Studies on isolation and susceptibility to antibiotics of the pathogens involved in *SCUD* etiology of aquatic turtles

George Cosmin NADĂȘ, Lucia Bel, Flore CHIRILĂ, Cosmina Maria BOUARI, Liviu Bogdan, Nicodim Iosif FIȚ
University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, 3-5 Calea Mănăştur street., 400372, Cluj-Napoca, Romania
cosmina.bouari@usamvcluj.ro

Abstract
The objectives of this study were to isolate and identify the bacteria present in the shell and plastron ulcers in a group of infected turtles compared with healthy turtles, and antibiotic susceptibility testing of bacterial species identified in order to recommend the appropriate treatment. A total number of 32 red-eared slider (Trachemys scripta elegans) with specific septicemic cutaneous ulcerative disease (SCUD) lesions were sampled from the shell and plastron, compared with 8 healthy turtles. Cotton swabs were used for sampling, and the inoculation was carried out on blood agar, XLD, MacConkey and SDA plates. Biochemical characterization used API Biomerieux 20 system. Susceptibility to antibiotics was evaluated using Kirby Bauer disk diffusion method on Mueller-Hinton agar. In the samples from turtles with lesions, predominantly Gram-negative bacteria were isolated, in particular Citrobacter freundii – 16 turtles, Escherichia coli – 13 turtles, Klebsiella – 10 turtles, Serratia – 9 turtles, Shigella – 8 turtles, Salmonella – 6 turtles, plus Gram positive bacteria such as Staphylococcus – 18 turtles, Micrococcus – 11 turtles and Bacillus – 9 turtles. Regarding the group of 8 healthy turtles ratio was significantly in favor of Gram positive, with Staphylococcus – 7 turtles, Micrococcus 6 and Bacillus 4 samples. Regarding the inhibition area diameter for infected turtles, the most efficient antibiotic was Doxycycline with the average value of 15.15 mm, Enrofloxacin with 14.95 mm and Florfenicol with 14.8 mm. Lower efficiency was observed for Ceftriaxone with 4.05mm and Colistin with 7.01 mm.
Keywords: *SCUD, turtles, ulcers, antibiotic susceptibility.*

Introduction

Septicemic cutaneous ulcerative disease (SCUD) is a shell disease of aquatic turtles caused by *Citrobacter freundii*; however, various bacteria have been isolated from diseased skin and shell. Anorexia, lethargy, and petechial hemorrhages on the shell and skin are seen; liver necrosis is also common. It is more common in soft-shelled turtles (*Appalone* spp.) (Jacobson, 2007).

SCUD is viewed more as a syndrome with many bacteria such as *Citrobacter freundii, Serratia anolium, Beneckea chitonovora* and other gram negative bacteria acting together with poor husbandry, poor water quality, abrasions and invertebrate predation to culminate in SCUD (Mader, 2006).

Shell ulceration can form when there is an injury to the shell in which the damaged area becomes infected. The initial injury could be minor and not easily noticeable or could be very obvious. It may have occurred in the form of an abrasion, scratch or even a burn. If left untreated or improperly cared for, this lesion could be penetrated and lead to a number of diseases such as fungal and bacterial infections and septicemia (Joyner, 2006).

The objectives of this study were to isolate and identify the bacteria present in the shell and plastron ulcers in a group of infected turtles compared with healthy turtles, and antibiotic susceptibility testing of bacterial species identified in order to recommend the appropriate treatment.

Materials and methods

The study was conducted in the laboratory of Microbiology at the Faculty of Veterinary Medicine Cluj-Napoca, Romania, between March and July 2015. A total number of 32 red-eared slider (*Trachemys scripta elegans*) with specific SCUD lesions were sampled from the shell and plastron, compared with 8 healthy turtles. The provenience of the turtles was Târgu Mureş ZOO. Cotton swabs were used for sampling, and the inoculation was carried out on blood agar, XLD, MacConkey and SDA plates. Biochemical characterization used API Biomerieux 20 system. Susceptibility to antibiotics was evaluated using Kirby Bauer disk diffusion method on Mueller-Hinton agar.

Results and discussions

In the samples from turtles with lesions, predominantly Gram-negative bacteria were isolated, in particular *Citrobacter freundii* – 16 turtles, *Escherichia coli* – 13 turtles, *Klebsiella* – 10 turtles, *Serratia* – 9 turtles, *Shigella* – 8 turtles, *Salmonella* – 6 turtles, plus Gram positive *Staphylococcus* – 18 turtles, *Micrococcus* – 11 turtles and *Bacillus* – 9 turtles.

