*J. Protozool. Res., 4, 56-61 (1994)*


# Heterogeneity among Strains of *Eimeria tenella* Isolated from Bangladesh

M. J. KARIM, N. BEGUM AND M. S. R. KHAN

*Department of Parasitology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh*

Received 8 February 1994 / Accepted 17 March 1994

Key words: *Eimeria*, strains, heterogeneity, immunity, precocious

ABSTRACT

Success of the recently developed precocious vaccine for controlling coccidiosis in chicken largely depends on the extent of immunological heterogeneity among strains. In this study, seven single-oocyst-derived strains of *Eimeria tenella* isolated from Dhaka (D91/1), Mymensingh (M91/1, M91/2 and M91/3), Chittagong (Ch91/1 and Ch91/2) and Sylhet (S91/1) districts of Bangladesh were compared for pathogenicity and cross-protection in vivo. Strain M91/1 was found to be more pathogenic compared to M91/2 and M91/3, and caused a significant ($P<0.05$) depression in weight gain and blood haematocrit values, and a higher mortality. None of these three strains were different from other strains. Two immunising doses produced a solid homologous immunity. Following heterologous challenge with strain Ch91/1, there was an increased oocyst output and significant ($P<0.05$) depression in weight gain in chicks immunised with S91/1. The results suggest that S91/1 was less immunogenic than strain Ch91/1, although neither of these two strains were different from other strains. It is suggested that this difference was not due to a difference in fecundity or pathogenicity, rather it was more likely to be a fundamental difference in antigenic repertoire.

## INTRODUCTION

Immunological diversity among strains of *Eimeria acervulina*, *Eimeria maxima* and *Eimeria mitis* has been reported by a number of workers (Joyner 1969, Long 1974, Norton and Hein 1976, Jeffers 1978, McDonald et al. 1985, and Karim and Trees 1989). But studies comparing field strains of *Eimeria tenella* are few. Joyner and Norton (1969) made a comparative study between two strains of *E. tenella* and reported a difference in pathogenicity, although the strains were not immunologically different. Chapman (1986) demonstrated that the difference in pathogenicity was related to a difference in Glucose phosphate isomerase (GPI) isoenzyme type, but Shirley et al. (1989) did not find any relationship between isoenzyme type and pathogenicity. Although immunological diversity in *E. maxima* was detected to an extent where inclusion of a second

line was considered necessary in precocious vaccine (Shirley and Bellatti 1988), immunological heterogeneity in *E. tenella* was found to be very minimal except when homologous immunity was incomplete by the criterion of oocyst output (Karim et al. 1991). However, Krylova and Dikovskaya (1975) reported that cross-protection between strains of *E. tenella* was incomplete. Development of precocious lines of coccidia offers an excellent promise of controlling coccidiosis in chicken by vaccination. To make a wide use of this vaccine a success, detailed study into immunological variations between strains of *Eimeria* spp. is necessary.

This paper describes a comparison of seven field strains of *E. tenella*, the most pathogenic species of chicken coccidia, for their pathogenicity and cross-protection in vivo.

## MATERIALS AND METHODS

### Chicks:

One -day- old Hysex cockerels were reared in a metal brooder on mesh floor. The chicks were fed a mash layer-ration without any anticoccidial drug. Strict precautions were taken to keep the chicks coccidia free, and to verify this faeces were examined twice weekly.

### Coccidia:

Seven field strains of *E. tenella* isolated from Dhaka (*E. tenella* D91/1), Mymensingh (*E. tenella* M91/1, M91/2 and M91/3), Chittagong (*E. tenella* Ch91/1 and Ch91/2) and Sylhet (*E. tenella* S91/1) districts of Bangladesh were used in this study. The strains were isolated by infecting 7 to 10 -day- old chicks with a single oocyst and maintained through passages in 7 to 10 -day- old chicks held in filter top polycarbonate cages (Karim and Trees 1990). For conducting the experiments, oocysts of comparable passage number, percentage sporulation and age were used.

### Studies on pathogenicity:

Six strains were examined in two experiments. The design is shown in Table 1. Twenty one -day- old chicks were weighed individually and grouped by ranking in groups of 10 chicks in experiment 1 and 12 in experiment 2. The chicks had access to a mash based layer-ration, and water 24 hours a day. The chicks were weighed every day till the end of the experiment on 11 days after infection. Daily oocyst output per bird was counted from 6 to 11 days post infection in some groups by a modified McMaster technique. Blood haematocrit values of five chicks randomly selected from each group were estimated on 0, 4, 5, 6 and 7 days post infection using a Microhaematocrit centrifuge (Hettich, Germany).

