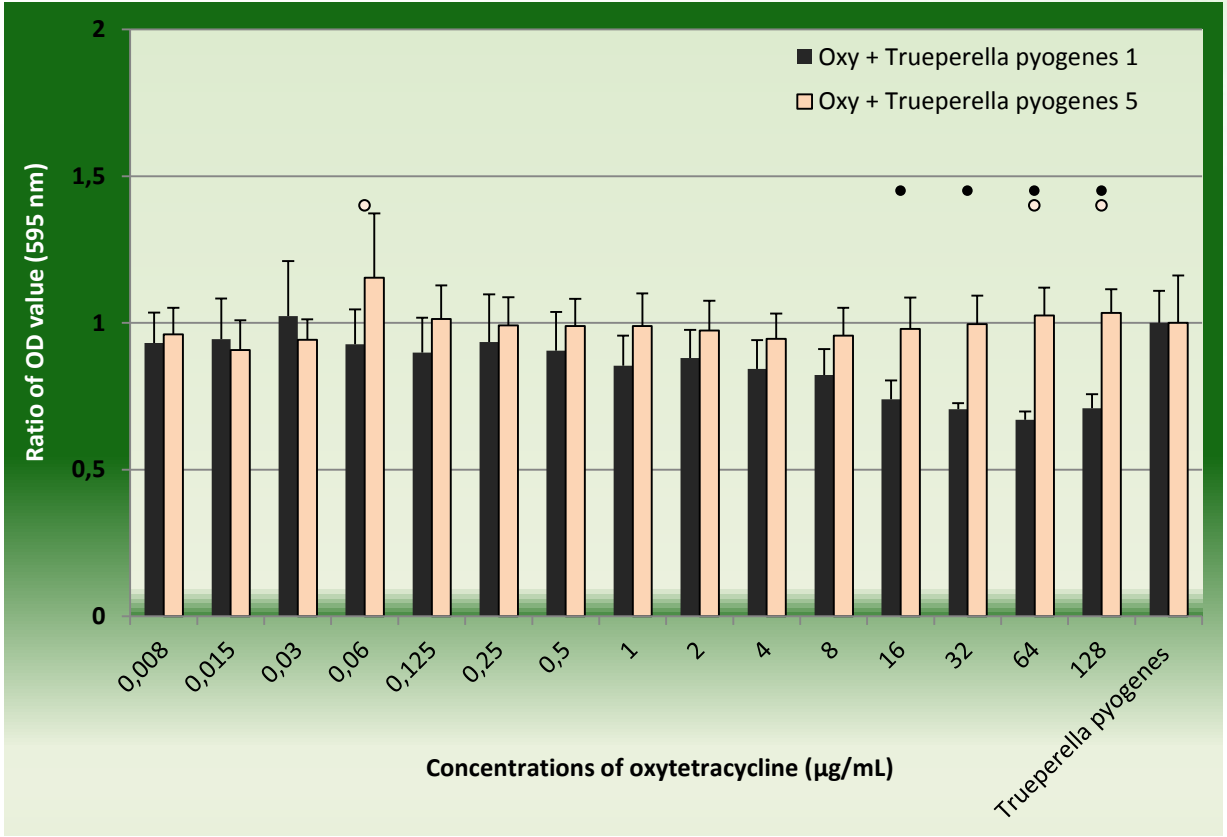
***Trueperella pyogenes 5 <2***

***Staphylococcus aureus* O74 > 4**



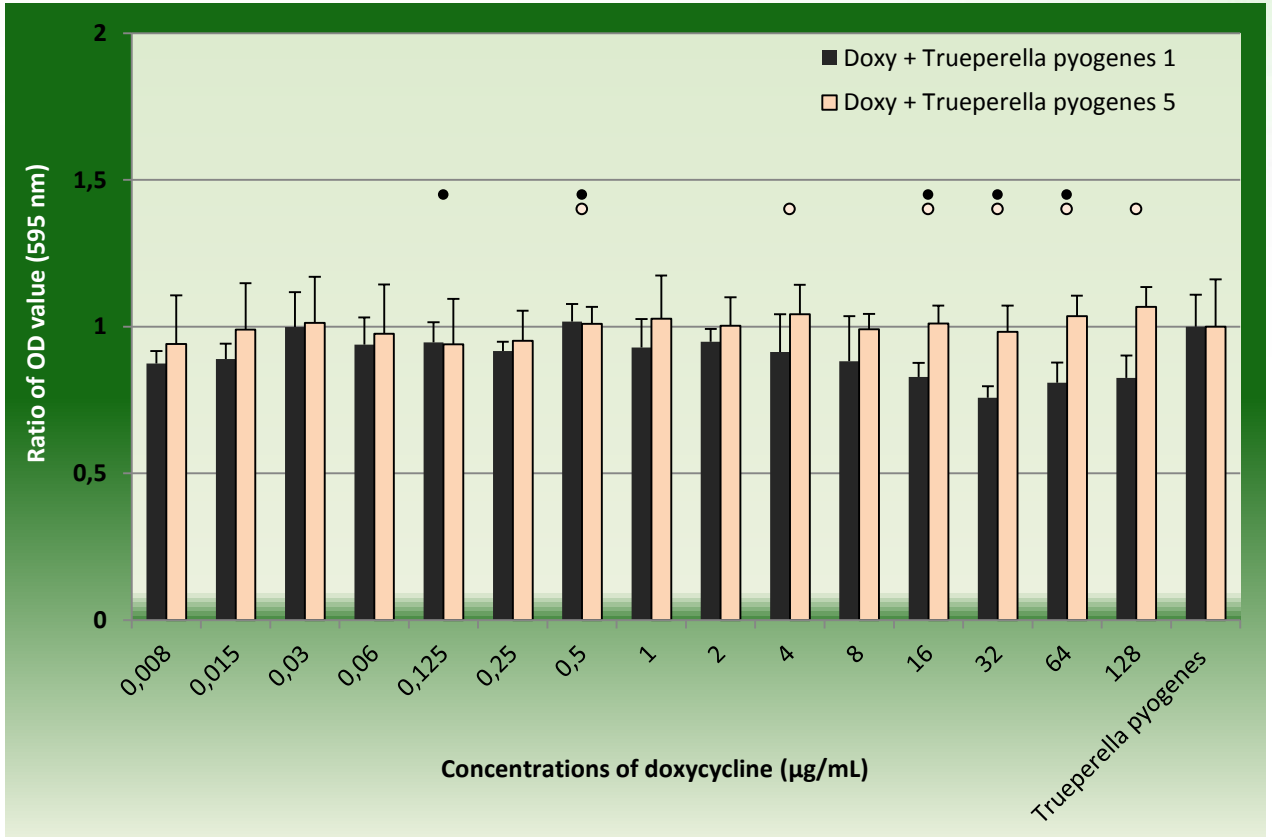
# Results

- Effect of antibiotic supplementation at different concentrations on ***Trueperella pyogenes 1*** and ***Trueperella pyogenes 5*** at the end of the experiment for biofilm formation, expressed as ratio $OD_{oxy\ suppl}/OD_{control}$
- Oxy decrease $OD_{oxy\ suppl}/OD_{control}$ *ratio at concentrations between 16 and 128 µg/mL in the experiment with* ***T. pyogenes 1***.



# Results

- Effect of antibiotic supplementation at different concentrations on ***Trueperella pyogenes 1*** and ***Trueperella pyogenes 5*** at the end of the experiment for biofilm formation, expressed as ratio $OD_{doxy\ suppl}/OD_{control}$
- Doxy did not decrease $OD_{doxy\ suppl}/OD_{control}$ *ratio, in contrary at some concentrations the ratio was significantly increased.*



# Results

The results from the metabolic assay tests show that the values of the fluorescence of the samples without/with Oxy or Doxy at a concentration range of 0.25 - 4 µg/mL were not statistically significantly different.

# Conclusions

- The investigated isolates of ***Trueperella pyogenes*** from cows with clinical metritis did not show potential for biofilm formation.
- The results confirmed that the positive control ***Staphylococcus aureus* O74** is a strong biofilm producer.
- The bacterial cells of ***Trueperella pyogenes 1*** and ***5*** cultured with Oxy/Doxy at a concentration range of 0.25 - 4 µg/mL show weak metabolic activity, compared to the positive control after the end of the biofilm assay which indicate presence of viable cells.

# Conclusions

- The growth of ***T. pyogenes 1*** cultured under conditions of biofilm assay was significantly inhibited by addition of Oxy at concentrations between 16 -128 µg/mL. Oxy had similar effect on ***T. pyogenes 5*** at concentrations between 64 -128 µg/mL;
- The growth of ***T. pyogenes 1*** and ***T. pyogenes 5*** cultured under conditions of biofilm assay was not inhibited by addition of Doxy. Higher OD of samples in comparison to positive controls shows that Doxy can potentiate the growth of bacteria at these experimental conditions.

# Acknowledgments

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Thank you for the attention!