PREVALENCE, IMMUNE RESPONSE AND CONTROL OF *Stomoxys spp.* OF FLIES

By

N. BASKAR

THESIS

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DEPARTMENT OF VETERINARY PARASITOLOGY
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
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DECLARATION

I hereby declare that this thesis entitled **"PREVALENCE, IMMUNE RESPONSE AND CONTROL OF *Stomoxys spp.* OF FLIES"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

Mannuthy
14.8.2000

N. BASKAR

CERTIFICATE

Certified that this thesis, entitled **"PREVALENCE, IMMUNE RESPONSE AND CONTROL OF *Stomoxys spp.* OF FLIES"** is a record of research work done independently by **Shri.N. Baskar,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

Dr.H. Subramanian
Chairman, Advisory Committee,
Associate Professor & Head,
Department of Veterinary Parasitology,
College of Veterinary and Animal Sciences,
Mannuthy.

14/8/2000
Mannuthy

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Shri.N.Baskar,** a candidate for the degree of Master of Veterinary Science in Veterinary Parasitology, agree that the thesis entitled **"PREVALENCE, IMMUNE RESPONSE AND CONTROL OF *Stomoxys spp.* OF FLIES"** may be submitted by Shri.N.Baskar, in partial fulfilment of the requirement for the degree.

14/8/2000

Dr.H.Subramanian
Chairman, Advisory Committee
Associate Professor & Head
Department of Veterinary Parasitology

14.8.2000

Dr. K.Devada
Assistant Professor
Department of Veterinary
Parasitology
(Member)

14/8/2000

Dr. G.Krishnan Nair
Associate Professor
Department of Veterinary
Microbiology
(Member)

14/8/2000

Dr.M.R.Saseendranath,
Associate Professor & Head
Department of Veterinary Epidemiology &
Preventive Medicine
(Member)

EXTERNAL EXAMINER
Dr. Placid E. D'Souza
Assoc. Professor
Dept. of Parasitology
Veterinary College

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TO

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INTRODUCTION

Livestock plays a significant role in the rural welfare and economy of Indian Agricultural sector. India has a cattle population of 196 millions of which 3.424 millions are in Kerala (India Livestock Sector Review, 1999). Ruminants are the precious possession of the peasants.

It is a reality that animals are always exposed to infection or infestation with one or other parasites. In Kerala, the geographical and climatic conditions are quite favourable for the proliferation and transmission of large variety of parasites and it is nearly impossible for any animal to be free from parasites.

Ectoparasites cause great losses which result from exsanguination, toxicosis, arthropod borne diseases, reduced animal production and performance. Among arthropods, *Stomoxys* infestation is second to none. Freebarn *et al.* (1925) estimated that stable fly causes 9.26 per cent of loss in milk production during one month infestation. Byford *et al.* (1992) estimated that the stable fly causes loss in cattle production to the tune of $ 607.8 millions/year.

Males and females of the *Stomoxys* genus are blood suckers and cause severe blood loss and annoyance by their painful bites. They also act as a vector for protozoans like *Trypanosoma sp.* (Saarma, 1979; Batra *et al.*, 1994 and Samba *et al.*, 1998) and *Anaplasma marginale* (Potgieter *et al.*, 1981) and as intermediate host for nematodes *Habronema* and *Stephanofilaria sp.* (Khronova, 1977 and Soulsby, 1982).

Stomoxys has been incriminated as an agent in transmission of *Bacillus anthracis* (Turell and Kundson, 1987), causative organisms of equine infectious anaemia (Cupp and Kemen, 1983), summer mastitis (Tarry and Carroll, 1988) and bovine viral diarrhoea (Tarry *et al.*, 1991). It also causes sweet itch in horses (Braverman *et al.*, 1983).

Campbell *et al.* (1977) reported that an average infestation of 100 stable flies per calf reduced weight gain by 0.22 kg/day and feed efficiency by 10.9 per cent.

Wieman *et al.* (1992) found that the direct and the indirect effects due to *Stomoxys* fly infestation caused 28.5 per cent and 71.5 per cent reduction in weight gain respectively.

Apart from direct and indirect effects, their attack may also lead to death. Negaty (1965) reported the death of two calves due to severe attack by *Stomoxys nigra*.

Until now no systematic study has been conducted on *Stomoxys* fly infestation in cattle in Kerala. Taking into consideration the favourable environment for fly proliferation and the great economic loss they produce, the present study was undertaken,

1. to study the prevalence of *Stomoxys* fly infestation in cattle in Thrissur.
2. to speciate the flies encountered.
3. to study the biology of the fly.
4. to assess the immune response of rabbit for the thoracic muscle antigen of the pharate adult fly and
5. to compare the efficacy of Diazinon, Deltamethrin, Ethofenprox and Carbaryl against *Stomoxys* infestation.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 Prevalence

Stomoxys fly is a common ectoparasite of cattle and is distributed throughout the world. Sen and Fletcher (1962) have given an account on several species of this fly and the area of distribution in India.

Dipeolu (1975) studied the prevalence of biting flies on cattle and found that *Stomoxys nigra* and *Stomoxys calcitrans* were the most abundant flies in Western Nigeria. He also noted that the flies were more numerous during rainy season and the intensity of the early rain influenced the subsequent abundance of the fly population.

A study by Kunz and Monty (1976) on the ecology of *Stomoxys* fly in Mauritius revealed that the proliferation of *Stomoxys nigra* and *Stomoxys calcitrans* were highest during warm-wet and cool-dry seasons respectively.

Sardey (1976) noted the prevalence of flies feeding on buffaloes in Nagpur, India. He collected 3,819 flies between July 1973 and June 1974, of which 56.47 per cent were non-biting muscids. Among the biting muscids *Stomoxys calcitrans* represented 12.09 per cent. He also observed that the first rise in population of *Stomoxys* fly was during the months of July and August and a second rise in November and December.

Hayakawa (1978) observed the seasonal occurrence of biting muscids associated with cattle in pasture at Taneyama and showed that the *Stomoxys calcitrans* was present from early May to late November and that the population increased rapidly in October.

Khan and Patnaik (1978) recorded the seasonal incidence of muscid flies associated with buffaloes at Izatnagar, U.P. and observed that *Stomoxys calcitrans* and *S. nigra* preferred cool and moderately humid weather.

Miranpuri and Lahkar (1980) reported the incidence of 20 species of Diptera in a cattle shed at a farm in Assam. The overall number was highest in the months of June to September and *Stomoxys calcitrans* represented 12.98 per cent of the total flies collected.

