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■ Keywords

Antimicrobial, gene, resistance, *Salmonella*.

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Phenotypic and Genotypic Resistance Profile of *Salmonella* Typhimurium to Antimicrobials Commonly Used in Poultry

ABSTRACT

Isolates of *Salmonella* sp (104) from poultry samples were isolated and serotyped where eleven were identified as *Salmonella* Typhimurium (ST). ST isolates were phenotypically tested by disk diffusion and minimum inhibitory concentration (MIC). Four genes related to enrofloxacin (GyrA, GyrB, ParC and ParE), two to gentamicin (AadA and AadB) and two to ceftiofur (BlaCMY-2 and AmpC) resistance were searched by PCR. Our results showed ST resistance to all three antibiotics tested (18.1% for ceftiofur, 45.4% for gentamicin, and 18.1% for enrofloxacin) according to the diffusion test. In the MIC test, the ST isolates showed higher levels of resistance (27.2% for ceftiofur, 54.5% for gentamicin, and 18.2% for enrofloxacin). Three resistance genes out of four searched genes for enrofloxacin were found in the ST isolates. Regarding gentamicin and ceftiofur, resistance genes were found mainly in samples with resistant phenotypic profile. Interestingly, some phenotypically-resistant strains did not present the resistance gene, which suggests an alternative route of resistance. Also, sensitive strains had the presence of the gene. It is possible to conclude that the ST isolates evaluated in this study have a multidrug resistance profile to the antibiotics routinely used in poultry production, and potential of greater levels of resistance in the near future.

INTRODUCTION

Salmonellosis is considered a common cause of foodborne illnesses in humans, representing a significant public health problem in many countries. Studies show that poultry products have been recognized as a major transmitter of these bacteria, taking an important role in disease control (Carrasco *et al.*, 2012).

Due to the increasing incidence of infections by *Salmonella* sp and frequent reports of multiresistant strains, it is necessary to investigate the mechanisms of resistance used by this microorganism. According to Guerra *et al.* (2000), the spread of genes that confer resistance to microorganisms is due to the indiscriminate use of antibiotics in veterinary medicine. Those authors found that 31% of the isolates tested were resistant to all antimicrobials tested, and the species with the highest resistance was *S. Typhimurium*.

The main objective of this study was to investigate the genotypic and phenotypic antimicrobial resistance profile to gentamicin, ceftiofur and enrofloxacin of *S. Typhimurium* of poultry origin.

MATERIALS AND METHODS

Isolation

Salmonella sp isolates (104 samples) of poultry origin from Brazil were obtained from a private and accredited laboratory. These isolates

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**Effect of vitamin E on the immune system of SPF birds vaccinated
against infectious bronchitis virus****Efeito da vitamina E sobre o sistema imunológico de aves SPF
vacinadas contra o vírus da bronquite infecciosa**

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Abstract

In order to analyze the influence of vitamin E on the immune response of birds vaccinated and challenged with Infectious Bronchitis Virus in poultry (IBV), and which dose of vitamin E is the best to answer this, an experiment was conducted using 50 SPF birds housed with one day of life at Embrapa – Suínos e Aves. All birds were divided into 10 groups of five birds each, including positive and negative control groups, supplemented with 15, 50, and 200 IU of vitamin E/Kg added into the feed. On the 14th day of life the birds were vaccinated with a commercial vaccine for infectious bronchitis (H-120) and after 28 days, one group for each treatment was challenged with IBV, strain classical (M-41). Five days after challenge birds were necropsied and abdominal macrophages were collected for analysis of microbicidal activity and measurement of nitric oxide (NO). The lung was collected for cell count by flow cytometry and also for NO dosage. Organs of predilection were also collected for histological analysis and virus isolation to analyze the efficiency of the vaccine. The results were evaluated by ANOVA and Student t-test. An increased of microbicidal activity of abdominal macrophages was observed in the groups of birds with higher levels of vitamin E (200 IU/Kg) supplementation when compared with groups that did not receive or received minor amounts (0, 15, 50 IU/Kg), indicating an improvement in the innate immune response influenced by vitamin E supplementation in the diet. The supplementation of this vitamin in high doses also increases the ability of macrophages to produce NO. And, by flow cytometry, suggests that the macrophages are the primary cells recruited in the lung tissue to combat infectious bronchitis and this action was potentiated by the addition of vitamin E, independent of the dosage.

