ANTIBODY AND INTERLEUKIN- 6 RESPONSE IN LOCAL CHICKENS INFECTED WITH SALMONELLA GALLINARUM AND TREATED WITH CRUDE EXTRACT OF ALOE SECUNDIFLORA

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SUMMARY

A study was conducted to establish the effect of crude extract of *Aloe secundiflora* on antibody and interleukin-6 response in local chickens experimentally infected with *Salmonella gallinarum*. At five months old, the chickens were screened for *S. gallinarum* antibodies and those found negative were randomized into five groups namely G1, G2, G3, G4 and G5. Birds of G1 (n=21), G2 (n=21) and G3 (n=21) were experimentally infected with $5.0x10^8$ c.f.u/ml of *Salmonella gallinarum* (RD 8 strain). G1 birds were treated with 200 mg/kg *Aloe* extract two weeks prior to infection and were continued with 400 mg/kg up to day-7 post infection (pi), G2 were untreated while G3 were treated with 400 mg/kg as from day-0 pi. G4 (n=10) were uninfected and untreated while G5 (n=10) were uninfected but given treatment as in G1. Antibodies against *Salmonella gallinarum* were detected in all infected groups by day-6 pi. From day-9 pi, G2 (infected and untreated) showed significant increase in antibody titre ($p < 0.05$). The treated groups G1 and G3 showed lower antibody levels with G1 (pre-treated) showing significantly lower level than G3 ($p<0.05$). All groups showed an increment in the levels of IL-6, however, levels in the uninfected groups remained below the cut off point during the experimental trial. Between day-3 and day-6 pi, levels of IL-6 in the infected and treated groups (G1 and G3) were significantly high ($p<0.05$) as compared to G2. This experiment showed that administration of *Aloe* extract in chickens infected with fowl typhoid resulted into lowering of antibodies against *Salmonella* and an increase in IL-6 levels before the peak of the antibody production. It is concluded that the mechanism triggerring antibody production was suppressed in *Aloe*-treated birds while the cellular response leading to IL-6 production was enhanced during the initial stage of infection. Therefore, *Aloe* extract could play a role in the immunoprotection to fowl typhoid. This phenomenon could be incorporated into the control strategy of fowl typhoid.

INTRODUCTION

Fowl typhoid, caused by *Salmonella gallinarum* has been categorised as a number two cause of mortality in village chickens in Tanzania (Minga, 1986). Drugs used in the treatment of fowl typhoid include oxytetracycline, sulphonamides and nitrofurazoline. Attenuated strains of *S. gallinarum* have been used successfully as live vaccines for chickens over a long time (Silva *et al.*, 1981). The drugs and the vaccines are only used in the commercial chicken industry since the rural economy cannot meet the demands of these current control measures that are expensive.

Where agroecological conditions are favourable, ethnoveterinary plant products with recognized medicinal properties are far more feasible and accessible to rural population than the western veterinary treatments. Morover they can be collected at little or no cost and are easily available. In ethnoveterinary practice, a lot of herbal preparations have been used to manage various chicken diseases. (Qureshi and Sabri, 1967; Prasad *et al.*, 1982; Minja, 1989; Bizimana, 1994; Agbede *et al.*, 1995; ITDG and IIRR, 1996). In Senegal, for example, barks of *Azardirachta indica* (neem) have been used extensively on chicken diseases (Agbede *et al.*, 1995). Bizimana (1994), reported that *Aloe* species (Aloaceae) have been used for control of fowl pox and enteritis in chickens in Zimbabwe. In Kenya, ITDG and IIRR (1996) also reported a wide use of *Aloe* species for chicken diseases including Newcastle disease, coccidiosis and fowl typhoid.

Although there is clear evidence on the extensive local use of herbal medicines against a variety of chicken diseases, scientific verification of the potential through well-designed studies is lacking. Information on the efficacy, modes of action, effect on the immune system and toxicity is very scanty. The present study was designed to investigate the immune response of chickens infected with fowl typhoid and treated with the extract of *Aloe secundiflora* which was found to have some antibacterial effects against *S. gallinarum* in our preliminary *in vitro* studies. The immune parameters measured were antibodies to fowl typhoid and interleukin-6 levels.