A study carried out by Bielenin *et al.* (1982) in Southern Poland between 1977 and 1979 on seasonal dynamics of Diptera occurring in cattle sheds showed that 50 to 75 per cent of the flies collected were *Stomoxys calcitrans* or *Musca domestica*. The flies appeared in very small numbers in March or April and disappeared in November. Mass emergence occurred in July and August.

Ito *et al.* (1982) studied the prevalence of muscid flies attacking cattle in Tochigi, Japan, during grazing season in 1978 and 1979. Among the four species of muscids collected, the most predominant was *Musca spp.* followed by *Stomoxys calcitrans*. The peak activity of *Stomoxys* was found in October and November.

Seasonal abundance of adult muscid species on equine premises was observed by Burg *et al.* (1990) in Kentucky horse farms from late April to mid October 1987 and 1988. They found that the peak stable fly activity was during mid-June, July and August of both the years.

The seasonal incidence of stable flies was studied by Lysyk (1993) at four dairies in Canada from May to October during 1989 to 1991 and he found that these flies were active from May to October during all years and showed population peak in August and September.

The fly population in two beef production units and two pig farms in Sweden, during 1983 to 1986 were observed by Chirico (1998) who reported that the stable flies were more prevalent during September to December.

Karunamoorthy and Chellappa (1999) studied the muscid flies infesting buffaloes in Namakkal, Tamil Nadu and showed that the overall stable fly prevalence was 1.54 per cent. The fly was not encountered during February to July and the peak incidence was observed in September.

2.2 Identification and speciation

Sen and Fletcher (1962) have given the diagnostic keys for identification of generic and specific details of *Stomoxys* fly. They also described the morphology of egg, larva and pupa of stable fly.

Cantrell (1978) described *Stomoxys* fly along with the identification characters for the flies of order Diptera.

The identification keys for larvae and puparia of the dung breeding muscid flies were given by Ferrar (1979) along with notes on the species.

A detailed account on morphology and identification features of stable fly was given by Soulsby (1982), Smith and Howard (1986) and Kettle (1995).

Patton and Cragg (1984) provided a detailed key for identification of the genus *Stomoxys* and to differentiate the different species.

2.3 Biology of the fly

Glasser (1924) evolved a simple, inexpensive and effective method of rearing and handling large number of stable flies by using horse manure and fermenting straw as larval medium. Flies were fed with warm whole horse or cattle blood, by depositing drops of blood over the surface of wire gauze on top of the

breeding jar. The life cycle parameters given by him were: 1 to 3 days for egg, 11 to 30 days for larva, 6 to 20 days for pupa, 18 to 53 days for egg to adult, 3 to 46 days of longevity and 9 to 13 days of preoviposition period.

Dotty (1937) described a convenient method for rearing the stable fly in which he fed the flies by keeping the petridishes containing cheese cloth soaked in warm citrated blood inside the cage. He prepared a medium (wheat bran, alfalfa meal, yeast suspension and water) for both oviposition and larval development. The life cycle outlined by him was: 1 to 3 days for egg, 9 to 15 days for larva, 4 to 14 days for pupa, 7 to 14 days of preoviposition period and a longevity of 2 to 30 days. Campau *et al.* (1953) followed the method described by Dotty (1937) to feed the flies. The life cycle parameters as to the appearance of pupae, adult emergence and first egg laying were 9, 14 and 21 days respectively after seeding eggs.

Champlain *et al.* (1954) had suggested some modifications in Campau (1953) method of rearing. They kept moist cellulose sponges in plexiboxes which were placed in front corner of the breeding cage for the females to oviposit. They added sand over the surface of larval medium which reduced mould growth and moistening it prior to pupation helped in separation of pupae. They also refrigerated the pupae and eggs for a limited period of time.

McGregor and Dreiss (1955) prepared a larval medium using oat bran, alfalfa meal and wood shavings. They fed the flies using a glass tube equipped with rubber suction bulb. The glass tubes were held with the open ends resting on screen holding cage so that the flies can feed through the screen. They kept a damp cotton wrapped with black cloth to attract the flies for oviposition and also added few drops of five per cent ammonia solution over the cloth to stimulate oviposition.

Parr (1959) evolved a method to breed the fly under lab condition. He fed the flies twice daily, through citrated bovine blood soaked absorbant pads,

which were hung from the roof of the cage. He used a larval rearing medium containing dried cow dung, blood granules and sugar.

Sen and Fletcher (1962) gave the life history parameters of stable fly which was 36 to 48 h for eggs, 2 to 3 weeks for larvae and about 10 days for pupae. The best oviposition and larval medium according to the author was horse dung and urine impregnated straw.

Ramsamy (1979) established a laboratory colony of *Stomoxys nigra*. He maintained the fly cages at 29±1.5°C and at Relative Humidity (RH) of 78 to 80 per cent and fed the flies with stored citrated blood. The eggs were harvested on moist kakhi cloth kept in the bottom of adult rearing cage. The larval medium was composed of shredded sugarcane, wheat bran and water and the pupae were separated by floatation over water.

Bidgood (1980) observed that stable flies generally preferred the legs of cattle to feed, but females were found more on the body. Period of maximum activity commences after 16 h of fasting and lasts for about 14 h in both sexes. Males showed a second peak activity after 43 h of fasting and no flies survived after 48 h.

A technique of mass rearing of stable fly was described by Williams *et al.* (1981). They maintained the flies at 24 to 26°C and 70 to 75 per cent RH and kept moist cotton covered with black cloth to stimulate oviposition. Adult eclosion averaged above 90 per cent, mating commenced 48 to 72 h later and peak oviposition occurred on 8^{th} or 9^{th} day. Flies were fed by using cotton pads saturated with citrated bovine blood.

Soulsby (1982) gave details about life cycle of stable fly and mentioned that the flies preferred moist decaying vegetable matter and hay, especially when mixed with urine, for oviposition. He found that the period of egg, larva and pupa

were 1 to 4, 14 to 24 and 6 to 9 days respectively and the time to complete life cycle was about 30 days.

Abasa (1983) found that, most eggs hatched during the first and second day after oviposition when flooded and concluded that the contact with water or exposure to humid condition within first three days after oviposition was necessary for successful hatch.

Patton and Cragg (1984) had explained in detail about the breeding technique of stable fly and gave the life cycle parameters as to the incubation period of egg, period of larva and pupa as 12 h, 7 to 21 days and 4 days respectively.