Key words: Immunity, nutrition, vaccine, vitamin E

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Experimental poisoning in broiler chickens by *Senecio vernonioides*, *Senecio conyzaeifolius* and *Senecio paulensis*¹

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ABSTRACT. Biffi C.P., Savaris T., Parizoto L.H., Ogliari D., Boreli V., Emmerich T., Gardner D. & Gava A. 2018. Experimental poisoning in broiler chickens by *Senecio vernonioides*, *Senecio conyzaeifolius* and *Senecio paulensis*. *Pesquisa Veterinária Brasileira* 38(11):2065-2069. Laboratório de Patologia Animal, Centro de Ciências Agroveterinárias, Universidade do Estado de Santa Catarina, Av. Luiz de Camões 2090, Conta Dinheiro, Lages, SC 88520-000, Brazil. E-mail: aldo.gava@udesc.br

Plants of the genus *Senecio* sp. are known to produce hepatic lesions in different animal species, including man. To evaluate the toxicity of three species of *Senecio* found in regions where cattle are bred in the state of Santa Catarina, experiments were conducted on broiler chickens (*Gallus domesticus*). Green leaves of *S. conyzaeifolius*, *S. vernonioides* and *S. paulensis* were collected and dried in the shade. After drying, the leaves were ground, mixed into the feed and fed to the chickens divided into 3 groups. Group 1 and Group 2 received single doses of 5g/kg and 20g/kg, respectively. Group 3 was given daily doses of 1g/kg for 20 days (values corresponding to green plant) and Group 4 (control) received free feed of the plant. For each group, five chickens were submitted to necropsy at 30 days and five to 60 days after planting, and five chickens that consumed *S. vernonioides* and *S. conyzaeifolius* at the dose of 1g/kg for 20 days were necropsied at 90 days after the beginning of the experiment. Macroscopic lesions were observed in chickens that consumed *S. vernonioides* at a dose of 1 g/kg for 20 days in birds necropsied at 30, 60 and 90 days and were characterized mainly by liver of firm consistency, diminished in size with yellowish coloration and evident lobular pattern, ascites and hydropericardium. In microscopy the main lesions observed were megalocytosis, hepatocyte swelling, fibrosis and biliary hyperplasia and were more intense at the dosage of 1 g/kg for 20 days for *S. vernonioides* and less severe in the chickens that ingested *S. conyzaeifolius*. The chickens that received *S. paulensis* showed no macroscopic and microscopic lesions.

INDEX TERMS: Poisonous plants, broiler chickens, poisoning plants, *Senecio conyzaeifolius*, *S. vernonioides*, *S. paulensis*, toxicoses.

RESUMO. [Intoxicação experimental em frangos de corte por *Senecio vernonioides*, *Senecio conyzaeifolius* e *Senecio paulensis*.] As plantas do gênero *Senecio* sp. são conhecidas

por produzirem lesões hepáticas em diferentes espécies de animais, inclusive no homem. Para avaliar a toxicidade de três espécies de *Senecio* encontradas em regiões onde são criados bovinos no estado de Santa Catarina, foram conduzidos experimentos em frangos de corte (*Gallus domesticus*). Folhas verdes de *S. conyzaeifolius*, *S. vernonioides* e *S. paulensis* foram coletadas e secadas a sombra. Após a secagem, as folhas foram trituradas, misturadas na ração e fornecidas aos frangos divididos em 3 grupos. O Grupo 1 e o Grupo 2 receberam doses únicas de 5g/kg e 20g/kg, respectivamente. Ao Grupo 3 foram fornecidas doses diárias de 1g/kg por 20 dias (valores correspondentes a planta verde) e o Grupo 4 (Controle) recebeu ração livre da planta. Para cada grupo, cinco frangos

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ADVANCE ACCESS

PROCESSING, PRODUCTS, AND FOOD SAFETY

Trimming and washing poultry carcass to reduce microbial contamination: A comparative study

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ABSTRACT The objective of this study was to compare the efficiency of washing and trimming broiler carcasses to reduce bacterial contamination. At the post-evisceration site, 100 broiler carcasses were collected during 4 visits to a slaughterhouse in Santa Catarina State, Brazil. Birds were from the same flock, age, and approximately 2.4 kg of weight. Groups were as follows: group 1, with fecal contamination; group 2, without fecal contamination; group 3, with fecal contamination and trimmed; group 4, with fecal contamination and washed; group 5, with fecal contamination, and washed and trimmed. Carcass washings were performed with at least 1.5 L/bird of potable water (0.5 to 1 mg/kg of residual chlorine) at room temperature (20–25°C) using spray cabinets with 44 spray nozzles distributed into 2 chambers (pressure of 2 kgf/cm² and 4 kgf/cm²).

