Immunization of young chicks using graded dose of wild strain of *Eimeria tenella*

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ABSTRACT

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A wild strain of *Eimeria tenella* was isolated and utilized for immunization studies. Its optimal sporulation was attained at room temperature 24–25 °C after 24–48 h. Two groups of chicks were immunized by dosing a graded dose of five oocysts/chick/day for 6 days followed by 50 oocysts/chick/day for 7 days. A third group was not immunized and served as a negative control. Immunized chicks gained mass at the same rate as unimmunized ones, but when challenged with 200 000 oocysts/chick, mass gains declined in the unimmunized group. The growth rate of immunized chicks was not affected by challenge ($P > 0.05$). Upon challenge, unimmunized chicks produced significantly more oocysts than immunized chicks ($P < 0.005$). Immunized chicks withstood a challenged with 200 000 oocysts/chick without developing any clinical signs whereas the unimmunized chicks developed typical clinical signs of coccidiosis. Unimmunized chicks had significantly more severe lesions in the caecum than any other group ($P > 0.005$) and also produced significantly more oocysts than any other group ($P > 0.005$).

Keywords: Chickens, *Eimeria tenella*, immunization

INTRODUCTION

Coccidiosis is one of the most commonly reported diseases of chickens in Tanzania and causes losses through poor performance, mortality and costs of prophylaxis. In the industrialized nations, coccidiosis is controlled by use of chemotherapy and vaccines, but only the former has been used in Tanzania. The anticoccidial drugs commonly used in Tanzania include sulphaquinoxaline, amprolium, ethopabate and sulphadimidine. However, development of drug resistance and drug residues in poultry meat are two factors against long-term reliance on drugs for the control of coccidiosis. Vaccination seems to be the only reliable long-term preventive measure. The commercial vaccines that are available have not been tested in much of Africa probably because of the market size. The objective of the current paper was to explore the possibility of utilizing a local strain of *Eimeria tenella* parasites for immunizing chickens against coccidiosis.

Eimeria tenella is the most pathogenic coccidian species of domestic poultry and it develops in the caecum. Caecal coccidiosis most frequently occurs in young birds, those aged 4 weeks being most susceptible. Clinical caecal coccidiosis is produced only when heavy infections are acquired over a period not exceeding 72 h (Davies, Bowman & Smith 1963). If birds acquire small doses of *E. tenella,* the parasite elicits an immune response. The most effective protection is provided by trickle infections

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with oocysts. Cell mediated immunity, secretory antibodies in the gut and circulating antibodies all appear to play a part in protection, probably in that order of significance. "Coccivac" (Schering-Plough Animal Health, USA) is a live vaccine consisting of non-attenuated oocysts, which are delivered in drinking water. It has been commercially available for some years but only in the USA. On the other hand, *Eimeria* spp. have been attenuated by selection for precocity (McDonald & Shirley 1987) or through passage in embryonated chicken eggs (Gore, Long, Kogut & Johnson 1983). "Paracox" (Schering-Plough Animal Health, UK) is a commercial vaccine, which consists of seven attenuated species of *Eimeria* that infect chickens and which is available in the European Union. The commercial vaccines have not been tested for use in Tanzania and it is therefore not known if they will protect chickens against local strains of *E. tenella*. This paper reports early attempts to isolate a local strain of *E. tenella* and to utilise it for immunization purposes.

MATERIALS AND METHODS

Isolation of parasites

Eight-week-old broiler chickens were stunned and then killed. The caeca were removed, opened and the contents collected into a flask containing saturated sodium chloride solution. The suspension was thoroughly homogenized, strained to remove unwanted solid material and left to settle for 30 min. The top layer of the suspension was harvested and placed in a test tube in which it was diluted ten times with tap water. The suspension was centrifuged at 50 x *g* for 30 min. The supernatant was discarded and water was added to the sediment, then the process of centrifugation was repeated to obtain a cleaner sediment. A drop of the sediment was placed on a microscope slide, covered with a cover slip and examined under a standard microscope at 40X magnification. The shape and size for the oocysts were recorded.