According to Kettle (1995) the period of egg, first stage larvae, second stage larvae, third stage larvae at 26.7°C and pupae at 30 to 31°C were 23 h, 23 h, 27 h, about 7 days and 5 days respectively. Lifespan of adult was 3 to 4 weeks during summer.

2.4 Immunological study

Several studies have been conducted in the aspect of controlling haematophagus arthropods through immunological means. Trager (1939a) presented the first experimental evidence on development of acquired resistance against a blood sucking arthropod. Subsequently, Trager (1939b) was able to induce resistance against ticks in guinea pig by injecting larval extracts from same species.

Alger and Cabrera (1972) immunized rabbit with *Anopheles stephensi* using triturated gut and whole mosquitoes as antigens and detected the immune response using double diffusion test. They found that feeding of *A. stephensi* on rabbit immunized with whole mosquito antigen did not increase their mortality but

those fed on rabbits immunized with gut antigens resulted in a significant increase in their mortality.

Schlein *et al.* (1976) showed that the meal proteins can enter the haemolymph and tissues of insects without any destruction. Immunoglobulins raised in rabbit against fly tissue were found in tissues of flies fed on rabbit serum, with specificity to muscles and nerve tissues.

Schlein and Lewis (1976) used different tissues of pharate *Stomoxys* fly like cuticle and adhering hypodermal cells, thoracic muscles, abdominal tissues and wing buds as antigen to raise antibodies in rabbit. The titres of precipitating antibody were determined by capillary precipitin test. The result of feeding normal flies on rabbits immunized with above antigens showed higher mortality compared to control flies. Abnormal effects like paralysis of leg or wing and difficulty in probing were observed. Mortality after 15 days of daily feeding of flies on rabbit immunized with thoracic muscle antigen was twice that of the control flies fed on control rabbit. The antibody raised was non-specific, as it also produced mortality in *Glossina morsitans* fed on immunized rabbits.

Antigens prepared from whole flies, abdominal sections and dissected gut materials of stable flies were used for immunization by Webster *et al.* (1992) and the serological response was detected using immunoblotting. Normal flies were fed with blood from immunized rabbit and the mortality was highest in the group fed with blood from rabbit immunized with gut antigen. There was also a significant difference in the egg viability between the test and control groups.

Opdebeeck (1994) and Wikel *et al.* (1996) gave details about control of haematophagus arthropods by immunological means in which they have described about antigens that can be used against *Stomoxys* fly.

Ravindran *et al.* (2000) detected the immune response in rabbit immunized with *Psoroptic* mite antigen using agar gel precipitation test, counter immuno electrophoresis and passive haemagglutination assay and obtained good results.

2.5 Insecticidal study

2.5.1 Diazinon

Dahm and Raun (1955) evaluated the residual effects of diazinon against stable fly by spraying on both actual and potential fly resting places and obtained good initial reduction of fly population following application. The range of effective fly control for diazinon at one per cent spraying varied between 7 and 35 days.

Johnston and Blakeslee (1961) studied the tolerance of stable fly against diazinon by confining 4 to 7 days old engorged flies for 15 min in pint manson jars, the interior of which were treated with known concentrations of diazinon and recorded the mortality after 24 h. The LC_{50} and LC_{90} for diazinon were 0.23 mg/sq ft and 0.98 mg/sq ft respectively.

Harris (1964) determined the susceptibility of adult stable fly to diazinon by applying the compound topically over the fly using microapplicator and recorded the mortality after 24 h. The LD_{50} and LD_{90} were found to be 0.21 µg/fly and 0.43 µg/fly respectively.

Mount *et al.* (1965) designed a wind tunnel apparatus in which 3 to 5 days old engorged stable flies were exposed to contact spray of diazinon and the mortality was recorded after 24 h. They obtained 99 per cent, 86 per cent and 48 per cent mortality at 0.25 per cent, 0.1 per cent and 0.05 per cent concentration respectively.

Face fly control experiment on horses using smear type mixture containing diazinon was done by Dorsey (1966). The mixture was applied on the face of horses which were released into pastures and it was found that diazinon at one per cent concentration failed to reduce the actual number of flies on the horses.

Control measure taken against the flies like *Musca spp.* and *Lyperosia sp.* on cattle in pasture showed that diazinon at one per cent spray was most effective (Razimuradov, 1978).

Perez *et al.* (1987) proved that diazinon at therapeutic concentration was non-toxic to cattle through biochemical and clinical evaluation of the animal.

Anziani *et al.* (1998) showed that ear tag containing 40 per cent diazinon provided more than 80 per cent protection against hornfly for 15 weeks.

2.5.2 Carbaryl

Roberts (1959) evaluated the effectiveness of carbaryl as 0.5 per cent spray against hornfly and found that it failed to give protection in the first week itself.

Blume *et al.* (1973) tested the effect of carbaryl at 0.5 per cent concentration for the control of biting flies and found that the spray was not effective against stable fly after one day post-treatment.

Susceptibility of lab reared horn flies to topical application of several insecticides were determined by Frazer and Schmidt (1979) and found that carbaryl (99.7%) showed LD_{50} and LD_{90} values of 1.187 ng/fly and 3.191 ng/fly respectively.

Galowalia (1980) evaluated the effect of carbaryl on three types of surface for control of *Stomoxys nigra*. An aqueous spray of the compound at 1 g a.i/m^2 was applied. On the formica and straw surface 100 per cent mortality was

produced up to 14 days and on plaster surface the mortality declined from 100 per cent on third day to less than 33 per cent on the seventh day. Subsequently Galowalia (1981) concluded that carbaryl was most effective for area spraying against *Stomoxys nigra*.

Srivastava (1988) studied the effect of different insecticides against stable fly by topical application using Aglamicrometer syringe. Mortality count made after 24 h showed that sevin (carbaryl) was more effective than diazinon.

A study on control of *Haematobia irritans* using self application dust bag containing 10 per cent carbaryl showed that animals treated four times after four consecutive milkings gave 99 per cent, 39 per cent and zero per cent protection after 24 h, 96 h and 114 h of last treatment respectively. The animals treated for 14 consecutive milkings gave 99 per cent, greater than 90 per cent and greater than 80 per cent protection after 24 h, 72 h and 96 h after last treatment respectively. There was no effect after eight days (Guglielmone *et al.*, 1997).

2.5.3 Deltamethrin

Morgan (1980) briefed that the contact-repellant activity of synthetic pyrethroid was characterized by brief duration of visit and high kill of flies immediately after treatment but later on only the lethal effect persisted.