### In vivo cross-protection experiments:

Seven strains were examined in three experiments. The design is shown in Table 2. Each group had a total of 10 chicks grouped by ranking. The chicks received a first immunising dose of $0.5 \times 10^3$ sporulated oocysts at 21 days followed by a second immunising dose of $10^3$ oocysts 10 days later. The chicks were challenged with $10^4$ sporulated oocysts 10 days after the second immunising dose. The chicks were weighed on the days of immunising and challenge infections, and then every day till the end of the experiment on 12 days after challenge. Daily oocyst output per bird was counted by a modified McMaster technique from 6 days after the first immunising dose. Faeces of control chicks were examined to detect any accidental infection.

### Statistics:

Group mean blood haematocrit values and weight gain were compared by students 't' tests (Bailey 1981).

RESULTS

Pathogenicity:

The results of the studies on pathogenicity are presented in Table 1. The chicks produced oocysts on 6 days after infection with

Table 1. Pathogenic difference in *E. tenella*: oocyst output, blood haematocrit values and weight gain following infection with different strains

| Strain | No. of oocyst | Haematocrit[+] (%) at days post infection 0 | 7 | Weight[@](g) | Oocyst[#] ($x10^6$) |
|---|---|---|---|---|---|
| Experiment 1: | | | | | |
| M91/1 | $10^3$ | 35.8 ± 2.5 | 26.0 ± 1.2[a*] | 37.1 ± 10.0[a] | 39.4 |
| M91/2 | $10^3$ | 35.8 ± 1.6 | 28.4 ± 0.9[b] | 47.8 ± 11.2[b] | 36.2 |
| M91/3 | $10^3$ | 36.0 ± 2.4 | 28.8 ± 1.3[b] | 42.0 ± 7.9[a,b] | 38.0 |
| M91/1 | $10^4$ | 36.0 ± 0.7 | 16.4 ± 1.1[a] | 14.9 ± 7.7[a] | ND |
| M91/2 | $10^4$ | 35.4 ± 2.3 | 18.6 ± 1.1[b] | 26.0 ± 6.6[b] | ND |
| M91/3 | $10^4$ | 36.0 ± 1.2 | 18.4 ± 1.1[b] | 32.1 ± 5.4[b] | ND |
| M91/1 | $10^5$ | 35.6 ± 1.8 | ** | 6.7 ± 4.7[a] | ND |
| M91/2 | $10^5$ | 35.8 ± 2.2 | 16.0 ± 2.9[a] | 13.0 ± 7.1[a,b] | ND |
| M91/3 | $10^5$ | 35.6 ± 2.3 | 16.5 ± 1.3[a] | 21.4 ± 3.7[b] | ND |
| None | | 36.0 ± 2.0 | 35.8 ± 1.3[c] | 57.8 ± 4.3[c] | -ve |
| Experiment 2: | | | | | |
| M91/1 | $10^4$ | 35.6 ± 0.7 | 15.2 ± 0.8[a] | 28.9 ± 15.1[a] | 64.9 |
| M91/2 | $10^4$ | 35.4 ± 1.1 | 18.2 ± 0.8[b] | 37.7 ± 17.4[b] | 56.1 |
| D91/1 | $10^4$ | 35.0 ± 1.6 | 16.6 ± 1.7[a,b] | 33.8 ± 10.8[a,b] | 68.3 |
| Ch91/1 | $10^4$ | 35.0 ± 1.9 | 16.2 ± 2.5[a,b] | 31.6 ± 10.6[a,b] | 63.3 |
| S91/1 | $10^4$ | 35.2 ± 2.1 | 16.6 ± 1.5[a,b] | 31.3 ± 11.2[a,b] | 59.0 |
| None | None | 35.4 ± 2.1 | 35.8 ± 1.5[c] | 78.4 ± 11.3[c] | None |

[+] Mean ± SD; n=5.
[@] Mean ± SD; n= 10 in experiment 1 and 12 in experiment 2.
[#] Total output per bird from days 6 to 11 after infection as detected by daily counts in groups.
* Mean in the same column followed by same letters are not significantly different (P>0.05) for each experiment/oocyst dose.
** All the chicks selected for bleeding were dead.
ND: Not done

all the strains. Depression in blood haematocrit values was noticed first on day 5 post-infection with $10^4$ oocysts and started to regain from day 7 post-infection. With $10^4$ and $10^5$ oocyst doses the depression was first noticed on 4 days after infection and was in lowest level on day 7 post-infection. The first sign of effect on weight gain was seen on day 5 after infection with all the strains and doses. Strain M91/1 caused a significant ($P<0.05$) depression in blood haematocrit values and weight gain, and also caused a higher rate of mortality (results not shown in Table 1) compared to strains M91/2 and M91/3. None of these strains were however different from other strains in this study.