Washed carcasses (trimmed or not) showed significantly ($P < 0.05$) lower counts of aerobic mesophiles (plate count agar) on the third evaluation, and even lower ($P < 0.01$) counts for total coliforms (CT) and fecal coliforms (*Escherichia coli*). Trimmed carcasses showed significantly lower counts ($P < 0.05$) for plate count agar; however, we observed higher counts for *E. coli* ($P < 0.05$). The association of both treatments (washing and trimming) showed significantly higher ($P < 0.05$) counts for coliforms (CT and *E. coli*). We can conclude that the washing method is overall more efficient than the trimming method to decontaminate chicken carcasses at the post-evisceration site. Hopefully, our findings can help poultry companies to minimize production costs by applying the washing method for carcass decontamination.

Key words: chicken meat, decontamination, microbial contamination, trimming, washing

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INTRODUCTION

Brazilian poultry production has grown considerably in recent years, keeping Brazil as the third largest producer in the world and the largest exporter of chicken meat (UBABEF, 2012). Even with this high production of excellent quality, approximately 8% of total poultry production is wasted due to strict sanitary measures related to the slaughter process (Santana et al., 2008). Among these losses, the most relevant is carcasses slightly contaminated by fecal material. According to Silva and Pinto (2009), such contamination corresponds to 22.84% of all carcasses condemned by the Brazilian Federal Inspection Service.

The bacterial flora of processed poultry carcasses consists of foodborne pathogens, bacterial indicators of fecal contamination, and spoilage microorganisms (Barnes, 1972). Some important pathogens found in poultry products considered pathogenic to humans are *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli*, and *Campylobacter* spp. Therefore, ways to minimize poultry contamination and ensure food safety are needed.

Currently, the method employed to remove fecal contamination allowed by Brazilian law is trimming using knives, where parts slightly contaminated are removed manually, which increases labor, and consequently, costs. The use of spray showers was recently approved in Brazil to reduce microbial contamination (Brazil, 2011). However, there is a lack of studies comparing the efficiency of this new spray method, called the washing method, and in addition, some officials responsible for poultry inspection are still reluctant to approve it.

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Development and evaluation of a novel subunit vaccine for *Mycoplasma gallisepticum*

[Desenvolvimento e avaliação de uma nova vacina para *Mycoplasma gallisepticum*]

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ABSTRACT

Adhesion proteins from *Mycoplasma gallisepticum* (MG) encoded by cytoadhesion genes *mgc1* and *mgc2* were cloned into plasmid vectors and transformed into *E. coli*. Seventeen groups of specific-pathogen free (SPF), birds at four weeks of age were used to inoculate these two proteins (MGC1 and MGC2) mixed into an oil emulsion creating a novel MG vaccine. Six different protein concentrations (50, 100, 200, 400, 800, and 1000 µg/bird) were tested with two equal concentration doses at four and seven weeks of age. In addition, many control groups were needed such as bacterin, membrane, no vaccine or challenge, oil emulsion alone, and no vaccine but challenged. Three weeks following the second vaccination, 50% of the birds in each treatment group were challenged with MG strain S6. The remaining birds were left as contacts to verify protection against horizontal transmission. All birds were bled before vaccinations, challenge and euthanasia. Birds were negative for MG at the first vaccination, as shown by serum plate agglutination test. At necropsy, tissue samples (trachea, lungs, and air sacs) were collected for histopathological examination. Swabs from trachea were used for PCR analysis. ELISA results showed a strong immune response to both protein preparations and almost the same response level for different doses tested, proving the immunogenic features of MGC1 and MGC2. However, humoral responses failed to prevent MG infection and disease when challenged as demonstrated by PCR and histopathology. MGC1 contact birds showed some degree of infection by PCR analysis. In addition, histopathological and ELISA results suggest that contact birds did not have enough time to develop lesions and to mount an immune response.