Fig. 1. Lesions at the level of the plastron (arrow)

Fig. 2. Lesions at the level of the shell (arrow)

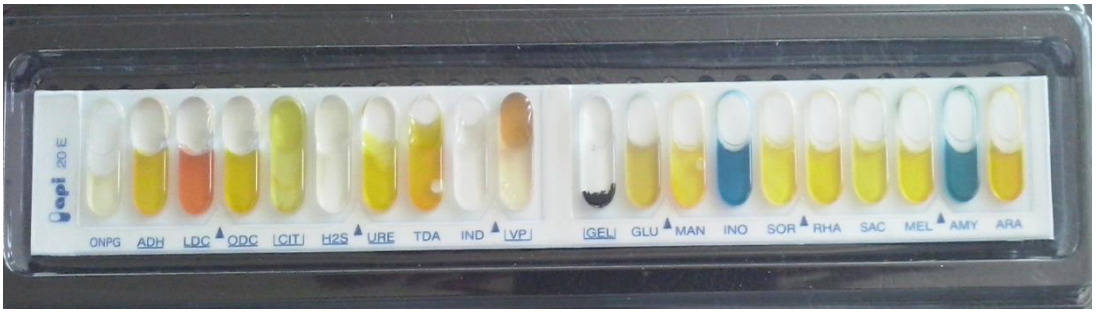

Fig.3. *Citrobacter freundii* strain identification – API 20 E gallery

Concerning the group of 8 healthy turtles ratio was significantly in favor of Gram positive, with *Staphylococcus* – 7 turtles, *Micrococcus* 6 and *Bacillus* 4 samples.

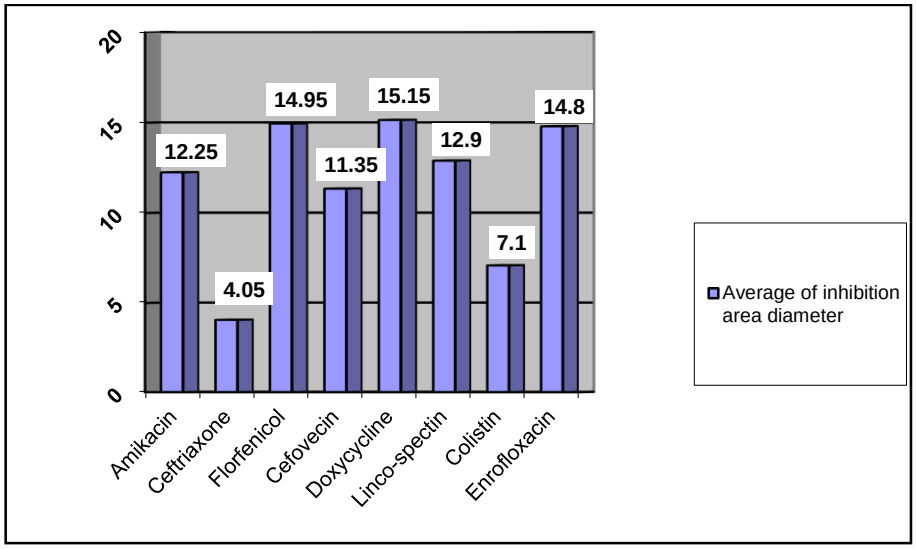

Fig.4. Average of inhibition area diameter for bacteria isolated from turtles with lesions

Regarding the inhibition area diameter for infected turtles, the most efficient antibiotic was Doxycycline with the average value of 15.15 mm, Enrofloxacin with 14.95 mm and Florfenicol with 14.8 mm. Lower efficiency was observed for Ceftriaxone with 4.05mm and Colistin with 7.01 mm.

The practice of feeding crayfish is often implicated in the ethiology of SCUD and should be discouraged.

Conclusions

1. *Citrobacter freundii* is not exclusively involved in SCUD etiology but predominates associated with other Gram negative bacteria.
2. Sanitation improvement and antibiotic administration improve turtle health status.
3. The overall susceptibility to antibiotic was only moderate probably due to previous treatments and difficult antibiotic penetration.

