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Short Communication

EFFECT OF REPEATED INFESTATION OF *CHRYSOMYIA BEZZIANA* ON WEIGHT OF ITS LARVAE

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ABSTRACT: There are about 20 species of obligate and 50 species of facultative myiasis producing flies found in India. Out of these, the Old World screwworm fly *Chrysomyia bezziana* is the most important obligate myiasis-causing fly. Three repeated infestations at an interval of one month each, larvae of *C. bezziana* developed in the clinically abraded wounds in cattle. Average weights of the larvae during the 1st, 2nd and 3rd infestation were 62.18±0.441, 56.53±0.389 and 56.45±0.485 mg, respectively. Statistical analysis revealed significant (P<0.05) reduction in larval weight during the 2nd and 3rd infestations compared to the larval weight of the initial infestation.

Key Words: *Chrysomyia bezziana*, larval weight, repeated infestation.

Myiasis is a disease condition caused by the invasion of living tissues of vertebrates by the larvae of dipterous flies (Soulsby 1982; Hall and Wall 1995). All the domesticated, pet and wild animals as well as human beings are susceptible.The Old World screwworm fly *Chrysomyia bezziana* is the most important obligate myiasis-causing fly in India and has been reported from 99% of traumatic myiasis cases in cattle (Narayan and Pillay 1936; Reddy and Krishna 1995; Das 1998; Baidya *et al.* 2006). In addition to India, *C. bezziana* is also a major endemic pest in Southern and South East Asia, parts of Africa, the Middle East and Papua New Guinea (Norris and Murray 1964) with the potential for serious outbreaks throughout the region (Navidpour *et al.* 1996; Reichard 1999). According to Spradbery (1994), on the event of its inadvertent introduction into Australia it may pose a serious threat to the pastoral industry and this pest alone would be responsible for major economic loss amounting to $430 million at 1990 values. Hence, the larvae of *C. bezziana* were allowed to develop in the wounds to study the

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development of acquired immunity on the weight of matured third instar larvae. Aim of the study was to observe whether repeated infestation of the larvae of *C. bezziana* can responsible for development of immunity to block or cure future infestation via retarded growth of the mature larvae.

The study

Two calves were bought to clinics having abraded skin and underlying fascia about 1.5-2 inch length. The animals were housed and fed properly and examined daily. These wounds were examined subsequently every day for the presence of egg masses. Flies laid their eggs at the edge of the wounds. The eggs hatched on the very next day and the first instar larvae entered into the wounds. On the sixth day of infestation mature larvae were collected from the wound by means of a forceps and transferred into normal saline solution (NSS). These larvae were washed in NSS vigorously to remove the host materials attached to larvae. Then these were subjected to five changes of PBS pH 7.2 and washed thoroughly. Out of these, fifteen larvae were separated randomly, blotted thoroughly on blotting paper and individual larvae were weighed with the help of electronic balance (METLER AE 240). Few larvae were fixed in simmering 70% alcohol. To identify the fly larvae, the first three segments containing the cephalopharyngeal skeleton and the anterior spiracles, and the last segment bearing the posterior spiracles were dissected out from the larvae and mounted on glass slides in Hoyer's Berlese mounting medium (Baker and Wharton 1952).

Few batches of fly eggs were also collected from the barb wire wound of calf bought to clinics. The 1st instar *C. bezziana* larvae were harvested by collecting the egg masses and incubating these egg masses in petridishes at 37°C with provision of sufficient moisture. The petridish containing the egg masses was incubated until the first instar larvae hatched out.

These first instar larvae of *C. bezziana* were challenged in the experimental calves, one month interval for two times. From the wounds, mature larvae were collected and processed as mentioned earlier.

After collecting the egg masses and mature larvae, the wounds were treated for killing the remaining larvae by application of turpentine oil, and healing of the wounds by antibiotic therapy and regular wound dressing with proper supportive treatment.

Table 1. Weights of *C. bezziana* larvae during repeated infestations.

	Weights of Larvae in mg		
Larva No	**1st infestation**	**2nd infestation**	**3rd infestation**
1	61.23	58.64	54.64
2.	64.46	55.43	57.74
3.	63.49	57.34	55.62
4.	60.53	56.73	58.32
5.	59.985	57.46	59.21
6.	60.39	55.91	57.58
7.	63.90	54.93	55.23
8.	63.70	58.48	58.29
9.	62.54	56.58	53.65
10.	59.53	57.61	55.61
11.	64.85	58.56	59.35
12.	61.25	56.68	56.34
13.	63.24	54.85	55.47
14.	62.43	53.88	53.49
15.	61.31	54.87	56.32
Mean ± S.E.	62.18^{a}± 0.441	56.53^{b}± 0.389	56.45^{b}± 0.485

Table 2. Analysis of variance of larval body weight in repeated infestations.

Source of Variance	Sum of square	Degree of freedom	Mean of square	F value	F critical
Between infestations	324.408	2	162.204	55.658*	3.21993
Within infestations	122.401	42	2.914		
Total	446.809	44			

*Significant ($P < 0.05$)

Findings of the study

These larvae were identified as *C. bezziana* larvae from their anterior end, posterior spiracles and cuticular spines with reference to the keys given by Zumpt (1965), Soulsby (1982) and Spradbery (1991).

The weights of individual larvae during the three repeated infestations have been summarized in Table 1. It was observed that the average weights of the larvae during the 1st, 2nd and 3rd infestation were 62.18±0.441, 56.53±0.389 and 56.45±0.485 mg, respectively. Statistical analysis (Table 2) revealed significant ($P<0.05$) reduction in larval weight during the 2nd and 3rd infestations compared to the larval weight in the initial infestation. However, DMR test (Kramer 1956) revealed no significant difference between the larval weights of 2nd and 3rd infestation.