Keywords: evaluation, development, mycoplasmas, poultry, vaccine

RESUMO

Os genes *mgc1* e *mgc2*, codificadores de duas proteínas de adesão (MGC1 e MGC2) da bactéria *Mycoplasma gallisepticum*, foram clonados em *E. coli*. Dezessete grupos de aves livres de patógenos específicos (SPF), com quatro semanas de idade, foram inoculados com uma emulsão oleosa contendo as proteínas MGC1 e MGC2 purificadas. Seis concentrações (50, 100, 200, 400, 800, e 1000 µg/ave) foram testadas com duas doses idênticas, às quatro e sete semanas de vida, respectivamente. Além disso, grupos controles foram avaliados com uma vacina comercial contra micoplasmose aviária, membrana de MG, grupo sem vacina/sem desafio, grupo vacina oleosa de MGC1 sem desafio, grupo com vacina oleosa de MGC2 sem desafio, grupo desafiado mas sem vacina. Três semanas após a segunda e a última vacinação, 50% dos animais dos grupos tratamentos foram desafiados com a cepa S6 de MG. O restante dos animais foi deixado como contato para averiguar proteção contra a transmissão horizontal da doença. Amostras de sangue de todas as aves foram coletadas antes das vacinações, do desafio e da eutanásia. As aves eram negativas para MG às quatro semanas de vida, conforme visto na aglutinação em placa. Na necropsia, tecidos (traqueia, pulmão e sacos aéreos) foram coletados para exame histopatológico. Suabes da traqueia foram utilizados para a PCR. Os resultados do ELISA demonstraram forte resposta imune contra as duas proteínas testadas e resposta similar independentemente do número de doses, provando a sua capacidade imunogênica. Porém, esta resposta humoral gerada foi incapaz de prevenir a infecção e a doença após desafio, conforme demonstrado pelos exames PCR e histopatológico. Aves-contato, inoculadas com MGC1, demonstraram estar infectadas nas

Intoxicação espontânea e experimental por frutos de *Eugenia uvalha* Cambess. (Myrtaceae) em ovinos¹

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ABSTRACT. Emmerich T., Borelli V., Cardoso T.C., Wisser C.S., Wicpolt N.S., Biff C.P., Traverso S.D. & Gava A. 2014. [Spontaneous and experimental poisoning by fruits of *Eugenia uvalha* Cambess. (Myrtaceae) in sheep.] Intoxicação espontânea e experimental por frutos de *Eugenia uvalha* Cambess. (Myrtaceae) em ovinos. *Pesquisa Veterinária Brasileira* 34(5):438-442. Laboratório de Patologia Animal, Centro de Ciências Agroveterinárias, Universidade do Estado de Santa Catarina, Av. Luiz de Camões 2090, Bairro Conta Dinheiro, Lages, SC 88520-000, Brazil. E-mail: aldo.gava@udesc.br

We describe the epidemiological, clinical signs and lesions of a disease of sheep characterized by apathy, sialorrhoea, teeth grinding, circling, blindness, incoordination, seizures and opisthotonos usually followed by death. The disease occurred in February and March 2009 of 2013, in the West highlands and mountainous regions of Santa Catarina State and was associated with the falling fruits of "uvaieira" (*Eugenia uvalha* Cambess). In farms where outbreaks occurred had loads of fruit fallen to the ground and the sheep ate them hungrily. Those who survived remained with intermittent seizures. No gross or histological changes were observed. However, the whole fruits or their fragments mixed in the content of the pre-stomachs and bowels were found. The disease was reproduced providing the uvaieira fruits to three sheep, in daily doses of 45,45 g/kg, 68,18 g/kg and 82,35 g/kg each, for until six days. The main way to diagnose the uvaieira poisoning is the observation of the clinical and epidemiological data, associated with the presence of fruits and seeds in the gastrointestinal tract.

INDEX TERMS: Poisonous plants, *Eugenia uvalha*, Myrtaceae, uvaieira, plant poisoning, sheep.

RESUMO. Descrevem-se os dados epidemiológicos, sinais clínicos e lesões de uma enfermidade de ovinos caracterizada por apatia, sialorréia, ranger de dentes, andar em círculos, cegueira, incoordenação motora, opistótono e convulsões, geralmente seguidos de morte. A doença ocorreu nos meses de fevereiro e março de 2009 a 2013, nas regiões Oeste e Planalto do Estado de Santa Catarina e está associada à queda dos frutos da "uvaieira" (*Eugenia uvalha* Cambess). Nas propriedades onde ocorreram os surtos havia grande quantidade de frutos caídos ao chão e os ovinos os consumiam avidamente. Os que sobreviveram permaneceram com convulsões intermitentes. Não foram observadas alterações macroscópicas ou histológicas. No entanto, fo-

ram encontrados frutos inteiros ou fragmentados, misturados ao conteúdo dos pré-estômagos e intestinos. A doença foi reproduzida a partir do fornecimento de frutos maduros de uvaieira a três ovinos, nas doses diárias de 45,45g/kg, 68,18g/kg e 82,35g/kg por até seis dias. A principal forma de diagnóstico de intoxicação por "uvaieira" é a observação do quadro clínico e epidemiológico, associado à presença de frutos e sementes no trato gastrointestinal.