MATERIALS AND METHODS

EXPERIMENTAL CHICKENS

Eighty one -week old local chicks were purchased from Morogoro rural district, Tanzania. The chicks were reared at the Sokoine University of Agriculture (SUA) in a chicken house where they were stabilized for five months using antibiotic oxytetracycline, anticoccidials (Amprolium 200®) Diocare Vet Ltd., AV Lab. South Africa) and antihelmintic (Kukuzole®(Mebendazole) Interchem Pharma Ltd., Moshi, Tanzania. The birds were also

vaccinated against Newcastle disease vaccine (Lasota® strain, Lohman Animal Health GmbH & Co KG, Heinz- Lohman, Cuxhaven, Germany). During the five months period and thereafter, the birds were fed *ad libitum* on locally available commercial feeds and water.

PREPARATION OF *ALOE* SAP

Mature plants of *Aloe secundiflora var. secundiflora* obtained from the Moshi district in Tanzania, which is a semi-arid habitat at an altitude of 914 m were used in the study. The voucher specimen is available in the herbarium of Botany Department, University of Dar es Salaaam, indexed as BJH 4828. *Aloe* exudate was obtained from transverse sections of 10 leaves each of 30cm long obtained from different *Aloe* plants. The sap was allowed to drain into a bucket for 30 minutes then 1 L of water was added to hasten the efficiency of obtaining the exudate. The harvested sap was preserved at 4°C before being lyophilised into a yellow-orange powder that had a concentration of 400 mg/ml.

PREPARATION OF *S. GALLINARUM* INOCULUM

The original stock of *Salmonella gallinarum* RD strain 8 (Mdegela, 1998) was cultured on MacConkey medium overnight. Thereafter three colonies were selected and inoculated iinto liquid broth medium (LB) and incubated for six hours at 37°C with shaking of the universal bottles at 10 minutes intervals. The broth was then diluted with phosphate buffered saline (PBS) to obtain the infective dose of 5.0 $x10^8$c.f.u/ml.

INFECTION AND TREATMENT OF THE CHICKENS

Prior to the experiment, the chickens were screened for antibodies against *Salmonella gallinarum* infection using Rapid Plate Agglutination Test (RPAT) (O I E, 1996). A locally- prepared antigen from the same strain that was used as inoculum was used for the screening. Chickens that showed negative results were randomly assigned into five experimental groups, namely G1, G2, G3, G4 and G5. Birds of G1 (n=21), G2 (n=21), and G3 (n=21), were experimentally infected with *Salmonella gallinarum* (RD 8 strain). G1 birds were given an oral dose of 200 mg/kg *Aloe* extract two weeks prior to infection and then continued with 400 mg/kg up to day-7 post infection (PI), G2 were untreated and G3 were treated with 400 mg/kg. *Aloe* extract as from day-0 to day-7 PI. G4 were uninfected and untreated, while G5 were uninfected but given *Aloe* extract treatment as in G1.

SERUM INVESTIGATIONS ANTIBODY LEVELS AND INTERLEUKIN –6 (IL-6) ANALYSIS

Following inoculation, 2ml of blood was obtained from the wing vein of each chicken on days 0, 3, 6, 9, 12 and 15. The serum was screened for antibodies against *S.*

gallinarum using Serum Agglutination Test (SAT). About 100µl of serum of each sample was frozen at -80°C before the analysis of IL-6 at the Institute of Physiology, Faculty of Vveterinary Medicine, University of Munich. IL-6 bioactivity was measured by its ability to stimulate proliferation of the IL-6-dependent murine hybridoma cell line 7TDI (Van Snick *et al.*, 1986). The 7TDI cell line was kindly provided by J. Van Snick, Ludwig of the Institute for Cancer Research, Brussels.