During the three repeated infestations at an interval of one month each, larvae of *C. bezziana* developed in the wounds. Record of weights of 15 larvae collected on the 6th day of each infestation showed significant reduction of larval weight during 2nd and 3rd infestation compared to the initial one. This indicated the development of acquired immunity in calves exposed to natural infestation with screwworm myiasis. However, the correlation between development of immunity and antibody titer was not studied.

Reports on naturally acquired immunity to the larvae of *C. bezziana* are scanty in India. Panda and Panda (2001) observed reduction in size of the larvae of *C. bezziana* during subsequent infestations in claves. Naturally acquired immunity to the larvae of *L. cuprina* in the sheep host has been reported (Watts 1979). Sandeman *et al.* (1986) and Baidya *et al.* (2006) also reported that acquired resistance resulted in a lower yield of larvae only in a proportion of animals. Circulating antibody titers to whole fly antigen extracts increased in response to both field strikes and artificial infestations and the predominant antibody was found to be IgG (O'Donnell *et al.* 1980). These antibodies were directed to many different components of the larvae particularly against salivary glands and larval secretary/ excretory (S/E) products (Sandeman *et al.* 1985). Positive correlation between total antibody levels and inhibition of larval growth in *in vitro* assay has also been reported (Eisemann *et al.* 1990; Seaton *et al.* 1992). The inhibition in larval growth and reduction in larval weight during subsequent infestations observed in this study might be due to the effect of circulating antibodies against the S/E products of *C. bezziana* larvae. However, further studies are necessary to correlate between the antibody titre and larval growth during repeated infestations in cattle and *C. bezziana* host-parasite system.

REFERENCES

Baidya S, Panda DN, Sahoo G, Mohanty J (2006) Antigenic characterization of *Chrysomyia bezziana* and *Chrysomyia megacephala* larvae and detection of cross reacting proteins. J Vet Parasitol 20(2): 109-113.

Baker EW, Wharton GW (1952) An introduction to Acarology. The Macmillian Company, New York. 10.

Das AK (1998) Studies on Myiasis and Myiasis causing Flies. M.V.Sc. Thesis submitted to the Orissa University of Agriculture and Technology, Bhubaneswar, India.

Eisemann CH, Johnston LAY, Broadmeadow M, O'Sullivan BM, Donaldson RA, Pearson RD, Vuocolo T, Kerr JD (1990) Acquired resistance of sheep to larvae of *Lucilia cuprina*, assessed *in vivo* and *in vitro*. Int J Parasitol 20: 299-305.

Hall M, Wall R (1995) Myiasis of humans and domestic animals. Adv Parasitol 35: 257-334.

Kramer CY (1956) Extension of multiple range tests to group means with unequal numbers of replications. Biometry 12: 307-310.

Narayan MA, Pillay MR (1936) Some notes on cutaneous myiasis in animals in the Madras Presidency. Indian J Vet Sci Anim Husb 6: 261-265.

Navidpour, S, Hoghooghi-Rad, N, Goodarzi, H, Pooladgar, AR (1996) Outbreak of *Chrysomyia bezziana* in Khoozestan province, Iran Vet Rec 139: 217.

Norris, KR, Murray MD (1964) Notes on the screw-worm fly, *Chrysomya bezziana* (Diptera: Calliphoridae) as a pest of cattle in Papua New Guinea. CSIRO Aust Div Entomol Tech Pap No. 6.

O'Donnell IJ, Green PE, Connell JA, Hopkins PS (1980) Immunoglobulin G antibodies to the antigens of *Lucilia cuprina* in the sera of flystruck sheep. Aust J Biol Sci 33: 27-34.

Panda DN, Panda MR (2001) Effect of repeated infestation of *Chrysomya bezziana* on the size of the larvae. Proceedings of Twelfth National Congress of Veterinary Parasitology, Tirupati, India. 63.

Reddy, PMT and Krishna, BVM (1995) Ivermectin in cutaneous myiasis of buffaloes. Buffalo Bull 14: 75.

Reichard R (1999) Case studies of emergency management of screw-worm. Revue Scientifique et Technique O.I.E. (Office International Des Epizooties) 18: 145-163.

Sandeman RM, Bowles VM, Stacey IN, Carnegie PR (1986) Acquired resistance in sheep to infection with larvae of the blow fly, *Lucilia cuprina*. Int J Parasitol 16: 69-75.

Sandeman RM, Dowse CA, Carnegie PR (1985) Initial characterization of the sheep immune response to infection with *Lucilia cuprina*, the sheep blowfly. Int J Parasitol 15: 181-185.

Seaton DS, O'Meara TJ, Chandler RA, Sandeman RM (1992) The sheep antibody response to repeated infection with *Lucilia cuprina*. Int J Parasitol 22: 1169-1174.

Soulsby EJL (1982) Helminths, Arthropods and Protozoa of Domesticated Animals. 7th edn. The English Language Book Society and Baillier Tindal, London. 809.

Spradbery JP (1991) A Manual for the Diagnosis of Screw-worm Fly. CSIRO Division of Entomology, Canberra, Australia. 64.

Spradbery JP (1994) Screw-worm fly: a tale of two species. Agric Zool Rev 6: 1-62.

Watts JE (1979) Body strike of sheep. In National symposium on sheep Blowfly and fly strike in sheep. Department of Agriculture, New South Wales. 113-119.

Zumpt F (1965) "Myiasis in Man and Animals in the Old World". Butterworths, London. 267.

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