TERMOS DE INDEXAÇÃO: Plantas tóxicas, *Eugenia uvalha*, Myrtaceae, uvaieira, intoxicação por plantas, ovinos.

INTRODUÇÃO

Uma enfermidade de ovinos foi observada na região do Planalto e Oeste Catarinense e caracterizou-se por apatia, sialorréia, ranger de dentes, andar em círculos, cegueira, incoordenação motora, opistótono e convulsões, muitas vezes seguidos de morte. Os animais que sobreviveram permaneceram com sequelas da doença, caracterizadas por convulsões intermitentes. A enfermidade foi observada durante

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Intoxicação experimental por folhas de *Crotalaria pallida* (*mucronata*) em ovinos¹

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ABSTRACT. Borelli V, Cardoso T.C., Biffi C.P., Wicpolt N., Ogliari D., Savari T., Traverso S.D. & Gava A. 2016. [Experimental poisoning by *Crotalaria pallida* (*mucronata*) in sheep.] Intoxicação experimental por folhas de *Crotalaria pallida* (*mucronata*) em ovinos. *Pesquisa Veterinária Brasileira* 36(10):935-938. Laboratório de Patologia Animal, Centro de Ciências Agroveterinárias, Universidade do Estado de Santa Catarina, Av. Luiz de Camões 2090, Bairro Conta Dinheiro, Lages, SC 88520-000, Brazil. E-mail: a2ag@cav.udesc.br

This paper describes the toxic potential of the green leaves of *Crotalaria pallida* and the clinical signs in experimentally poisoned sheep. Green leaves of *C. pallida* were collected in São João do Sul, Santa Catarina, and administered orally to six adult sheep, as single dose of 40, 20, 10, 5, 2.5 and 2.5g/kg, and for two sheep at doses of 0.63 and 1.25g/kg during 30 days. The poisoning was reproduced at lethal doses of 2.5g/kg. One of the sheep that received a single dose of 2.5g/kg and another that received daily doses did become ill. All sheep which developed clinical signs died between 13 and 22 hours after administration of the plant. Slight signs started in about 11 hours after ingestion of the plant, and clinical signs included loose stool, restlessness and increased heart and respiratory rates. A few minutes preceding death, pronounced abdominal breathing and severe dyspnea was observed. The main gross lesions were restricted to the thoracic cavity, as severe pulmonary edema and hydrothorax. Lung microscopy showed marked edema in the diffuse pleural surface, the interlobular spaces and around the peribronchial arteries. Multifocal edema was observed within alveoli and around the bronchi and bronchioles, and mild multifocal infiltration of neutrophils. The clinical and pathological alterations of the experimental poisoning was similar to that observed in the spontaneous poisoning, characterized by marked pulmonary edema and death shortly after the onset of clinical signs.

INDEX TERMS: Poisonous plants, animal pathology, *Crotalaria mucronata*, plant poisoning, sheep.

RESUMO. Descrevem-se o quadro clínico, patológico e o potencial tóxico das folhas verdes de *Crotalaria pallida* em ovinos. Para o experimento folhas verdes de *C. pallida* foram coletadas no município de São João do Sul em Santa Catarina e administradas por via oral para seis ovinos adultos, em doses únicas de 40, 20, 10, 5, 2,5 e 2,5g/kg, e para dois ovinos, em doses diárias de 0,63 e 1,25g/kg durante 30 dias. Reproduziu-se a intoxicação com doses letais a

partir de 2,5g/kg. Um dos ovinos que recebeu a dose única de 2,5g/kg e os que receberam as doses diárias, não adoeceram. Todos os ovinos que apresentaram sinais clínicos morreram entre 13 e 22 horas após a administração das folhas. Sinais clínicos leves como fezes pastosas, inquietação e aumento das frequências cardíaca e respiratória tiveram início cerca de 11 horas após a ingestão da planta. Minutos antes da morte observaram-se dispnéia intensa com respiração abdominal pronunciada. As principais lesões macroscópicas foram restritas à cavidade torácica, como hidrotórax e edema pulmonar acentuado. O exame microscópico revelou no pulmão, edema acentuado e difuso na superfície pleural, nos espaços interlobulares e ao redor das artérias peribronquiais e peribronquiolares. Edema multifocal foi observado no interior de alvéolos e ao redor de brônquios e bronquíolos, além de leve infiltrado neutro-

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