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[Short Communication]

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A Report on the Impact of a Microsporidian Parasite on Lamerin Breed of the Silkworm *Bombyx mori* L.

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Lamerin breed of the silkworm, *Bombyx mori* L. for Northeastern India hosts a vertically transmitted microsporidian parasite for generations, which does not harm significantly the cocoon production. The transversally infected progenies do not exhibit marked external sign or symptom. The microspordian causes inapparent infection and over (80%) of the infected progeny survives and spin cocoons. There is possibility of co-existence between the breed and the associated micosporidian parasite. To evaluate the impact of the microsporidian on breed the present study was conducted in respect of tranovarial transmitted (observed as T1), secondarily infected (observed as T2) and healthy silkworm (observed as T3). The larval and pupal mortality was 12% and 6% in T1 and 10% and 3% in T2 batch, while in case of T3 batch there was no mortality. Significant changes were also observed in single cocoon weight, single shell weight, denier, reelibility, raw silk recovery % and neetness. There is no significant impact of the infection on the fecundity and hatchability. The hatchability of the eggs laid by healthy or infected moths are equall as much as control but the progeny had the infection transmitted from the parent.

Key words: Microsporidian, Parasite, Co-existence, Transmission, Lamerin breed, Silkworm

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Introduction

Micosporidiosis (Pebrine) in silkworm *Bombyx mori* is dreaded diseases caused by a microsporidian an intracellular parasites that can cause sever disease in their hosts including reduction in larval development, growth and fecundity of adults. It infects all age, stage and breed of the silkworm by transovarial and secondary contaminations. The infected larvae become cholrotic, reduced in body size and remain in immature stage much longer than the uninfected individuals. Apart from *Nosema bombycis* several strains and species of the microsporidia have since been isolated from silkworm and other insects (Kishore *et al.*, 1994; Shabir Ahmad Bhat and Nataraju 2004). Most of them are less virulent and cause a chronic infection that often does not kill an insect. Such chronically infected insects frequently do not exhibit marked external sign or symptoms (*e.g.*, color changes, abnormal movement and behavior). Some of the microsporidia are not transmitted by transovarial means and other have low rate of transovarial transmission (Fujiwara, 1980; Fujiwara, 1984; Iwashita *et al.*, 1990; Ananthalakshmi *et al.*, 1994; Nageswara Rao *et al.*, 2004). The mortality caused by them also varies.However no silkworm race(s) are reported to be completely immune to pebrine. Certain races of silkworms were found to be more tolerant to the microsporidia where multiplication rate of the microsporidian is comparatively lower and incubation period is longer (Tanada, 1969; Weiser, 1969; Fujiwara, 1993). However a silkworm breed Lamerin is associated with microsporidian infection for several generations without causing much damage to silk cocoon production or silkworm seed production. There was no systematic study available on the impact of infection of microsporidian to Lamerin silkworm breed and the present report is the first part of investigation.

Materials and Methods

Collection of eggs of Lamerin breed of the silkworm

The healthy and infected eggs with the microsporidian of silkworm breed Lamerin were received from Regional Tasar Research Station, Imphal, Manipur, India during 2003 and reared in the laboratory following the standard procedure. The eggs from healthy and infected moths were prepared and incubated.

Microsporidian spores

Microsporidian spores (Lb_{ms}) were isolated from the infected larvae purified following the method suggested by Sato and Watanabe (1980). The spores were suspended in distilled water and were quantified using haemocytomater. The stock solution was diluted to obtain inoculum dosage of (1×10^7 spores/ml). The purified spores were stored at 4°C in physiological saline (0.85% NaCl).

Influence of the microsporidian parasite on larval development, survival and economic characters

To determine the Influence of the microsporidian parasite infection on larval development, survival and commercial characters of Lamerin breed, newly molted Ist instar larvae were divided into the following three treatments:

T_1 – Transversally infected

T_2 – Secondarily infected

T_3 – Healthy silkworm (as control)

Transversally infected and disease free laying were brushed separately obtained from infected and healthy mother moths. At the zero day of 2nd instar larvae were kept treatment wise. Transversally infected larvae were reared and observed as T_1. Secondarily inoculated by *per os* with purified Lb_{ms} spore 1×10^7 spores/ml and observed as T_2. The Lamerin larvae without any infection were observed as T_3. Each treatment had 5 replications of 100 larvae and was reared till cocooning following standard procedure. The larval weight, larval, pupal mortality, % of infected moths, % of infected layings and hatchability were recorded. Data on the post cocoon characters were also recorded as well. Larvae and Pupae that died were examined for the presence of spores and the individuals that died due to infection were included for data analysis. The larvae or pupae died due to other diseases were excluded from the statistical analysis.