Experimental sporulation of oocysts

Five mℓ of 4 % potassium dichromate was mixed in a petri dish with 5 mℓ of a suspension containing unsporulated oocysts. Three samples were used and labelled to indicate oocyst concentration, volume and date of sporulation. One sample was kept at room temperature (24 °C) and the other two were incubated at 25 °C temperature. The samples were examined for sporulation after 24 and 48 h.

Experimental infection of chicks

One-day-old broiler chicks were purchased from commercial suppliers. They were vaccinated against Newcastle and Gumboro diseases and were fed using commercial feeds without anticoccidial medication. Sixty chicks were randomly allocated into three groups of 20 each. A coloured leg band identified each chick and each group was identified by a different colour. The groups were reared in three separate cages with wire floor to minimize faecal contamination of the feed and water and their body mass were measured on alternate days for 30 days before they were challenged.

Two groups of chicks were immunized by trickle infection using a graded dose of sporulated oocysts of *E. tenella*. The initial infection involved five oocysts/chick/day for 6 days, followed by the higher dose of 50 oocysts/chick/day for 7 days given *per os* in water as a drench using a plastic syringe. Immunity was allowed to develop for 8 days before they were challenged. The third group of chicks was not immunized.

One week after the last immunization all three groups were challenged with 200 000 sporulated *E. tenella* oocysts, which were also given as a drench using a plastic syringe. After challenge, Group I was treated with amprolium (3 g/ℓ in the drinking water) for 7days, while the other two groups received no treatment.

For 8 days the chicks were monitored daily for mortality, mass gains and oocyst production. The number of oocysts was counted by using a McMaster counting chamber. After 8 days all 60 chicks in the three groups were stunned and killed to observe caecal lesions. Caecal lesions were scored according to the methods described by Johnson & Reid (1970), which are:

0 = No gross lesions

1+ = Very few scattered petechiae on the caecal wall, no thickening of the caecal walls, normal caecal content present

2+ = Lesions more numerous with noticeable blood in the caecal contents, caecal wall somewhat thickened, normal caecal contents present

3+ = Large amounts of blood or caecal cores present, caecal walls greatly thickened; little, if any, faecal contents in the caeca

4+ = caeca greatly distended with blood or large caseous cores, faecal debris lacking in caeca or included in cores.

Data analysis

Data on body mass and oocyst production were analysed by the Student T-test while the lesion scores were compared by ANOVA using the statistical software Statistix®.

RESULTS

The shape and size of oocysts

Eimeria tenella oocysts were observed and measured under the microscope. Two types of shapes were observed, i.e. round and ovoid. The majority (80 %) were ovoid and the remainder round. Their sizes ranged from 17-25 µm in length with a mean of 22.14 µm. The width ranged from 15–23.3 µm with a mean of 19.58 µm.

Oocysts count and sporulation

Fresh oocysts obtained from chicken caeca had not sporulated but the percentage sporulated had risen to above 70 % after 24 h for all oocysts in the suspensions samples held at either 24 °C or 25 °C. The highest sporulation of 82.5 % was achieved in the Group B sample that was kept at 25 °C for 48 h, but the difference between the groups was not statistically significant ($P > 0.05$).

Body mass measurements

The body mass were measured on alternate days for 30 days. The mean body mass for all three groups increased steadily until after the challenge when the control group showed slower mass gains. Immunized chicks gained mass even after the challenge with 200 000 sporulated oocysts. Overall comparison of the mass revealed no significant statistical differences ($P > 0.05$).

Oocysts output after challenge

Chicks that were immunized and treated with amprolium (Group 1) produced significantly the lowest numbers of oocysts ($P < 0.005$) while the unimmunized control group produced significantly more oocysts than either the other two groups ($P < 0.005$).

