South Asian Journal of Parasitology

3(3): 1-5, 2019; Article no.SAJP.53593

Ascaridia galli Protect *Salmonella typhimurium* from Antibiotics

E. I. Sop Foka[1], J. Yondo[2], B. Katte[3], C. N. Noumedem Anangmo[2*] and Mpoame Mbida[1]

[1]Department of Animal Biology, Faculty of Sciences, University of Dschang, Cameroon.
[2]Department of Biomedical Sciences, Faculty of Health and Pharmaceutical Sciences, University of Dschang, Cameroon.
[3]Department of Animal Production, Faculty of Agronomy and Agricultural Sciences, University of Dschang, Cameroon.

Authors' contributions

This work was carried out in collaboration among all authors. Authors EISF, JY, BK, CNNA and MM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author EISF managed the analyses of the study. Authors EISF and CNNA managed the literature searches. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Sirigireddy Sivajothi, Department of Veterinary Parasitology, College of Veterinary Science, Sri Venkateswara Veterinary University, Andhra Pradesh, India.
Reviewers:
(1) Immaculate Jeyasanta, India.
(2) Afroza Parvin, Jahangirnagar University, Bangladesh.
Complete Peer review History: http://www.sdiarticle4.com/review-history/53593

Original Research Article

Received 24 October 2019
Accepted 29 December 2019
Published 11 January 2020

ABSTRACT

Background: *Salmonella* bacteria and *A. galli* worms cause serious illness, pathological defects and economic losses even in modern poultry production systems. This study was undertaken to elucidate the antibiotic sensitivity of *Salmonella typhimurium* when associated to *A. galli*.
Materials and Methods: *Salmonella typhimurium* LT_2 was incubated *in vitro* in the presence and in the absence of *A. galli,* with three antibiotics: amoxicillin, cefepime and chloramphenicol.
Results: In the presence of *A. galli* worm the antibiotics showed a significant reduction in the ability to inhibit *Salmonella typhimurium* growth. Treatment of salmonellosis becomes less efficient when the bacteria are associated with *A. galli* worm.
Conclusion: *A. galli* may somehow protect the bacteria from the antibiotics through unknown mechanisms. However, further work is necessary to evaluate the mechanisms involved in the protection of *A. galli.*

**Corresponding author: E-mail: noums11@yahoo.fr, ericigor2006@yahoo.fr;*

Keywords: Salmonella typhimurium LT_2; Ascaridia galli; in vitro; antibiotics; salmonellosis.

1. INTRODUCTION

The occurrence of interactions between gram negative enteric bacteria and other parasites has recently begun to surface.

Some experimental studies have begun to demonstrate persistence and enhanced growth of *Salmonella* bacteria in chickens concurrently infected with *Ascaridia galli* worms [1]. The demonstration of *Salmonella* within the gut or on the body wall of *A. galli* worms further indicated an intimate association of the two pathogens [2]. Studies of the antibiotic inefficacy during the treatment of *Salmonella* and *Schistosoma* association were reported, despite drug sensitivity of the bacteria in an *in vitro* experiment [3].

The aim of this study was to test the *in vitro* antibiotic sensitivity of *Salmonella typhimurium* in the presence of *A. galli* worm. To assess the nature and extend of this phenomenon, we developed an *in vitro* antibiotic protection assay in which anti-*Salmonella* antibiotic efficacy is evaluated using *Salmonella typhimurium* LT_2 incubated with adult *A. galli* worm and a variety of three antibiotics. We used the experimental approach previously described for *Schistosoma*-associated *Salmonella* [4]. Based on the experiment with *Schistosoma*-associated *Salmonella,* the hypothesis was as follow: The presence of the *A. galli* worm considerably reduce the efficacy of the antibiotics.

2. MATERIALS AND METHODS

The study was carried out in the Biology and Applied Ecology Research Unit, and in the Animal Health and Physiology Research Unit at the University of Dschang, Cameroon.