Williams and Westby (1980) designed a pyrethroid impregnated ear tag for control of horn flies and reported that deltamethrin at 1.5 per cent concentration provided 95 per cent reduction of horn flies up to 13 weeks.

Appleyard *et al.* (1984) reported that deltamethrin at 0.01 per cent concentration applied to head of lamb and gimmer at 30 days interval failed to control the sheep head fly disease.

Dukes and Hallmon (1984) evaluated different insecticides for the control of adult stable flies and found that the synthetic pyrethroid were the most toxic, followed by carbamates and organophosphates.

Field trial conducted by Bertels and Robijns (1985) using 2 per cent deltamethrin as pour-on against *Haematobia irritans* showed a steady increase in fly number after six weeks and no effect after nine weeks.

Tymezyna and Majewski (1990) sprayed one per cent aqueous emulsion containing 0.25 g deltamethrin per litre in cow sheds and found that the number of flies/m^2 of wall was reduced from 11 to zero up to 16 days.

2.5.4 Ethofenprox

The discovery, mode of action, safety, metabolism and effects on beneficial insects of ethofenprox were dealt by Udagawa (1988).

Yoshimoto *et al.* (1989) investigated the insecticidal action of ethofenprox and found that it was very effective against Lepidoptera, Hemiptera, Coleoptera, Diptera and Orthoptera. Low mammalian toxicity, higher compatibility with other insecticides, low dermal and eye irritation and no cross resistance to carbamates and organophosphates were the other beneficial properties.

Putsintseva *et al.* (1992) studied the activity of ethofenprox at 10 per cent and 30 per cent flow concentration against a variety of common insects and against mosquito larvae and found that it was less effective than permethrin against fleas and house flies. He also observed that the residual effect was retained upto one month, according to the type of surface treated.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Prevalence of *Stomoxys* fly

The prevalence study was conducted on cattle brought to University Veterinary Hospitals at Mannuthy and Kokkalai and also on cattle in University Livestock Farm, Mannuthy from June 1999 to May 2000. Each month fifty numbers of cattle were observed and the average number of flies infesting per animal was calculated. Meteorological data were collected from Department of Agricultural Meteorology, College of Horticulture, Vellanikkara and were correlated with the prevalence of *Stomoxys* flies.

3.2 Collection and identification of flies

The flies were collected using polythene bags from different areas on the animal body and from animal dwellings and were identified using the keys provided by Sen and Fletcher (1962) and Patton and Cragg (1984) and also by observing the morphological features given by Soulsby (1982), Bowmann (1995) and Kettle (1995).

3.3 Rearing and colonisation of adult flies in laboratory (Fig.1)

The method of rearing *Stomoxys* fly described by Glasser (1924) was followed with slight alterations. The flies were caught from stables and cattle sheds or while feeding on the animal body, using polythene bags and were transferred to a breeding jar (Fig.2). Cattle blood (10 ml) was collected every week in sodium citrate added vials (2 mg/ml) and stored at 4°C. The flies were fed twice daily with citrated blood after warming to 37 to 40°C. Absorbant cotton pads soaked in the warm blood was kept over the cage for the flies to feed.

Fig. 1. Colonisation of adult flies in laboratory

Fig. 2. Single fly breeding cage


II
III

Fresh moist horse dung was kept inside the cage in a petridish for the gravid females to oviposit. The petridish with dung was removed every day and replaced with fresh dung. Small amount of sand was added over the dung in the petridish removed from the jar and kept moist by adding water. The matured larvae migrated over the sand and pupated. The pupae were collected and transferred to a fresh cage for adult emergence.

3.4 Antigen preparation

Thoracic muscle antigen was prepared according to the method described by Schlein and Lewis (1976) with slight modification. Pharate adult (24 h before eclosion) flies were used for antigen preparation. Thoraces were separated and stored at -70°C in sterile normal saline after removing the gut. Benzylpenicillin (1 lakh I.U.) and Streptomycin (1 g) in 40 ml sterile distilled water was added at the rate of 0.01 ml to 1 ml of the sample.

Tissues were homogenised using sterile glass tissue grinder kept over ice. The ground tissue was sonicated using four 20-S pulses with 30-S intervals and centrifuged at 13,000xg to remove cellular debris. The supernatant was centrifuged at 100,000xg for 1 h. The supernatant containing soluble proteins was removed and stored in cryovials at -70°C.

3.5 Protein estimation

The protein content of the prepared antigen was estimated by Biuret method using ready made kits available from new India Chemical Enterprises, Cochin, with the help of photometer 5010, Boehringer Mannheim. This antigen was used for immunization and in all serological tests.

3.6 Immunization

Two 3 month old healthy cross bred rabbits weighing 1 kg each were reared, of which one was used for immunization and the other was kept as control.

Rabbits were fed with compounded feed obtained from Small Animal Breeding Station, College of Veterinary & Animal Sciences, Mannuthy and with natural grass. Blood was collected before immunization, serum separated, centrifuged and stored at -20°C until used. The antigen was injected as per the following schedule.

Zero day

One and a half ml each of antigen (6 mg) and Freunds complete adjuvant were mixed and made into a emulsion and injected intramuscularly into leg muscles.

Seventh day

One and a half ml each of antigen (6 mg) and Freunds incomplete adjuvant were mixed and made into a emulsion and administered subcutaneously at different sites.

3.7 Blood collection and serum separation

The immunized rabbit was bled on 14th, 21st and 28th day, serum separated from clotted blood, centrifuged and stored at -20°C until used.

3.8 Serological tests

3.8.1 Agar gel precipitation test (AGPT)

The procedure of doing AGPT described by Kagan and Norman (1976) was followed with slight modifications.

a) Reagents

Agarose	- 0.8 g
Sodium chloride	- 0.8 g
Phenol	- 0.015 g
Distilled water	- 100 ml

Staining solution

Amidoblack 10 B	- 1 g
Sodium chloride	- 8.5 g
Distilled water	- 1000 ml

Decolouriser-I

Methanol	- 40 parts
Acetic acid	- 10 parts
Distilled water	- 10 parts

Decolouriser-II

Absolute alcohol	- 35 parts
Acetic acid	- 5 parts
Distilled water	- 10 parts

b) Test proper

Melted agarose was poured on to a clean grease free glass slide and allowed to solidify initially at room temperature and subsequently at 4°C for 15 min. Wells were cut in 5 well pattern and the central well was loaded with the prepared antigen and the peripheral wells with sera from preimmunized and immunized rabbit. The slides were kept in a humid chamber at room temperature for 48 h and examined against light for the development of any precipitation line.

c) Staining

The slides were soaked in two changes of normal saline 24 h each and then in distilled water for further 24 h to remove unreacted proteins. The slides were dried slowly by placing a moist filter paper over them. They were then stained with Amidoblack for 15 min and decolourised in decolouriser I and II for 20 min each. The slides were dried at 37°C for one hour and mounted in DPX mountant.

