International Journal of Livestock Production Vol. 3(2), pp. 21-24, February 2012
Available online at http://www.academicjournals.org/IJLP
DOI: 10.5897/IJLP11.045
ISSN 2141-2448 © 2012 Academic Journals

Full Length Research Paper

Salmonella control in poultry breeder farms in Sri Lanka: Effects of oral antibiotic treatment on whole blood agglutination test with *Salmonella pullorum* antigen

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Accepted 30 December, 2011

Salmonella pullorum **is a common disease in local poultry industry, which causes severe economic impact on the industry. Whole blood agglutination test (WBAT) is a screening test done to individual birds in a poultry breeder flock. It is compulsory to maintain a free status of this disease in line with the regulation declared by the Department of Animal Production and Health (2010). It is believed that of all the different methods used for reading whole blood agglutination tests, which can be misleading, prolonged treatment with antibiotics was the most. The objective of this study is to evaluate the effect of antibiotics on the results of whole blood agglutination tests in commercial chicken. This study was carried out with different antibiotics which are widely used in the field, such as Enrofloxacillin, Amoxycillin, Sulpa-Trimethoprim combination and Enro-Amoxycillin combination. The result indicated that antibiotics had no direct and significant effect on the reading of WBAT, although they were indirectly involved in the reading. In the field, antibiotics may flush out microflora, including *Salmonella,* from the GIT and prevent the development of high antibody titer in chicken. The study concluded that the isolation of *S. Pullorum* from the organs failed when the birds were infected at adult stage.**

Key words: *Salmonella*, antibiotic, agglutination test.

INTRODUCTION

Salmonella infection is one of the most important global poultry diseases which is caused by different Salmonella species (Kabir, 2010). More than 2,500 serotypes have been described mostly under the species, *Salmonella enterica* (Calenge et al., 2010). Salmonellosis, especially the one that manifests as Pullorum disease, is one of the commonest diseases of local poultry in Sri Lanka, which causes severe economic loss to the industry. *S. enterica* serovar, Gallinarum and Pullorum, are both normally reported to attack poultry, although they have been eradicated in commercial poultry of developed countries (Priyantha, 2009). From the point of view of public health, *Salmonella Enteritidis* is considered important due to its zoonotic nature (Quinn et al., 1994). In poultry, non-host specific salmonellosis increased between 1990 and 2000 as a result of the rapid growth of the industry (Priyantha, 2009). The Department of Animal Production and Health, authorized agency for animal health and production, initiated the Salmonella control program for poultry breeder farms. The program consists of periodical screening and culling of serologically positive birds from breeding followed by frequent testing of commercial hatcheries which includes isolation and identification of salmonella organisms by bacteriological methods (procedure for prevention/ control/eradication of pullorum and fowl typhoid in poultry breeder farm in DAP and H). Whole blood agglutination test (WBAT) has been identified as the screening test used for live breeds. The government also made *Salmonella pullorum* antigen and encouraged its use in order to maintain uniformity during testing, although commercial preparations are available.

There are 35 to 40 poultry breeders farms in Sri Lanka,

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Table 1. The concentration and doses of the different oral antibiotics used in the study.

Generic name	Dose for poultry
Each 100 mg contains 50 mg of Amoxycillin Trihydrate	100 mg to 600 L of water
Each 100 ml of solution contains Enrofloxacillin 10 g.	1 ml for 1L of water
Sulphadiazine 40% and Trimethprim 8%	1ml for 2 L of water
Amoxy-Enro combination	100 mg to 600 L water and 1 ml for 1 L of water

but only few of them are maintained by the government (DAP and H, Sri Lanka, www.daph.lk). Most of the large scale operations are run by private sectors and it is compulsory to register annually before the importation of a new stock. According to the current salmonella control program, farms' registration is allowed in a case where positive percentage is less than 1% at any given point. The objective of this regulation is to encourage poultry breeder farmers to control or eradicate salmonellosis in their farms in order to produce salmonella-free commercial chicks and safe guard the industry and consumer from zoonotic salmonellosis. Another objective of this regulation is to limit the use of antibiotics in commercial operations by minimizing the number of outbreaks in commercial poultry.