2.1 Isolation of Parasites

A. galli eggs were recovered from female mature worms, obtained from naturally infected local chickens. These were incubated for 30 days in 0.1 N sulfuric acid in order to obtain embryonated eggs as previously described by Permin, et al. [5]. Chickens of Arbor acres breed obtained from a commercial hatchery and previously raised up to 42 days, were then infected with *A. galli* embryonated eggs [5]. They were sacrificed 30 days post-infection and adult worms were removed from the intestines.

2.2 Antibiotic Protection Assay

To determine, if *Salmonella typhimurium* can evade antibiotics when associated with the nematode, *S. typhimurium* (LT_2 1344) sensitive to antibiotics, were incubated with a therapeutic concentration of three classes of antibiotics namely amoxicillin, cefepime and chloramphenicol (30 µg/ml). These antibiotics are currently recommended against systemic salmonellosis [6]. Chloramphenicol was chosen because it has been reported to be ineffective in the treatment of *Salmonella-Schistosoma* co-infection, although its sensitivity has been demonstrated in *Salmonella* isolates [3]. Approximately 10^4 CFU *Salmonella typhimurium,* were incubated for 2 hours with a single adult *A. galli* in tissue culture medium containing 1 ml of Roswell Park Memorial Institute (RPMI 1680) with 50% fetal bovine serum (difco) [4]. The co-incubation was followed by the addition of different concentrations of 30 µg / ml [7] of one of the antibiotics. After two hours, the antibiotics were washed, trypsin (0.05%) was then added in order to the cleave *Salmonella* fimbriae [8]. The media was then removed and plated on *Salmonella-Shigella* agar and incubated at 37ºC for 24 hours. *Salmonella typhimurium* colonies were then counted for the determination of the percentage of bacteria recovered, i.e. an indirect measure of the antibiotic efficacy. The percentage of the antibiotics efficacies equals to:

$100 \times (10^4 - \text{number of CFUs recovered}) / 10^4$) [4]

2.3 Statistics Analysis

Statistical analysis was performed using ANOVA at 5% with Scheffe's F test for multiple comparisons. Comparisons were made between antibiotics and incubation conditions.

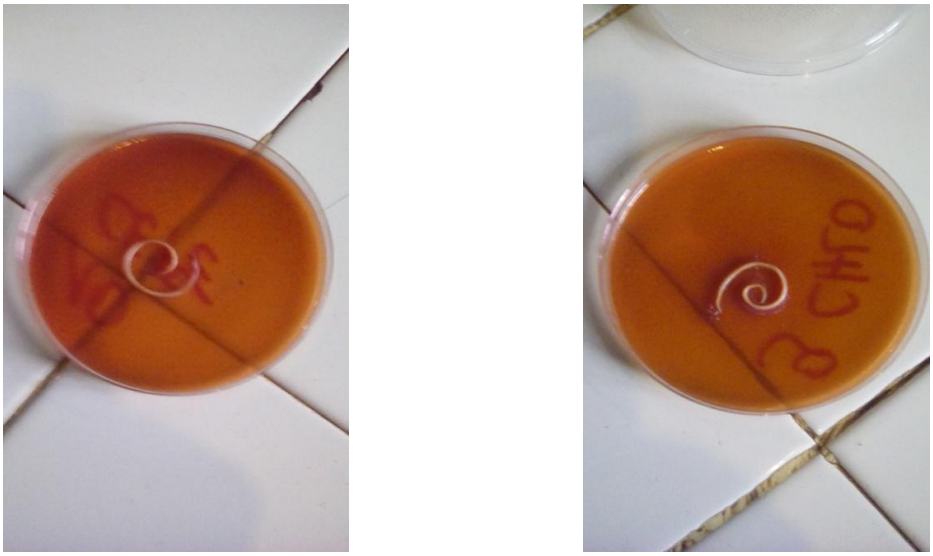
3. RESULTS

An antibiotic protection assay in which antibiotic sensitive *S. typhimurium* were incubated with a therapeutically relevant concentration of amoxicillin, cefepime and chloramphenicol known to be 100% lethal to these bacteria showed in Table 1 that: Bacterial survival was significantly elevated in the presence of *A. galli* compared to when the nematode was absent. Specifically, the three antibiotics killed more than 99% of *Salmonella typhimurium* when incubated in the absence of worms, while in their presence,