References

1. Jacobson E.R., 2007, Infectios diseases and pathology of reptiles, USA, 465, 491.
2. Joyner P.H., Brown J., et al., 2006, Characterization of the Bacterial Microflora of the Tympanic Cavity of Eastern Box Turtles With and Without Aural Abscesses, Journal of Wildlife Diseases, 42(4), 2006, pp. 859–864.
3. Kaplan H. M.,1957, Septicemic, cutaneous ulcerative disease of turtles. ProcAnim Care Panel, 7:273–277
4. Mader, D.R., 2006, Reptile Medicine and Surgery. 2nd Edition.
5. http://www.merckvetmanual.com/exotic-and-laboratory-animals/reptiles/bacterial-diseases-of-reptiles.

Retrospective analysis on somatic cells count trend in *Staphylococcus aureus* cows' mastitis

Ioan HUTU[1,3], Matiuti MARCEL[1,3], Camelia TULCAN[4], Simona MARC[2], Bianca LUNGU[1,3], Calin MIRCU[2,3*]

[1]Animal Productions and Public Veterinary Health Department, Preclinical[4] and [2]Clinical Department, Faculty of Veterinary Medicine, Banat University of Agricultural Science and Veterinary Medicine *King Michael I of Romania* – Timisoara, 119th Aradului Street, 300645, TM - Romania

[3] Experimental Unit, *Horia Cernescu* Research Experimental Units, Banat University of Agricultural Science and Veterinary Medicine *King Michael I of Romania* – Timisoara, 119th Aradului Street, 300645, TM - RO

*Correspondent author e-mail address: calinmircu@usab-tm.ro

Abstract

The study was carried on detection of mastitis, using increasing respectively decreasing trend of somatic cells count (SCC) of two successive samplings. The analysis was performed to identify the associations between SCC trend with retrospective and transversal milk quality indicators, and mastitis. From a BIOAMR database, 28 cows with ***a posteriori*** *diagnosed mastitis with Staphylococcus aureus (4/28 cases were methicillin-resistant Staphylococcus aureus – MRSA) were sampled. The trend of SCC was Spearman's rho correlated with previous lactose (r_s = +0.785, p=0.03), pH (r_s = +0.662 at p=0.019), and current SCC measurements (r_s = +0.781, at p=0.000). Increasing trend of SCC was retrospectively associated with lactose content (Z = -2.152 at p = 0.031), pH (Z = -2.152 at p = 0.031) SCC at first measurement (Z = -1.764 at p = 0.078) and currently associated with SCC (Z = -3.316 at p = 0.001), fat content (Z = -1.88 at p = 0.060) and fat/protein ratio (Z = -1.717 at p = 0.086). The 28 samples of the study did not revealed strong association between SCC trend and type of Staphylococcus aureus (p = 0.186 by Mann-Whitney test), even if MRSA had a higher increasing trend of SCC in comparison whit S. aureus (non-MRSA) infections (1403.5 vs. 288.2 thousands somatic cells). By preliminary results the trend of somatic cells could be an indicator in detection of mastitis but more case studies are necessary.*

Keywords: *Staphylococcus aureus, somatic cell count, cow mastitis and AMR.*

Introduction

Staphylococcus aureus is one of the main contagious pathogens responsible for the intra-mammary infection in dairy cattle and mastitis is one of the most economically important health traits for milk production *(6).* Detection of mastitis is generally based upon a number of indicators of mammary gland inflammation. The detection of the inflammation is based upon the response of the body to the mammary gland infection. The aim of the study is detection - diagnosed remarks in a retrospective study of the trend of somatic cells count (SCC) as two successive measurements in association with changes in milk, in order to improve future detection and treatment patterns.

Materials and methods

Farms and animals sampling: 15 partner farms of Extension unit in three counties of West Romania were stratified sampled (5 farms for each of the counties Arad, Bihor and Timiş) in a screening for dairy mastitis infection. All farms are included in the Official Control of Milk Production managed by Breed associations *(8,9,10,11)* - the last SCC value preview farm visit was considered. The SCC trend was calculated for in 11.2 ± 1.15 days' distance between two successive measurements. From overall *Bioeconomic approach to antimicrobial agents - use and resistance (acronym BIOAMR)* database 28 cases diagnosed with *Staphylococcus aureus* mastitis were

studied. In the study, from the sampled BIOAMR database, 28 cows with *a posteriori* diagnosed mastitis with *Staphylococcus aureus* (4/28 cases were methicillin-resistant Staphylococcus aureus – MRSA) were used.