To this end, the primary testing is carried out by the farm staff, while verification is done by the government authority. Most of the entrepreneurs and breeder farm owners support the program, while only a few of them try to manipulate the results of WBAT. There are a number of methods adopted to manipulate the results. One of the commonest methods is treating birds with antibiotics for 3 to 5 days just before the verification test. It was found that negative results were obtained by oral treatment, although the flock was positive for salmonella serologically. Hence the objective of this study is to determine the effect of antibiotics on WBAT in chicken.

MATERIALS AND METHODS

Three hundred salmonella-free commercial birds (Shaver Brown birds of 10 weeks old) were used in this experiment instead of a number of salmonella-free breeder hens from breeder flock. All the birds were subjected to WBAT at 10 weeks. This was followed by conventional culturing of pooled cloacal swabs of salmonella using specific media (Tetra-Thionate broth and brilliant green agar). At 16 weeks of age, all the birds were tested again and innoculated with S. pullorum (S. Pullorum reference culture at Veterinary Research Institute) broth culture (8 h incubated 37°C), in which contained 1 to 5 X 10^9 colony forming units (c.f.u.) /ml. Two weeks later, the blood samples of the birds were tested via WBAT and all the samples tested positive for salmonella. The whole birds were separated into 10 groups with 30 birds in each group. The common antibiotics used for the study included Enrofloxacillin, Sulphadiazine and Trimethprim and Amoxycillin.

The groups were numbered 1 to 10. Groups 1 and two were treated orally with Enrofloxacillin for 5 days. Groups 3 and 4 were treated with Sulphadiazine and Trimethprim for 5 days. Groups 5 and 6 were treated with Amoxicillin, while Groups 7 and 8 were treated with a combination of Amoxicillin and Enrofloxacillin. The remaining groups were not given any antibiotic for the five consecutive days (Table 1) following the day blood samples were collected from all birds. WBAT was carried out according to the method described in the Manual of Diagnosis and Terrestrial Vaccine, OIE. After that, birds were culled and organs (liver, spleen, intestine, heart, cloacal swabs, ceacal tonsil, ovarian follicle, gall bladder) were pooled from five birds and cultured on salmonella specific agar. During the WBAT, whole serum samples were diluted two fold with *Salmonella pullorum* antigen from *S. enterica* Pullorum reference strain in line with OIE standards. The isolation and identification of *Salmonella pullorum* was carried out according to the method described in the OIE Terrestrial Manual, 2009. The salmonella antigen used for the study was from polyvalent type D, which was positive for S. Pullorum, S. Gallinarum as well as S. Enteritidis (OIE, 2009). Data were analyzed separately by two samples "t" test on each antibiotic.

RESULTS AND DISCUSSION

Chicken is the natural host for *S. Pullorum* and *S. Gallinarum* (Bercheri et al., 2010). The results of the WBAT are summarized in Table 2). The p values of each treatment with the antibiotics Enrofloxacillin, Amoxycillin, Sulpha-Trimethoprim and Amoxy-Enrofloxacillin combination were 0.984, 0.999, 0.999 and 0.468 respectively. It is important to note that no significant difference was observed in the WBAT of chickens treated with antibiotics. This means that the treatment of oral antibiotics does not have a direct effect on the reading of screening test which is a critical step in salmonella control program of breeder farms in the country. Naturally, antibody response due to the salmonella infection caused by *Salmonella Enteritidis* and *S. Typhimurium* show a prolonged and high titer of specific antibody since these organisms persist in GIT, but this was not observed with *S. Gallinarum* (Wigley et al., 2005b). However, S. Pullorum caused low level, persistent, systemic infection and high titer of antibody response compared to S. Gallinarum (Wigley et al., 2001). Furthermore, in the field, antibiotics may flush out microflora, including Salmonella, from GIT and prevent the development of high antibody titer in chicken. This may be taken as an advantage in the reading of WBAT since higher antibody titer causes prominent slide agglutination than lower titer such that it becomes difficult to take readings in the field. This suggests that there is an indirect effect on the reading when antibodies are mixed with infection, although not such primary infections as those caused by S. Pullorum. The role of antibodies in salmonella infection in poultry is

Table 2. The agglutination shown after the dilution of the serum of chicken with different antibiotics.