Table 1. Antibiotic efficacy (Anti-*Salmonella*) at the dose 30 µg/ml in the presence and in the absence of *Ascaridia galli* worm

Antibiotics	Dose (µg/ml)	% of antibiotic efficacy	
		Presence of worm	Absent of worm
Amoxicillin	30	75.86	99.86
Chloramphenicol	30	45.46	99.46
Cefepime	30	60.70	99.70

NB: Each data represents the mean of at least three experiments. The experiments were made at 5% significance level



Before incubation **After incubation**

Fig. 1. Plate showing different incubation period for *Ascaridia galli*

the percentage of antibiotics efficacy was less than 76% for amoxicillin, 60.70% for cefepime and 45.46% for chloramphenicol. Amoxicillin appears to be the most effective antibiotic. This protective effect of *A. galli* doesn't depend on the ability of *Salmonella typhimurium* to invade eukaryotic cells because the strain is a non-invasive strain.

4. DISCUSSION

Ascaridia galli worms reduce antibiotics (anti-*Salmonella*) efficacy, when associated to *Salmonella typhimurium*. This situation reminds the experimentation with *Schistosoma*-associated *Salmonella* [4]. The experiment showed antibacterial treatment failures in *Schistosoma-Salmonella* co-infections despite *in vitro* sensitivity to the antibiotics in the absence of the trematode. This situation is not related to the invasiveness of bacteria strain used. Barnhill, et al. [4] reported that even non-invasive and hyper-invasive *Salmonella* strains were recalcitrant to antibiotics in the presence of schistosomes. This phenomenon was not observed with *Salmonella* that bind to other eukaryotic cells such as mammalian epithelial HEp-2 cells or tegument cells on the surface of free platyhelminths [4]. Additionally, several *Salmonella* serotypes have host-specific tropisms, such as *S. pullorum* and *S. gallinarum* are adapted to birds and benefit from *Schistosoma* protection [9]. Our study thus reveals that *A. galli* may possesses mammalian cell-like epitopes, which are *Salmonella* binding sites.

Since antibiotic resistance is only observed in the presence of the nematode, the mechanism of this resistance may be a physical barrier that could include a biofilm. Indeed, the glycocalyx of *A. galli* is thick, highly immunogenic and contains sugar. This suggests that antibiotics hardly penetrate the medium [10]. *Ascaridia galli* may have the capacity to chemically alter the antibiotics. Bacterial quiescence is another possibility, but *Salmonella* depends on folate self-depravity and the temporal nature of this

phenomenon [11]. The same argument may be used for the activation of other metabolic changes in the physiology of *Salmonella*, that is, the two-hour incubation period would be clearly insufficient to talk about resistance. However, this resistance may be due to the behavior of some breeders who often mix antibiotics with food, as an adjuvant, without any standard. The consequence is the selection of many resistant strains from the outset to several families of antibiotics that can infect animals and humans and make, all treatments with antibiotics impossible [12].

Amoxicillin was the most effective for the inhibition of *S. typhimurium* whether in the presence or absence of *A. galli.* This is quite normal because it is the third-generation amoxicillin that has been used [13], it is amoxicillin associated with clavulanic acid. This combination with clavulanic acid makes it possible to prevent the destruction of the antibiotic by certain pathogens. Chloramphenicol was the least effective of the three antibiotics. This could be explained by the fact that this drug has been abused in both animals and humans. In addition, this drug is not digested and it is also responsible for fatal aplastic anemia, a condition that appears to be 13 times more common after use of chloramphenicol [14,15]. Cefepime is an antibiotic molecule of the third-generation cephalosporin class. Further in-depth studies are required to shed light on the basic principles of the phenomena described above. This study provides plausible reasons that explain the difficulties of the antimicrobial therapy of the salmonellosis-helminthiasis association in humans and on the experimental models.

