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ORIGINAL PAPER

Evaluation of isopathic treatment of *Salmonella enteritidis* in poultry

A Berchieri Jr.[1,*], WCP Turco[2], JB Paiva[1], GH Oliveira[1] and EV Sterzo[1]

[1]*Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, 14870-000 Jaboticabal, São Paulo, Brazil*
[2]*DES-FAR Laboratórios Ltda. Rua Eponina, 412. 03426-010 São Paulo-SP, Brazil*

Background: **Salmonellosis is a common problem worldwide in commercially reared poultry. It is associated with human Salmonellosis. No fully satisfactory method of control is available.**
Method: **Nosodes to an antibiotic-resistant strain of *Salmonella enterica* serovar Enteritidis in D30 (30X) potency were prepared. One day old chicks ($N = 180$) were divided into four groups: two control and two different preparations of the nosode. Treatments were administered in drinking water for 10 days. The birds were challenged by a broth culture of the same *Salmonella*, by mouth, on day 17. Cloacal swabs were taken twice weekly for *Salmonella enterica* serovar Enteritidis.**
Results: **Birds receiving active treatment were less likely to grow the strain of *Salmonella* from cloacal swabs compared to control.**
Conclusion: **Isopathy is low cost and non-toxic. It may have a role to play in the widespread problem of Salmonella in poultry. Further research should be conducted.** *Homeopathy* (2006) **95,** 94–97.

Keywords: *Salmonella*; antibiotic resistance; poultry; nosodes; isopathy

Introduction

The poultry industry has to control Pullorum and Fowl Typhoid to produce meat and egg on a large scale.[1,2] Poultry is also susceptible to the infection by other *Salmonella* serotypes.[2] Some can be transmitted vertically among the birds and are excreted with the faeces.[3,4] They can be associated with human foodborne salmonellosis.[5]

Once infectious pathogenic microorganisms are introduced in a poultry flock they are easily disseminated throughout the farm.[6] Feed containing fishmeal introduced *Salmonella agona* to poultry farms of many countries and is responsible for some outbreaks of human foodborne salmonellosis.[3,7] Many other *Salmonella* serotypes can cause human salmonellosis including *Salmonella infantis*, *S. senftenberg,* and of course *S. typhimurium*.[8] *Salmonella enterica* serovar Enteritidis has been the focus of surveillance program in animal farms since it was responsible for several outbreaks of human foodborne salmonellosis related to food prepared with meat or eggs of chickens.[9–13]

Salmonella are still the most common agents of foodborne diseases and poultry products continue to be the main source of *S. enteritidis*.[14] This serotype may infect newly hatched chicks through vertical transmission and be disseminated to the poultry flock,[5] resulting in contaminated carcasses and eggs.[15] *S. enteritidis* is thought to reach poultry farm from rodents.[16] *S. enteritidis* has been detected in commercial broilers and laying hens worldwide.[15–19] Many countries have established surveillance for *S. enteritidis*; the Brazilian Government has ruled that imported birds must be free of *Salmonella* serotypes Gallinarum, Pullorum, Enteritidis and Typhimurium.[20] Nevertheless, *Salmonella* has been found in day-old birds and flocks may remain contaminated.[21,22]

Many methods to control *S. enteritidis* including monitoring breeding flocks have been tried, as have measures to avoid its introduction and dissemination in poultry flocks. The available methods are not fully

*Correspondence: Angelo Berchieri Junior, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Via de acesso Paulo Donato Castellane, s/n, 14884-900 Jaboticabal, SP Brazil.
E-mail: berchier@fcav.unesp.br
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effective and have negative aspects. Isopathy may be an alternative approach to prevent avian salmonellosis. This method has been used to control infectious diseases in animals, either individually or in herds, but there is no report of its use to control *Salmonella* infection in animals or humans.