Data collection and processing: the Californian Mastitis Test (CMT) and milk samples have been taken and primary analyzed (figure 1) on the farm for all dairy cows. Only positive sample to CMT were collected for chemical milk constituents (*Funke Gerber Lactostar Dairy Analyser*) and SCC analysis (*DeLaval cell counter DCC)*. Such chemical milk analysis device features fully automatic cleaning and rinsing system and zero point calibration for fast and accurate testing. The SNF (fat free dry matter), protein, fat, lactose and minerals with reproductibility maximum ± 0.04 % were measured and freezing point and density was calculated.

Microbiology analysis. The microbiological samples were collected by *COPAN's ESwab*^{TM} liquid collection and transport system form all positive quarters. Each infected quarter was considered an individual sample and only the most affected quarter (higher number of SCC) of one animal was included in the study. The germs were isolated and later, by microbiological exam, other 28 cases were used for the retrospective study. The typifying and antibiotic resistance was done by *Walk Away System* using *MicroScan® Dried Panels.*

Figure no. 1: Collecting and primary analysis of the samples at the farms level
CMT screening of the dairy cows in the milking parlor, in order to detect mastitis (*left*). Analyzing the milk positive sample for milk constituents *(Funke Gerber Lactostar Dairy Analyser)* and content, and somatic cells count (*DeLaval cell counter DCC*) – in the Animal Production Laboratory (*right*). *Source: UEX Media, 2019*

Statistical Analysis

The trend of SCC was considered positive or negative, depending on the value difference between the SCC measurement on the day of the farm visit, and the preview measurement, usually from results of Official Control of Milk Production. *SPSS® Statistics* software for *Spearman's* correlation, *Mann-Whitney U test* and nonparametric tests were used in order to do the analyses of association, frequency and differences between SCC trend and several groups and variables of the study. Significance value was accepted to be $\alpha = 0.05$.

Results and disscution

Milk somatic cells (considered as a Somatic Cells Count) are a mix of milk-producing cells and immune cells. Various factors and management practices modulate SCC and hematological parameters in a dairy herd: udder inflammation, parity, stage of lactation, unhygienic and incomplete milking, hot-humid climate, change in housing and feed or distress increase the SCC. Otherwise, healthy udder, antioxidants, hygienic milking, proper cow therapy, selection against mastitis, lower the *SCC (3,7,12,13,14).*

Staphylococcus aureus classified as a contagious pathogen, which can efficiently adapt to the environment of the mammary gland and spread cow to cow during milking, was considered in association with SCC. Several changes occur in blood *(4),* tissues and in milk *(5),* as a reaction to infection, including infiltration of leukocytes (measured by somatic cells content - SCC) and increased vascular permeability, resulting alterations in the chemistry of the milk resulting from hydrolysis of milk proteins by hydrolytic enzymes and oxidative substances released from phagocytes, alterations in milk pH and ionic solutes, and ingestion of milk components by phagocytes.

The trend of SCC was positive *Spearman's rho* correlated with previous lactose (r_s = +0.785, p=0.03), density (r_s = +0.662 at p=0.019), and with the SCC measurements on the day of the farm visit (r_s = +0.781, at p=0.000). Increasing trend of SCC was retrospectively associated with lactose content (Z = -2.152 at p = 0.031), density (Z = -2.152 at p = 0.031) SCC at first measurement (Z = -1.764 at p = 0.078) and currently associated with SCC at farm visit time (Z = -3.316 at p = 0.001), fat content (Z = -1.88 at p = 0.060) and fat/protein ratio (Z = -1.717 at p = 0.086). The 28 samples of the study did not reveal strong association between SCC trend and type of *Staphylococcus aureus* (p = 0.186 by *Mann-Whitney* test), even if MRSA had a higher increasing trend of SCC in comparison whit *S. aureus* (non-MRSA) infections (1403.5 vs. 288.2 thousands somatic cells).

The lower SCC trend can be associated with the capacity of the body to react to infection; the higher trend can be associated with infection. In fact, the percentage of noninfectious and infectious cells from SCC is, and can be, established. The percentage of leukocytes in SCC in milk is different in healthy cows vs. infected. *Alhussien et. al. 2016 (1,2)* reported 19% vs. 75% neutrophils (diameter 12-15 μm, nucleus is multi-lobed with bridges), 66% vs. 17% macrophages (diameter 20-30 μm, the largest cell type in milk) and lymphocytes 15 vs. 8% (diameter 9-16 μm, deeply stained round nucleus with low cytoplasm).