3.8.2 Counter immunoelectrophoresis

Test was done as per the method described by Kagan and Norman (1976) with slight modifications.

a) Reagents

1. Tris-Barbital Buffer

Barbitone sodium	- 9.9 g
Tris (Hydroxy Methyl amino methane)	- 17.7 g
Sodium azide	- 0.3 g
Distilled water	- 2000 ml

pH was adjusted to 8.6 with 1N hydrochloric acid

2. Agar coated slides

Clean grease free microscopic slides were dipped in one per cent melted agarose in distilled water and dried in air by keeping the slides horizontally over glass rods. Dried slides were stored at room temperature until used.

b) Test Proper

The melted agarose (0.8 per cent in Tris barbiturate) was poured on to each slide kept on levelled surface. The agar was allowed to solidify and wells of 5 mm diameter were cut in parallel rows 3 mm apart.

The electrophoresis tank was filled with the buffer. The wells were filled with antigen and serum and slides were loaded in the tray in such a way that the antigen was near to cathode of the power supply.

The filter paper wicks were placed in position, power was supplied at rate of 5 mA per slide and the electrophoresis was continued for 45 min. The slides were examined for precipitation line.

Slides were removed from the tray, incubated for 6 h at room temperature and stained as in the case of AGPT.

3.8.3 Passive haemagglutination assay (PHA)

The test was done with tanned sheep erythrocytes as per the method of Kagan and Norman (1976), with slight modification.

Reagents

1. Anticoagulant solution

Sodium citrate	- 3.8 g
Distilled water	- 100 ml

Sterilized at 15 lbs pressure for 15 min and stored at 4°C.

2. Phosphate buffered saline (PBS)

Stock solution (0.15 M) was prepared in distilled water.

Disodium Hydrogen phosphate anhydrous (Na_2HPO_4)	- 21.3 g/litre
Potassium dihydrogen phosphate (KH_2PO_4)	- 20.4 g/litre
Sodium chloride (NaCl)	- 8.8 g/litre

PBS, pH 6.4 (0.15 M)

Na_2HPO_4	- 32.3 ml
KH_2PO_4	- 67.7 ml
NaCl	- 100.0 ml

PBS, pH 7.2 (0.15 M)

Na_2HPO_4	- 76.0 ml
KH_2PO_4	- 24.0 ml
NaCl	- 100.0 ml

Diluent: One per cent Normal Rabbit Serum (1 per cent NRS)

Serum from the control rabbit was inactivated at 56°C for 30 min, stored frozen and reinactivated for 10 min immediately before use. Mixed one ml of the inactivated serum with 99 ml of PBS, pH 7.2.

Tannic acid 1:20,000 dilution

Fresh solution of 1:1,000 dilution of tannic acid was prepared by dissolving 10 mg of reagent grade tannic acid in 10 ml of PBS, pH 7.2.

Diluted the 1:1,000 stock solution, 1:20 for the 1:20,000 dilution used in the test.

Preparation of tanned sensitized red cells

1. Sheep blood was collected in 3.8 per cent sodium citrate solution and then washed three times with PBS pH 6.2 by centrifugation at 800xg for five min twice and for 10 min for third wash. Adjusted to 5 per cent suspension.
2. Added an equal volume of 1:20,000 tannic acid solution and mixed well. Incubated the mixture in water bath at 37°C for 10 min.
3. Tannic acid treated cells were removed from the water bath and centrifuged for 5 min at 800xg. Supernatant fluid was decanted, washed once with PBS, pH 7.2 and resuspended the cells to a 5 per cent suspension with PBS, pH 6.4.
4. Sensitized the tanned cells by adding an equal volume of the optimal dilution of the antigen in PBS, pH 6.4, to the cell suspension. Incubated the mixture in 37°C water bath for 15 min. The optimum dilution was predetermined for each lot of antigen by box titration of dilution of positive sera of known titre as shown below.
5. Removed the antigen treated cells from the water bath and centrifuged for 5 min at 800xg. Decanted the supernatant fluid and washed the cells twice with 1 per cent NRS.
6. The cells were adjusted to a 5 per cent suspension in one per cent NRS after a final pack by centrifugation at 800 xg for 10 mins.

Determination of optimal concentration of antigen

1. Prepared four two fold dilutions of antigen in PBS pH 6.4 (1:25, 1:50, 1:100, 1:200)
2. Sensitized the cells with each dilution of antigen as described above in preparation of tanned sensitized red cells, step 4, 5 and 6.
3. Checked one negative and one positive serum with each dilution by the test procedure given below. The lowest concentration of antigen giving the highest titre with the immune serum and no reaction with the negative serum was considered optimal.

Test procedure

Inactivated the serum specimen for 30 min at 56°C. Serial dilution of the serum in one per cent NRS was prepared, as follows:

1. Transferred 0.05 ml of one per cent NRS with a micropipette to all wells of microtitration U-plate in which serum dilutions would be made.
2. Transferred 0.05 ml of test serum to the first well containing 0.05 ml of one per cent NRS, mixed thoroughly and 12 two fold serum dilutions were prepared.
3. Added 0.025 ml of 5 per cent sensitized cell suspension to each serum dilution and mixed well by tapping at the sides of U plate.
4. The cells were allowed to settle for 2-3 h at room temperature.
5. The pattern of cells on the bottom of the wells was read. A positive reaction (4+) was indicated by a mat or carpet of cells covering the bottom of the well. In strong reactions the edges were folded. A negative reaction was one in which the cells had settled to form a compact button or ring at the centre of the well. Titre was the end point dilution factor of the highest dilution showing a 4+ reaction.

Control

Transferred 0.05 ml of one per cent NRS to several wells in a U plate and added 0.025 ml of the five per cent suspension of sensitized cells to all control wells. These reactions should be negative.

3.9 Feeding of *Stomoxys* flies with immunized rabbit blood

The method described by Webster *et al.* (1992) was followed with slight modifications to study the effect of immunized rabbit blood on normal flies.