In vivo cross-protection experiments:

The results of the cross-protection experiments are presented in Table 2. Two immunising dose produced a complete homologous immunity, and did not cause any significant depression ($P>0.05$) in weight gain compared to the unimmunised control groups. The oocyst

output following second immunising dose was much reduced and was not detectable in faeces on and from 12 or 13 days after the second immunising dose (results not shown in Table 2). In experiment 1 the

Table 2. Cross-protection between strains of E. tenella: oocyst output and mean weight gain.

| Immunising strain | Challenge strain | Oocyst[@] ($\times 10^6$) | Weight[#] (g) following Immunisation | Challenge |
|---|---|---|---|---|
| Experiment 1: | | | | |
| M91/1 | M91/1 | -ve | 200.8 ± 15.5[a*] | 95.6 ± 6.0[a] |
| M91/1 | D91/1 | 1.4 | 198.8 ± 15.4[a] | 91.2 ± 10.6[a] |
| D91/1 | D91/1 | -ve | 205.3 ± 31.5[a] | 93.6 ± 12.3[a] |
| D91/1 | M91/1 | 1.5 | 200.1 ± 30.3[a] | 92.7 ± 10.4[a] |
| None | M91/1 | 70.9 | 206.6 ± 23.1[a] | 45.1 ± 8.5[b] |
| None | D91/1 | 79.1 | 207.7 ± 18.9[a] | 50.6 ± 9.2[b] |
| None | None | -ve | 204.7 ± 30.2[a] | 96.7 ± 10.5[a] |
| Experiment 2: | | | | |
| Ch91/1 | Ch91/1 | -ve | 157.8 ± 32.4[a] | 81.4 ± 7.2[a] |
| Ch91/1 | S91/1 | -ve | 155.0 ± 31.4[a] | 77.4 ± 8.7[a,b] |
| S91/1 | S91/1 | -ve | 147.0 ± 25.9[a] | 77.9 ± 6.6[a] |
| S91/1 | Ch91/1 | 9.8 | 151.2 ± 18.7[a] | 71.3 ± 4.6[b] |
| None | Ch91/1 | 73.5 | 152.8 ± 23.4[a] | 49.6 ± 10.7[c] |
| None | S91/1 | 65.9 | 149.6 ± 12.4[a] | 47.4 ± 7.1[c] |
| None | None | -ve | 153.8 ± 24.5[a] | 83.7 ± 11.2[a] |
| Experiment 3: | | | | |
| M91/1 | Ch91/1 | 1.4 | 144.9 ± 13.5[a] | 79.3 ± 10.0[a] |
| M91/2 | Ch91/1 | 1.8 | 143.1 ± 9.5[a] | 76.8 ± 7.0[a] |
| M91/3 | Ch91/1 | 1.7 | 147.8 ± 12.8[a] | 76.4 ± 10.8[a,b] |
| D91/1 | Ch91/1 | 0.3 | 142.6 ± 19.5[a] | 73.7 ± 9.5[a,b] |
| S91/1 | Ch91/1 | 7.3 | 141.2 ± 6.1[a] | 68.4 ± 5.3[b] |
| Ch91/1 | Ch91/1 | -ve | 146.7 ± 12.2[a] | 77.4 ± 8.1[a] |
| Ch91/2 | Ch91/1 | 2.2 | 145.6 ± 8.9[a] | 78.7 ± 8.5[a] |
| None | Ch91/1 | 60.1 | 147.4 ± 8.1[a] | 40.9 ± 10.2[c] |
| None | None | -ve | 142.2 ± 10.2[a] | 80.0 ± 10.4[a] |

[@] Total output per bird from days 6 to 12 after challenge as detected by daily counts in group.

[#] Mean ± SD; n=10.

* Means in the same column followed by the same superscripts are not significantly ($P>0.05$) different for each experiment.

chicks passed a few oocysts in the faeces following heterologous challenge, although there was 98% reduction compared with respective unimmunised challenged groups. In experiment 2 and 3 the immunised chicks which received heterologous challenge with Ch91/1 suffered a significant ($P<0.05$) depression in weight gain compared to the homologous challenged groups and the sentinel. However strain Ch91/1 afforded a total protection against heterologous challenge with strain S91/1.

## DISCUSSION

The results of the present study indicate that intraspecific difference in pathogenicity exists in *E. tenella*, and demonstration of this difference in two separate experiments suggests the reproducibility of the results. Differences in pathogenicity between strains of *E. tenella* has also been reported earlier (Joyner and Norton 1969, Shirley et al. 1989), which were not related to

immunogenicity or isoenzyme type. The oocysts used for obtaining strains M91/1, M91/2 and M91/3 were from the same isolate obtained from a sample collected from the caeca of a chick at post-mortem examination. This suggests that any single species in an isolate may contain heterogeneic populations with more than one inherent properties.