By preliminary results and corroboration with other study *(5),* the trend of somatic cells was proven to possibly be an indicator in detection of mastitis, but more studies are necessary.

Conclusions

- The higher trend of somatic cells count was associated with persistency of mastitis caused by '*S. aureus*
- The trend of somatic cells count was retroactively and positive correlated with milk lactose content and density.
- Trend of somatic cells count is associated with SCC, fat content and fat/protein ratio, on the day of the on-farm visit.

Acknowledgments

Costs of the research were covered under project *Bioeconomic approach to antimicrobial agents - use and resistance*, Contract no. *7PCCDI, PN-III-P1-1.2-PCCDI-2017-0361* financed by *UEFISCDI* and research was run within Animal Production Laboratory, part of *Horia Cernescu*

Research Unit in *Banat University of Agricultural Science and Veterinary Medicine "King Michael I",* Timisoara.

References

1. Alhussien M, Manjari P, Mohammed S, Sheikh A.A, Reddi S, Dixit S, Dang A.K. *Incidence of mastitis and activity of milk neutrophils in Tharparkar cows reared under semi-arid conditions.*Trop. Anim. Health Prod.2016, 48:1291–1295.
2. Alhussien M, Manjari P, Sheikh A.A, Seman S.M, Reddi S, Mohanty A.K, Dang A.K. *Immunological attributes of blood and milk neutrophils isolated from crossbred cows during different physiological conditions*. Czech J. Anim. Sci. 2016, 61:223–231.
3. Alhussien, M.N., Dang, A.K., *Milk somatic cells, factors influencing their release, future prospects, and practical utility in dairy animals: An overview*, Vet World. 2018,11(5):562–577.
4. Bobbo, T., Fiore, E., Gianesella, M., Morgante, M., Gallo, L., Ruegg, P.L., Bittante, G., Cecchinato, A., *Variation in blood serum proteins and association with somatic cell count in dairy cattle from multi-breed herds*. Animal., 2017, 11(12):2309-2319.
5. Botaro, B.G., Cortinhas, C.S., Dibbern, A.G., e Silva, L.F., Benites, N.R., dos Santos, M.V., *Staphylococcus aureus intramammary infection affects milk yield and SCC of dairy cows.* Trop Anim Health Prod. 2015; 47(1):61-6.
6. Davies G, Genini S, Bishop SC, Giuffra E. *An assessment of the opportunities to dissect host genetic variation in resistance to infectious diseases in livestock*. Animal.2009, 3:415–436.
7. Dufour S, Fréchette A, Barkema H.W, Mussell A, Scholl D.T. *Invited review:Effect of udder health management practices on herd somatic cell count*.J. Dairy Sci. 2011; 94:563–579.
8. Hutu I., *Considerations on milk production in west Romania dairy farms*, Lucrări Ştiinţifice Medicină Veterinară , 2012, 45(1):137-142.
9. Hutu, I., Adelina Proteasa, Irina Patras, Maria Serb, F. Ionescu, *Considerations on dairy farms in West Romania*, Lucrări Ştiinţifice Medicină Veterinară, 2012, 45(1):133-136.
10. Hutu, I., Mircu, C., *A study on formally inspected dairy cows' milk - a case study*, Lucrari Stiintifice - Universitatea de Stiinte Agricole a Banatului Timisoara, Medicina Veterinara, 2015, 48(1):67-73.
11. Hutu, I., *Considerations on lactation in cattle under Romanian formal recording* Lucrari Stiintifice - Universitatea de Stiinte Agricole a Banatului Timisoara, Medicina Veterinara, 2015, 48(1):74-80.
12. Morar, D., Ciulan, V., Simiz, F., Moţ, T., Hutu, I., Văduva, C., *Effect of heat stress on haematological parameters in dairy cows,* Lucrari Stiintifice - Universitatea de Stiinte Agricole a Banatului Timisoara, Medicina Veterinara, 2018, 51(2):65-70.
13. Sharma N, Singh N.K, Bhadwal M.S. Relationship of somatic cell count and mastitis:An overview. Asian Aust. J. Anim. Sci. 2011, 24:429–438.
14. Syridion D, Layek S.S, Behera K, Mohanty T.K, Kumaresan A, Manimaran A, Dang A.K, Prasad S. *Effects of parity, season, stage of lactation, and milk yield on milk somatic cell count, pH and electrical conductivity in crossbred cows reared under subtropical climatic conditions*. Milchwissenschaft. 2012, 67:362–365.