Thirty numbers each of newly emerged flies were put in two cages. One group was fed with citrated blood from immunized rabbit and the other was fed with the control rabbit blood. Fly mortality was assessed by counting the dead flies after removing them from the cage at the end of every 24 h period. The total eggs oviposited was calculated by collecting the deposited eggs at the end of each 24 h period and their viability was determined by allowing them to develop and counting the number of larvae hatched. Pupal development and fly emergence from them were also observed and compared. The cumulative percentage of mortality of flies in both cages were also calculated and compared.

3.10 Evaluation of insecticides on stable fly

The method of evaluation described by Roberts *et al.* (1960) was followed with slight modifications.

Thirty calves of 6 to 12 months of age were divided into five groups of six each. Group I was applied with Diazinon - 0.3 per cent (Neocidol 20 per cent EC), Group II with Deltamethrin - 0.0025 per cent (Butox 1.25 per cent EC), Group III with Carbaryl 0.1 per cent (Sevin 50 per cent WP), Group IV with Ethofenprox 0.002 per cent (Trebon 10 per cent EC) and Group V was kept as control which was treated with water alone. The compounds were evaluated for their insecticidal and knockdown effects.

Test proper

An area of 5 inches diameter was marked with black dye on upper half of the body just behind the shoulder. Hair was clipped closely in order to facilitate easy feeding of the flies. Insecticides were diluted with water to arrive at the required concentrations and applied topically over the area without run-off.

Twenty newly emerged flies not fed for 16 to 18 h were transferred to a tube of four inches length and two inches diameter and covered with mosquito net. The mouth of the tube was kept over the treated area after removing the cover and the flies were exposed for 20 min. After exposure the flies were transferred to a holding cage.

Mortality was assessed by counting the dead flies after 24 h of exposure and the knockdown effect by noting the percentage of the flies that were unable to fly or walk immediately after exposure. The mortality was also expressed in percentage and compared with the mortality and knockdown effects obtained in the control flies which were exposed to the area treated with water alone.

The comparative efficacy of different insecticides tested were analysed statistically. Analysis of variance was done by following the method of Snedecor and Cochran (1967).

RESULTS

4. RESULTS

4.1 Prevalence of the fly

The study on the prevalence of *Stomoxys* infesting cattle during June 1999 to May 2000 showed a comparatively a higher incidence in the months of June (94.4 per cent), July (100 per cent), August (91 per cent) and September (92.8 per cent) than in other months. The mean monthwise prevalence rate of infestation varied between a maximum of 10.9 flies/animal in July '99 to a minimum of 0.7 fly/animal in February 2000. The mean prevalence rate in other months were, June - 7.7 flies/animal; August - 9.3 flies/animal; September - 9 flies/animal; October - 3.6 flies/animal; November - 3.9 flies/animal; December - 2.6 flies/animal; January - 0.8 fly/animal; March - 1.6 flies/animal; April 1.8 flies/animal and in May - 1.4 flies/animal (Graph 1).

The effect of seasonal and climatic variation on the prevalence of fly infestation was noted during the period (Table 1).

Higher prevalence of the flies during the month of July was associated with lowest mean atmospheric temperature (25.7°C), high mean RH (89 per cent) and highest rainfall (823.3 mm). During February, the lowest prevalence was in relation with high mean atmospheric temperature (28°C) and low mean RH (68.5 per cent) and mean rainfall (4.6 mm).

4.2 Species of the fly collected

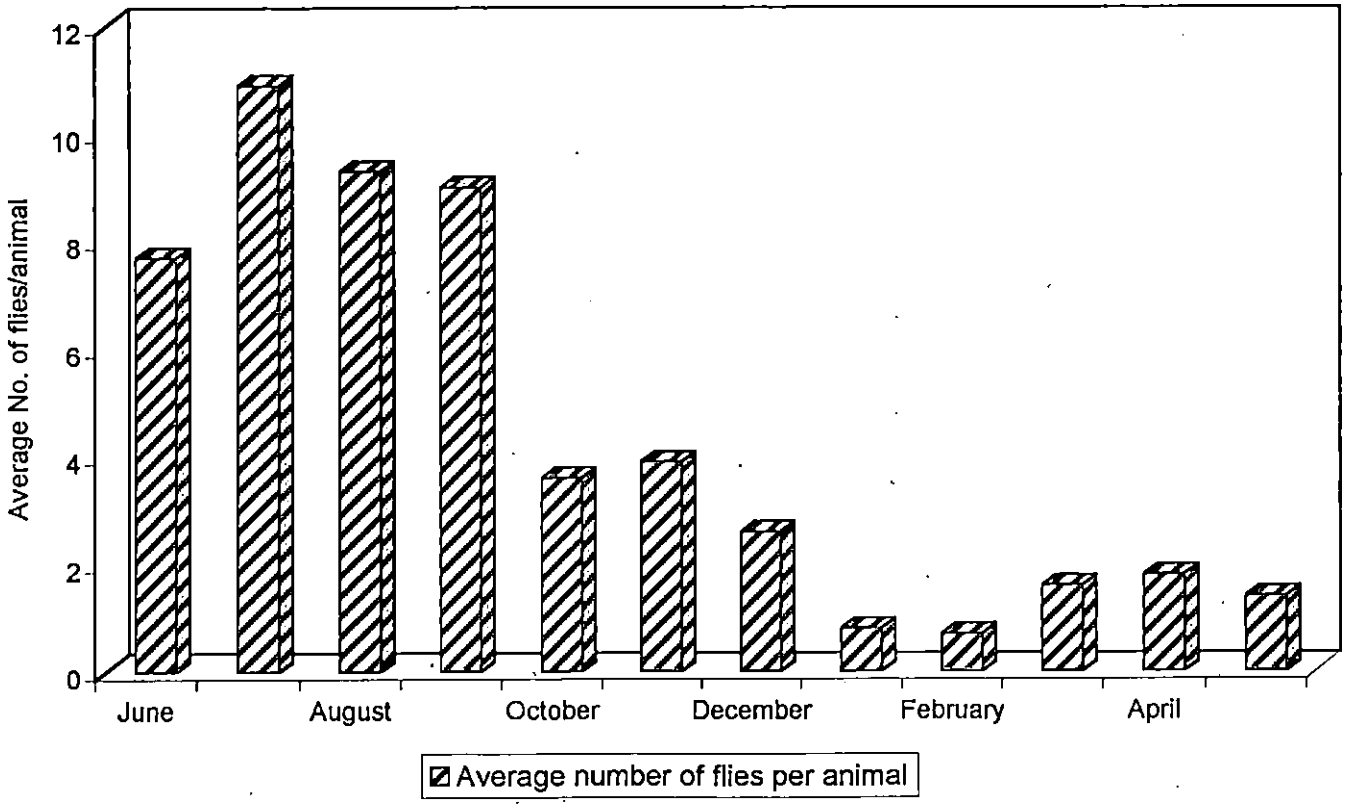
The flies collected from various animals and animal dwellings were speciated and were found to be *Stomoxys calcitrans*.