In this study it is evident that immunogenic diversity exists between strains of *E. tenella* even when the homologous immunity was complete. In earlier studies however (Joyner and Norton 1969) it was shown that strains of *E. tenella* afforded a total cross-protection when the homologous immunity was complete, and heterogeneity was demonstrated only when the homologous immunity was incomplete by the criterion of oocyst output (Karim et al. 1991). The strain S91/1 was appeared to be less immunogenic than strain Ch91/1, although none of these two strains were different from other strains in this study. As shown by the results of the studies on pathogenicity and from the results of the unimmunised challenged chicks it can be concluded that this immunogenic difference was not due to a difference in fecundity or pathogenicity, which was also observed earlier (Karim et al. 1991). Although Karim et al. (1991) hypothesised that this heterogeneity may reflect a biological difference between the strains, the detection of incomplete cross-protection even when the homologous immunity was complete possibly suggests that a fundamental difference in antigenic repertoire is more likely to be the cause of this difference. Similar environmental conditions and the management practices to which the strains were exposed further suggests that this was not due to any selection pressure.

The results of this study confirm that intraspecific diversity existing in *E. tenella* has to be considered in successful use of precocious vaccine.

ACKNOWLEDGEMENTS

This study was financed by the Bangladesh Livestock Research Institute. Thanks are due to Mr. Nur Mohammed Sarkar for the technical assistance.

REFERENCES

Bailey, N. T. J. 1981. Statistical Methods in Biology. 2nd edn. Hodder and Stoughton, London, United Kingdom.

Chapman, H. D. 1986. Isolates of *Eimeria tenella*: Studies on resistance of ionophorous anticoccidial drugs. *Res. Vet. Sc. 41*: 281-282

Jeffers, T. K. 1978. Genetics of coccidia and the host response. pp. 51-125. *In*: Avian Coccidiosis. Proceedings of the Thirteenth Poultry Science Symposium, 14-16 September, 1977. Long, P. L., Boorman, K. N. & Freeman, B. M. (Eds). British Poultry Science Ltd, Edinburgh, United Kingdom.

Joyner, L. P. 1969. Immunological variation between two strains of *Eimeria acervulina*. *Parasitol. 59*: 725-727.

Joyner, L. P. & Norton, C. C. 1969. A comparison of two laboratory strains of *Eimeria tenella*. *Parasitol. 59*: 907-913.

Karim, M. J. & Trees, A. J. 1989. Immunogenicity of a hybrid line of *Eimeria acervulina*. pp. 639-643. *In*: Coccidia and Intestinal Coccidiomorphs. Proceedings of the Vth International coccidiosis conference, 17-20 October, 1989. Yvore, P. (Ed), Tours, France.

Karim, M. J. & Trees, A. J. 1990. Isolation of five species of *Eimeria* from chicken in Bangladesh. *Trop. Anim. Hlth. Prodn. 22*: 153-159.

Karim, M. J., Hoare, J. A. & Trees, A. J. 1991. Limited heterogeneity between strains of *Eimeria tenella* isolated from Britain and Bangladesh. *Res. Vet. Sc. 51*: 29-33.

Krylova, N. P. & Dikovskaya, V. E. 1975. Immunogenic properties of coccidia from fowls (comparison of strains of *Eimeria tenella*) (In Russian). *Vet. Moscow. 11*: 58-60. (*Vet. Bull.* 1976. *46*: 505, abstract 3725).

Long, P. L. 1974. Experimental infection of chickens with two species of *Eimeria* isolated from Malaysian jungle fowl. *Parasitol. 69*: 337-347.

McDonald, V., Shirley, M. W. & Chapman, H. D. 1985. Attenuation of *Eimeria* species: further characterisation of two lines of *Eimeria mitis*. *Res. Vet. Sc. 39*: 328-332.

Norton, C. C. & Hein, H. E. 1976. *Eimeria maxima*: a comparison of two laboratory strains with a fresh isolate. *Parasitol. 72*: 345-354.

Shirley, M. W. & Bellatti, M. A. 1988. Live attenuated coccidiosis vaccine: selection of a second precocious line of *Eimeria maxima*. *Res. Vet. Sc. 44*: 25-28.

Shirley, M. W., Chapman, H. D., Kuchera, J., Jeffers, T. K. & Bedrnik, P. 1989. Enzyme variation and pathogenicity of recent field isolates of *Eimeria tenella*. *Res. Vet. Sc. 46*: 79-83.