5. CONCLUSION

Treatment of salmonellosis becomes less efficient when the bacteria are associated with *A. galli* worm*. A. galli* may somehow protect the bacteria from the antibiotics through unknown mechanisms. However, further work is necessary to evaluate the mechanisms involved in the protection of *A. galli.*

ETHICAL APPROVAL

All authors hereby declare that “principles of laboratory animal care” (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

ACKNOWLEDGEMENT

The authors wish to thank LoVerde Philip (University of Texas Health, San Antonio, USA) who kindly provided *Salmonella typhimurium* strain used in the study and NFOR Brian for editing the manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Eigaard NM, Schou TW, Permin A, Christensen JP, Ekstron CT, Ambrosini F, Cianci D, Bisgaard M. Infection and excretion of *Salmonella enteritidis* in two different chicken lines with concurrent *Ascaridia galli* infection. Avian Pathology. 2006;35(6):487-493.
2. Sop Foka EI, Yondo J, Agyingi L, Tsila HG, Mpoame Mbida. Transport capacity of *Salmonella typhimurium* by *Ascaridia galli* body parts. International Journal of Science and Research. 2019b;8:768-770.
3. Njunda A, Oyerinde J. *Salmonella typhi* infection in *Schistosoma mansoni* infected mice. West African Journal of Medicine. 1996;15:24-30.
4. Barnhill EA, Novozhilova E, Day TA, Carlson SA. *Schistosoma*-associated *Salmonella* resist antibiotics via specific fimbriae attachments to the flatworm. Journal of Parasites and Vectors. 2011;4: 123,8.
5. Permin A, Pearman M, Nansen P, Bisgarrd M, Frandsen F. An investigation on different media for embryonation of *Ascaridia galli* eggs. Helminthologia. 1997b;34:75-79.
6. Baltazar M, Ngandjio A, Holt KE, Lepillet E, Pardos de la Gandara M, Collard JM, Bercion B, Nzouankeu A, Le Hello S, Dougan G, Fonkoua MC, Weill FX. Multidrug-resistant *Salmonella enterica* serotype typhi, Gulf of Guinea Region, Africa. Emerging Infectious Diseases. 2015;21(4):655-659.
7. CLSI. Performance standards for antimicrobial disk and dilution susceptibility test. Wayne, PA. 2008;2:29.
8. Melhem R, LoVerde P. Mechanism of interaction of salmonella and *Schistosoma species*. Infection and Immunology. 1984; 44:274-281.

9. Wilson R, Elthon J, Clegg S, Jones B. *Salmonella enterica* serovars gallinarum and *pullorum* expressing *Salmonella enterica* serovars *Typhimurium* type1 fimbriae exhibit increased invasiveness for mammalian cells. Infection Immunity. 2000;63:4782-4785.
10. Page AP, Hamilton AJ, Maizels RM. *Toxocara canis:* Monoclonal antibodies to carbohydrate epitopes of secreted (TES) antigens localize to different secretion related structure in infective larva. Parasitology. 1992;75:56-71.
11. Keren I, Shah D, Spoering A, Kaldulu N, Lewis K. Specialized persisted cells and the mechanism of multidrug tolerance in *Escherichia coli.* Journal of Bacteriology. 2004;186:8172-8180.
12. Dinh Nam Lâm, Carles M, Tripodi A, Brugère-Picoux J, Bodin G. Étude bactériologique des infections par le genre *Salmonella* chez le canard dans la province de Can Tho (Viet Nam). *Revue de Médecine Vétérinaire.* 2000;151(10): 955-964.
13. Cuneyt OK, Mehmet ZO, Ilknur KA, Hacer E. Comparison of amoxicillin with second and third generation cephalosporins in the treatment of acute otitis media. Le Innfezioni in Medicina. 1998;2:93-95.
14. Wallerstein RK, Condit PK, Kasper JWB, Morrison FR. Etude à l'échelle de l'Etat du traitement au chloramphénicol et de l'anémie aplasique fatale. Jama. 1969;208 (11):2045-2050.
15. Fraunfelder FT, Bagby GC, Kelly DJ. Fatal aplastic anemia following topical administration of ophthalmic chloramphenicol. American Journal of Ophthalmology. 1982;93(3):356-360.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/53593