Table 1

Effect of seasonal and climatic variation on the prevalence of *S. calcitrans* in cattle

Period	Mean Temperature (°C)	Mean Relative Humidity (%)	Rainfall (mm)	Total number of animals observed	Total number of animals infested	Prevalence (%)	Total number of flies noted	Average number of flies per animal
June 99	26.2	84.5	500.2	54	51	94.4	418.	7.7
July 99	25.7	89.0	823.3	71	71	100	773	10.9
Aug 99	26.4	83.5	260.1	67	61	91.0	624	9.3
Sep 99	27.5	76.0	28.4	70	65	92.8	633	9.0
Oct 99	26.9	84.5	506.2	76	68	89.5	273	3.6
Nov 99	27.0	69.0	9.1	69	61	88.4	268	3.9
Dec 99	26.7	60.0	0.0	75	61	81.3	193	2.6
Jan 2000	28.0	59.6	0.0	74	46	62.2	58	0.8
Feb 2000	28.0	68.5	4.6	73	45	61.6	51	0.7
Mar 2000	29.8	66.5	0.0	75	62	82.6	116	1.6
Apr 2000	29.3	74.0	67.9	74	62	83.8	131	1.8
May 2000	29.0	72.0	117.2	70	58	82.9	95	1.4
Total/Mean	-	-	-	848	711	83.8	3633	4.3

Graph 1. Monthwise prevalence rate of *S.calcitrans* infestation in cattle from June 1999 to May 2000



4.3 Morphology of *Stomoxys calcitrans*

4.3.1 Egg

Egg measured 1.3 mm x 0.3 mm and was creamy white when fresh but turned brownish on drying. The shape was elongate and had narrow anterior and round posterior ends, round on one side and almost straight on other side. A narrow longitudinal ribbon ran along the entire length of the straight side (Fig.3). The incubation period of egg was one to three days.

4.3.2 Larva

There were three larval instars and the larvae had tapering anterior and rounded posterior ends. The first instar measured 1.8 by 0.25 mm to 2.3 by 0.4 mm and the second instar measured 2.5 by 0.5 mm to 4.0 by 0.7 mm. The segmental divisions and locomotor pads were well marked. The matured larva measured 4.2 by 0.8 to 12 mm by 1.3 mm and was dull white to creamy white in colour (Fig.4). The anterior spiracles were yellowish and had five papillae (Fig.5). The posterior spiracles were wide apart and not conspicuously raised and had a triangular peritreme. There were three 'S' shaped slits and a central button in each spiracle (Fig.6). The cephalopharyngeal skeleton (Fig.7) in the gross specimen was like a dark line in the anterior end. The period of development of first, second and third stage larvae were 22 to 25 h, 26 to 29 h and 9 to 13 days respectively.

4.3.3 Pupa (Fig.8)

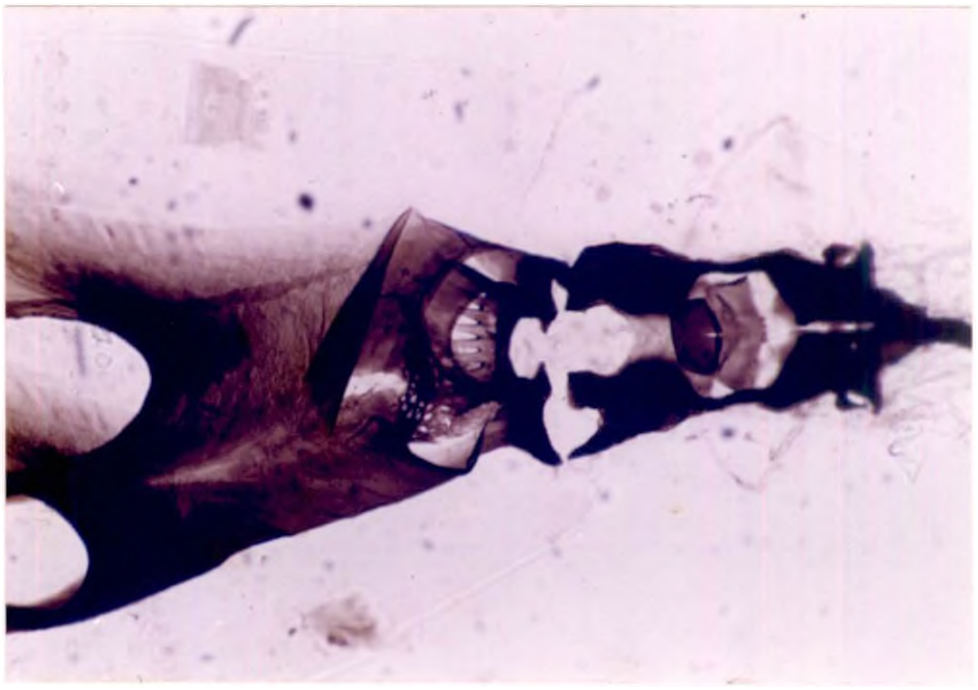
The coarctate pupa was mahogany brown in colour and had transverse striations on its surface (Fig.9). It measured 5 by 1.5 mm and had a development period of 5 to 8 days.