RESULTS

ANTIBODY LEVELS

Geometric mean titre of the antibody levels against *S. gallinarum* obtained from the serum samples from G1, G2 and G3 are shown in Fig. 1. There were no detectable antibodies in all three infected groups until day 6 pi. On day-6, RPAT results showed that 48% of chickens in G1 were positive, 50% in G3 and 100% in G2. On the following days all the chickens except the uninfected controls were positive. The antibody levels increased in all groups with a significance divergence on day-9 pi between the groups ($p=0.001$), and which remained until the end of the experiment. G1 remained lowest with a decreasing trend in antibodies between day 12 and 15. G2 showed an increasing trend attaining maximum levels by day 15 pi. G3 maintained a steady increasing trend although the antibody levels remained lower than those of G2 ($P=0.01$). There were no antibodies detected in the sera from G4 and G5 (uninfected groups).

INTERLEUKIN-6 (IL-6)

Results of the levels of IL-6 levels in all the groups are shown in Fig. 2. There was an increasing trend in the level of IL-6 for all the groups. All the groups started at an average level below the cut off point (0.1). The IL-6 levels of the infected groups (G1, G2 and G3) over the 15 days trial period had a higher increment as compared to the uninfected groups (G4 and G5) ($p<0.05$). G1 and G3 showed increasing trends up to day-9 after which they decreased. In the uninfected groups (G4 and G5), IL-6 levels only increased slightly up to day 3 after which they levelled off to day 9, but remained below the cut off point until day 15.

DISCUSSION

The low antibody response among the *Aloe* treated groups, G1 and G3 indicates that antibacterial activity of *Aloe* reduced the bacteria load that could have enhanced marked immune response. Chickens of G1 had been on a prophylactic dose of *Aloe* for two weeks prior to infection. The prophylactic treatment with *Aloe* could have contributed to high accumulation of *Aloe* in the chickens leading to a stronger antibacterial effect. This could explain the low levels

of antibodies in G1 as compared to the other groups. In contrast, there was high antibody level to *Salmonella gallinarum* in G2 (infected and untreated) suggesting a higher bacterial load in circulation. The prophylactic activity of *Aloe* extract was also demonstrated by Solar *et al.* (1979) who found that mice inoculated with a leaf preparation from *Aloe vahombe* were protected from infection by *Klebsiella pneumoniae*, through stimulation of the immune system. In another study, it was shown that mice, infected with *Escherichia coli* gained protection on injection of extracts from *Aloe barteri* and *Aloe ferox* through stimulation of the phagocytic activity (Delaveau *et al.*, 1980).

Interleukin- 6 response is an important factor in the immune responses to infections in chickens such as *Eimeria* and *Salmonella* (Kaiser *et al.*, 2000; Lynagh *et al.*, 2000). The present study also demonstrated that the infection resulted into high levels of IL-6. Studies elsewhere have shown that infection with *S. gallinarum* caused no increase in IL-6 (Kaiser *et al.*, 2000). The *Aloe* treated groups showed an increasing trend attaining