Results and Discussion

The result indicated that the microsporidian isolate from Lamerin breed of the silkworm infects by *per os* means and is also transmitted to the progeny by transovarial means. The virulence was low and cause chronic infection that does not have much impact on various character of the breed. However larval and pupal mortality caused by the infection was low (Table 1). 12% and 6% in T_1 batch due to the transovarial tranmission while 10% and 3% in T_2 batch due to the secondarily infection. 82% and 87% larvae survived to form cocoons in T_1 and T_2 while in case of T_3 it was 93%. There was 74.32% infection in moths obtained from tranovarilly infected batch while 59.38% from secondary infected batch. 49.74% and 40.13% infected layings were obtained from T_1 and T_2 batch while in T_3 did not produce infected eggs. Eggs laid by healthy or infected moths are equall in hatchability. Results of cocoon characters are presented in (Table 2). It is evident

Table 1. Impact of microsporidian infection on the development and survival of the Lamerin breed of the silkworm *Bombyx mori* L.

Treatment	Larval wt. (g)	Mortality due to infection		No. of moths emerged	% of infected moths	Total laying obtained	% of infected layings	Hatching %
		Larva	Pupa					
T1	26.00	12.00	6.00	82.00	74.32	45.00	49.74	75.38
T2	26.33	10.00	3.00	87.00	59.38	47.00	40.13	74.33
T3	26.33	0.00	0.00	93.00	0.00	51.00	0.00	76.11
CD at 5%	-	1.0	1.3	3.0	5.14	3.6	-	-

Table 2. Impact of microsporidian infection on the commercial characters of the Lamerin breed of the silkworm

Treatment	Single cocoon wt. (g)	Single shell wt (g)	SR%	Single cocoon filament length (mtrs.)	Denier	Reelibility	Raw silk recovery %
T1	1.056	0.12	11.30	193	2.0	38.42	33.31
T2	1.194	0.14	11.86	199	1.7	37.38	26.29
T3	1.472	019	13.06	212	1.9	31.60	21.89
CD at 5%	0.201	0.02	-	-	0.1	3.03	5.08

from the data obtained that significant changes were observed in single cocoon weight, single shell weight, denier, reelibility and raw silk recovery %. Percentage of silk content and single cocoon filament length and raw silk were not affected significantly by the microsporidian infection.

The result of the study is clear indicator of the close association of the microsporidian with Lamerin breed of the silkworm *Bombyx mori* for generations and transmits infection vertically but does not cause much damage to the cocoon production. The infected larvae do not exhibit visible sign of the infection externally but differs in larval size. The microsporidian cause inapparent infection in its host, which does not, causes much larval mortality. The larval and pupal mortality were low 12% and 6% in T_1 and 10% and 3% in T_2. The microsporidian isolate cause low mortality to the host. The larvae infected through transovarial transmission by *N. bombycis* show irregular molting and growth becomes tiny and dies during 3rd and 4th instar after discharging spores. However in Lamerin larvae infected through transovarial transmission 82% of the progeny survives and spins cocoons. The secondary infected larvae also survive and 87% of progenies spin cocoons and the moth lays infected eggs. Eggs laid by healthy or infected moths are equal to hatch. However the infection influences the some of the commercial characters of the host. It is possible that the either breed is most tolerant to the infection or the associated microsporidian causing infection may not infect tissues that greatly affect the health status of the breed that finally leads to death of the host as is observed in *N. bombycis* infection. *N. bombycis* causes acute infection and larva dies before spining. Further investigation on the pathogenicity of the microsporidian and susceptibility of the Lamerin breed to the microsporidiosis will throw light on the host pathogen relationship.

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