Lesion scores

During the immunization process of the chicks in the two groups that were immunized showed no obvious change in their health. However, upon challenge the unimmunized group developed clinical signs of coccidiosis, which included huddling together, ruffled feathers, lack of appetite and severe bloody diarrhoea. No mortalities occurred in either of the three groups of chicks. Eight days after challenge all 60 chicks were stunned and killed in order to score the caecal lesions. None of the 20 chicks in groups which had been immunized and treated chicks developed gross lesions in the caeca, and in group II only one of the 20 immunized but untreated chicks developed minor lesions in the caeca while all 20 unimmunized chicks had severe lesions including caecal cores containing blood. The differences in the lesion score between the three groups are statistically significant ($P < 0.005$).

DISCUSSION

In the current study, a graded immunizing dose consisting of five oocysts per day for 8 days followed by 50 oocysts per day for 7 days induced protective immunity in the young chicks. This dose could appear low if compared to that in the work of Nakai, Uchida & Kanazawa (1992) who used 50 oocysts daily for 13 days or that of Maes, Vanparijs & Marsboom (1991) who used 2 000 oocysts on Days 4, 6, 8, 11 and 13. Despite the low immunizing dose the chicks did develop a protective immunity and could withstand being challenged with 200 000 oocyst as did those immunized with higher doses in the trial of Maes *et al.* (1991) and Nakai *et al.* (1992).

The body mass of the three groups of chicks increased steadily until when they were challenged with 200 000 sporulated oocysts of *E. tenella* after which the growth of the unimmunized birds was retarded. Poor growth in unimmunized chickens which were challenged with *E. tenella,* has also been reported (Maes *et al.* 1991). The challenge infection in the current study had no effects on the growth of the chicks in the immunized groups. The immunized chicks in Group I which had received amprolium after being challenged showed higher mass gains than the other two groups although the differences were not statistically significant. The cumulative effects of reduction in the growth of broiler chicks can cause very serious economic loss because they will consume more food to reach slaughter mass.

Protective immunity is generally manifested by the presence of a reduction in the severity of lesions, lower oocyst output and reduced mortality. In this study, the immunized and medicated chicks in Group I produced the least number of oocysts, which was significantly lower than that in the chicks in Group II that had received immunization alone.

On the other hand, the unimmunized group produced significantly more oocysts than the other two groups. It is generally agreed that trickle immunization leads to a low number of oocysts (Nakai *et al.* 1992). The observation that the immunized medicated group produced the least number of oocyst is in agreement with the findings of Ruff, Garcia, Chute & Tamas (1993) that amprolium medication reduced the number of oocysts shed in *E. tenella, Eimeria acervulina, Eimeria maxima* and *Emeria necatrix* infections. Production of low levels of oocysts by the immunized and medicated groups may provide trickle infection, which could be useful for maintenance of immunity in the flock. Large doses, however, such as those produced by the unimmunized chicks in this experiment may lead to outbreaks of clinical disease which can have serious consequences including mortality.

In this study, the immunized chicks did not develop any clinical signs of coccidiosis. All unimmunized chicks developed clinical disease which culminated in the development of severe caecal lesions with cores and blood. The lesion scores for the unimmunized group were significantly higher than the other two groups. The immunized, amprolium-medicated group did not develop any gross caecal lesions, which contrasts with the findings of Maes *et al*. (1991) who found that lesions did develop in diclazuril-medicated, trickle-infected chickens. Some lesions were observed in 5 % of the chickens that received immunization alone, which also contrasts with the results of Nakai *et al*. (1992) who observed caecal lesions in almost all chickens that were immunized for 1 week from either 0–6 or 7–13 days of age. Thus, the graded dose administered during the first 2 weeks of life appears to confer more protection than a fixed dose given for 1 week.

This study has shown that trickle infection using a graded dose of oocysts conferred protective immunity against caecal coccidiosis, which was manifested by absence of disease, good growth performance, low oocyst production and low lesion scores. If trickle immunization is ever fully developed in Africa, it will enhance commercial poultry production by reducing losses associated with coccidiosis such as mortalities, reduced growth performances, reduced feed conversion efficiency and costs of medication. Widespread use of immunization against coccidiosis will in addition, reduce drug residues in poultry products as well as the problems associated with drug resistance.

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