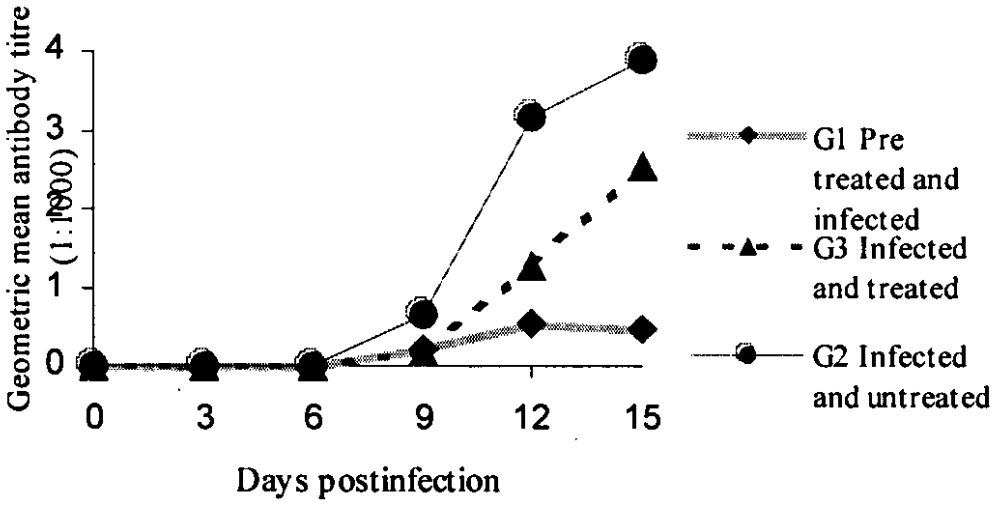

Fig. 1. Trends in antibody levels in the three infected trial groups of chickens over the 15 days experimental period.

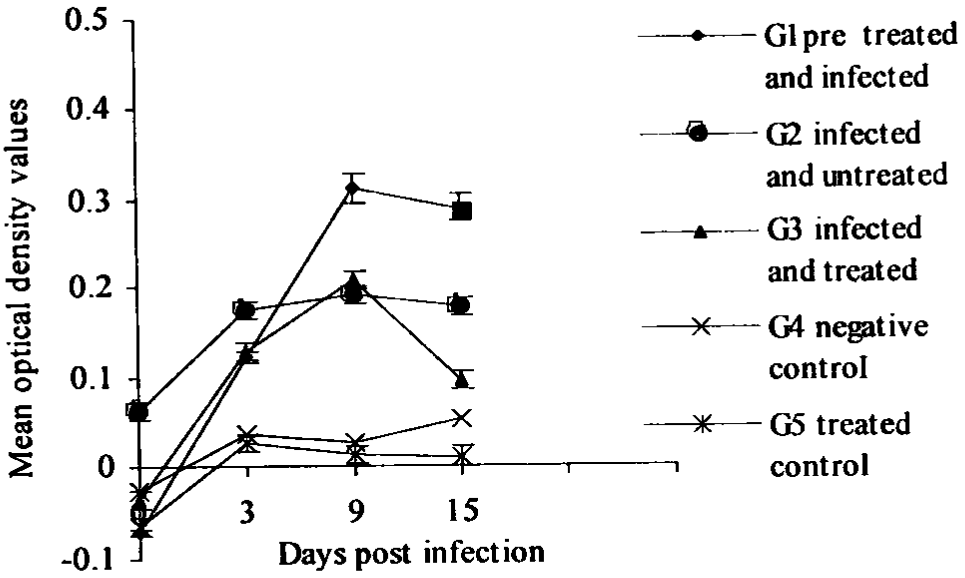

Fig. 2. Trends in interleukin 6 (IL-6) levels of all experimental groups.

highest levels by day-9. The decrease in IL-6 by day-15 could be associated with the clearance of the bacteria from the tissues. The drastic drop in levels of IL-6, which was recorded in G3 could be attributed to the complete clearance in the tissues.

Although the role of IL-6 in this study cannot be clearly defined, studies elsewhere have shown that acemannan, which is a major carbohydrate fraction obtained from the gel of *Aloe vera* stimulated *in vitro* macrophage cytokine production of TNF and IL-6 in a dose-dependent relationship (Zhang and Tizard, 1996). More experimental studies are suggested in order to investigate the role and the mode of action of IL-6. There is high possibility that the crude extract of *Aloe secundiflora* used in this study could induce IL-6 production in a similar manner like the purified acemannan. From the results of this study it was speculated that IL-6 together with other fractions conferred protection in the *Aloe* treated groups. Following this, the crude *Aloe* extract was fractionated using (High performance liquid chromatography (HPLC). Compounds identified included aloenin, aloin, aloinside and other aloin derivatives (Waihenya *et al.*, 2003). These compounds were found to have antibacterial effect against *Salmonella gallinarum in vitro.*

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