A report from Cuba demonstrated the effectiveness of isopathy in reducing sub clinical bovine mastitis.[23] In Germany domestic animals were submitted to homeopathic treatment for 5 years. According to the author, morbidity rate was reduced among newly housed fattening pigs; treatment of respiratory illness were comparable to drug therapy using antibiotics, allowing reduction in the use of allopathic drugs.[24] In pigs, homeopathic treatment of respiratory tract diseases was more effective than using low-dose antibiotics, but less effective than using therapeutic doses of the same antibiotic.[25]

Intensive rearing of poultry is necessary but favors dissemination of infectious disease agents like *Salmonella*. The incorporation of antimicrobial substances in animal feed is now banned in animal production. Some antimicrobial drugs like furazolidone and chloramphenicol, which are very active against *Salmonella*, cannot be used now. These substances are either deleterious to human health or responsible for acquisition of drug-resistance by microorganisms, associated with human hospital acquired infection.[26] Homeopathic products have no contraindication and may contain several nosode (biotherapies). This work evaluated the use of isopathic products prepared from a *S. enteritidis* strain to control the infection of commercial birds by the same strain.

Material and methods

Bacterium

A nalidixic acid and spectinomycin resistant spontaneous mutant of *Salmonella enterica* serovar Enteritidis (SE Nal^rSpec^r) was isolated from birds from a commercial poultry flock. The mutant was prepared according to the method described previously.[27]

The nosode was prepared as described in the Brazilian Homeopatic Phamarcopoeia.[28] Broth culture of SE Nal^rSpec^r was made in 10 ml nutrient broth (Oxoid CM 67) incubate for 24 h at 37 °C in a shaking incubator (100 strokes/min). Such culture contained approximately 2.5×10^9 UFC/ml. The broth culture was centrifuged at 4000 rpm for 30 min and the pellet resuspended in 1 ml fresh nutrient broth to concentrate the culture.

To obtain a final dilution in 70% ethanol the solution was prepared by mixing 4.41 l of 99.0% alcohol with deionized water treated by UV light to a final volume of 6.3 l. To obtain the final 30% concentration it was mixed 2.4 l of 99.0% alcohol plus deionized water treated by UV light to a final volume of 8 l. Succussion was performed in a Dynamizer model 100P – 10F. The D1 (1X) solution was prepared by adding 1 g of broth culture of SE Nal^rSpec^r plus saline solution (9% sodium chloride in deionized water) to 10 g. The flask was dynamized with 100 succussions. This flask was labeled D1 and was used to prepare D2. The procedure was the same as used to obtain D1 but mixing 1 g of D1 with saline solution. The procedure was repeated until D10. From D11–D26 3.3 g of solution was mixed with 70% alcohol up to 33 g in a 60 ml flask. D27–D29 solutions were prepared as before but mixing 5.5 g of D26 solution with 70% alcohol up to 55 g in a100 ml flask. D30 was prepared from D29 but with 30% alcohol (SD30A) or 70% alcohol (SD30B).

Birds and challenge strain

SE Nal^rSpec^r was cultured as previously described in 100 ml to contain approximately 2.5×10^9 UFC/ml. From this, 1 ml of the broth culture was inoculated directly into the crop of the birds by gavage.

Birds used in this work were light white variety from a commercial hatchery. Some birds were bled for serological examination for evidence of *Salmonella* by slide agglutination test and by culture of swabs from the interior of the transport boxes.[20]

Experimental design

In each experiment, 90 1-day old chicks were separated in two groups, one being treated with the nosode, and the other with no treatment (control group). The birds received water and feed ad libitum and a heat source for the first 7 days of life.

The nosode was added to the drinking water at the concentration of 8.10 ml per 922.50 ml of water for the first 5 days of life and 8.10 ml per 1545.75 ml of water for the second 5 days of life, once a day. The nosode was shaken gently 10 times before use. The water offered to the chicks was placed in plastic drinkers. Seven days after finishing the treatment, the birds were challenged with the bacterial culture and cloacal cotton swabs were taken twice weekly.

Microbiological examination

The cotton swabs were placed into tubes containing selenite broth (Oxoid CM395) plus novobiocin (0.4%). The swab was streaked out on Brilliant green agar (Oxoid CM 263) containing nalidixic acid (100 μ/ml) and spectinomycin (100 μ/ml) (BG NalSpec) and incubated at 37 °C for 24 h. The result was considered positive when there was growth of at least one colony suggestive of *Salmonella*. The colony was submitted to agglutination test with *Salmonella* O antiserum factor 9 (Difco 2818-47) and with Salmonella H antiserum factor m (Difco 2546-47). When there was no growth, the swab was streaked out again on BG NalSpec agar incubated at 37 °C for 24 h.