Group	Dilution of serum 1:00	1:01	1:04	1:08
Enrofloxacillin A1	29/29	29/29	29/29	28/29
Enrofloxacillin B1	28/28	28/28	28/28	28/28
Amoxycillin A2	26/26	26/26	26/26	26/26
Amoxycillin B2	29/30	29/30	29/30	29/30
Sulpha-Trimethoprim A3	29/29	29/29	29/29	29/29
Sulpha-Trimethoprim B3	30/30	30/30	30/30	29/30
Enro-Amoxy combination A4	30/30	30/30	29/30	29/30
Enro-Amoxy combinationB4	26/27	26/27	26/27	26/27
No antibiotic A5	28/28	28/28	28/28	28/28
No antibiotic B5	27/28	27/28	27/28	27/28

not clearly known, although it has already been proven to serve as protection against systemic secondary infection (Wigley et al., 2005a).

Other important findings of the study were the isolation and identification of S. Pullorum from the different organs of the chickens. S. Pullorum was not isolated in both the treated or non-treated groups. Isolation was not successful even in cloacal swabs. Most of the birds showed no signs of gross pathological lesion except for the spleen which got swollen in few cases. There were no signs observed in rest organs and none of the birds from either group showed clinical signs of salmonellosis. It was observed that the viable organism of S. Pullorum was not further localized in these organs; it was possibly cleaned off by the natural immune system almost four weeks after the initial infection. However, the isolation of S. Pullorum as shown by different studies, was observed in the heart and spleen five weeks post infection when infected at 4 days of age (Wigley et al., 2001). It has been observed in previous studies that S. Pullorum cannot survive in most organs as immunological clearance occurs five weeks after oral infection when infected at four days of age (Wigley et al., 2001).This difference can be attributed to the infective age - birds were inoculated at 16 weeks of age in the present study instead of 4 days. Though S. Pullorum did not localize for 3 to 4 weeks in the organs, a slight difference was observed in other biovar like S. Gallinarum which could cause either mortality of susceptible birds or undergo bacterial clearance within 3 to 4 weeks after initial infection in resistant birds (Wigley et al., 2001).The isolation of S. Pullorum from the control groups was done at 20 weeks which was the laying period of commercial birds, although it was not successfully cultured.

The isolation of the organism from carrier birds is an essential part of the salmonella control program in the country (procedure for prevention/control/eradication of pullorum and fowl typhoid from poultry breeder farm in DAP and H), especially at the time of culling. In this study, S. Pullorum could not be isolated from any organ of slaughtered birds. The isolation of S. Pullorum by conventional methods was difficult in carrier birds. This fact is supported by a previous study which revealed that non-specific suppression of cellular immunity by S. Pullorum bacteria themselves during laying period resulted in an increase in the number of bacteria in the reproductive tract, liver and spleen (Wigley et al., 2005a). The infection in the reproductive tract led to subsequent vertical transmission followed by the production of infected chicks; this is a critical phenomenon (Wigley et al., 2005a). Since these bacteria survive in macrophages, and antibody response is affected by clearance of intra-cellular bacteria, culling of carrier birds is recommended whether the organism is isolated or not.

It is difficult to explain the association between WBAT and oral treatment with antibiotics in poultry, especially for S. Pullorum infection. They survive in macrophages as intracellular organisms, and appear in the spleen and liver during the time of laying (Wigley et al., 2001).

Conclusion

From this study, the conclusion is drawn that oral treatment with antibiotics does not affect the reading of the WBAT during the screening of *S. Pullorum* infected flocks, although it had indirect effect on antibody titer of GIT salmonella. With the established conventional methods, the isolation and identification of relevant bacteria from different organs poses a challenge at adult stage (16 weeks) and as such, the organism may not be affected by the antibiotics in both treated and non-treated birds. However, the situation may change if the birds are infected in the first two weeks of life when it is possible to isolate the organism from the organs.

ACKNOWLEDGEMENTS

We thank the Department of Animal Production and Health for providing funds for this trial research under project 292-2-2-7-2502(20), and all the staff in bacteriology

division, Veterinary Research Institute, Sri Lanka for their kind cooperation. The authors also acknowledge Mr. Jayantha, the manager, Marawill NLDB Farms for providing salmonella-free chicks and Mr. I. P. Ranathunga for technical support.

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