Fig.3. *S. calcitrans* - Egg (10x)

Fig. 4. Matured larva of *S. calcitrans*

Fig.5. Anterior spiracle of *S. calcitrans* larva (10x)

Fig. 6. Posterior spiracle of *S. calcitrans* larva (10x)



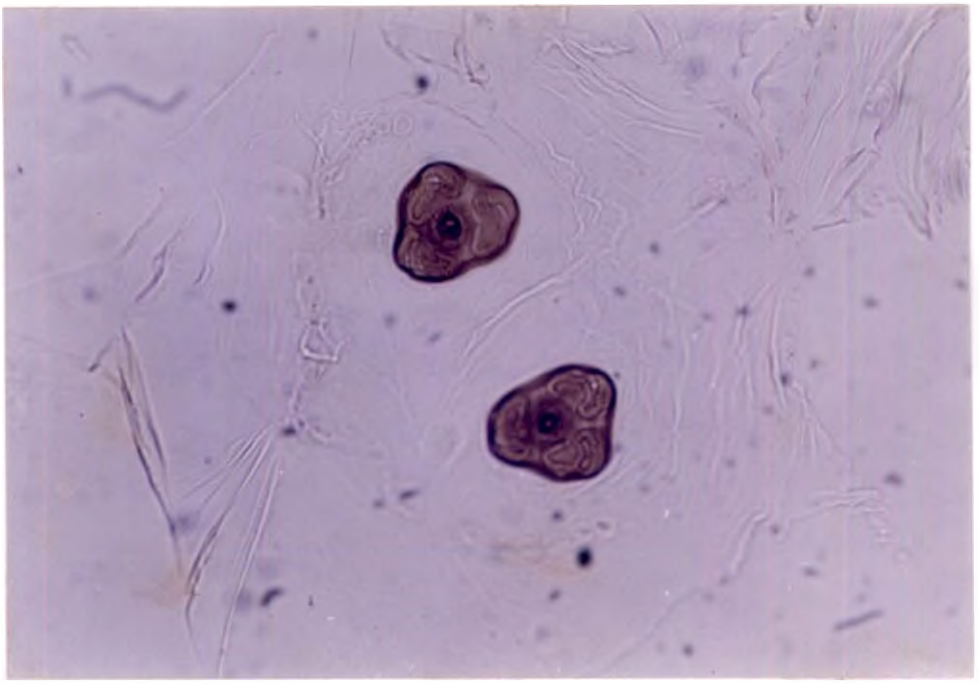


Fig.7. Cephalophanyngeal skeleton of *S. calcitrans* larva (4x)

Fig. 8. Pupae of *S. calcitrans*

4.3.4 Adult fly (Fig.10)

Head

Head in profile was straight. Eyes were holoptic in male and dicoptic in female. Frons in the male was 1/4th the width of head and in female 1/3rd. Bristles were seen on arista only on its upper surface (eight to nine in number). Palps were small and were about half the length of the proboscis (Fig.11) and concealed during rest.

Thorax

Thorax was yellowish to whitish grey with four brown to black longitudinal stripes and the lateral ones were interrupted at the suture. Dorsal stripe was narrow at anterior end and widened towards the posterior end. On thorax the chaetotaxy were Humeral-3; Posthumeral-1; Supra alar-1; inter alar-1; Post alar-2; acrostichal-1; mesopleural-12 and sternopleural-1. In the wing, the apical cell was open (Fig.12). The legs were black in colour.

Abdomen

The abdomen was grey in colour with a median stripe on the second and third segments and two lateral circular dark spots on each segment. The fourth segment normally had a triangular basal spot or band which may be very indistinct or absent. In some specimens the median stripes of second and third segment fused together and formed one band, extending up to the fourth segment.

4.4 Biology of the fly

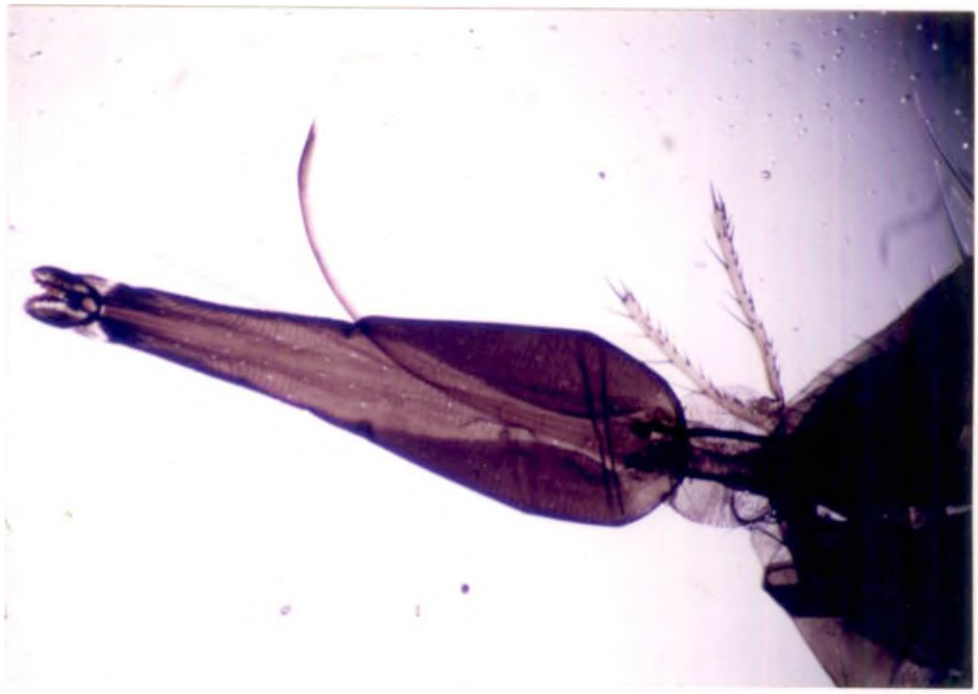
Males and females of *Stomoxys calcitrans* were blood suckers and were fed with citrated cattle blood which was safe compared to EDTA (Anticoagulant) added blood which was found to be toxic. The gravid females oviposited in the

Fig. 9. Single pupa of *S. calcitrans*

Fig. 10. Adult flies of *S. calcitrans* (Females and Males)

Fig. 11. Mouth part of *S. calcitrans* fly (4x)

Fig. 12. Wing of *S. calcitrans* (4x)



horse dung kept in the petridish. The same medium was found to be satisfactory for larval development also. The life cycle parameters of the fly are presented in the Table 2.

4.5 Immune Response

4.5.1 Preparation of the antigen

The thoracic muscle antigen prepared had a protein concentration of 4 mg/ml. All the tests were done using this antigen preparation.

Agar Gel Precipitation Test (AGPT)

The immunized rabbit serum showed a single clear precipitation line in AGPT. The immune serum could detect the antigen prepared on 14^{th}, 21^{st} and 28^{th} day of immunization (Fig.13).

Counter Immuno Electrophoresis (CIE)

The immunized rabbit serum showed a single clear precipitation line as in AGPT (Fig.14).

Passive Haemagglutination Assay (PHA)

The serum antibody titre of the rabbit immunized with the antigen prepared was assessed. The optimum concentration of the antigen used for the assay was found to be 1 in 100.

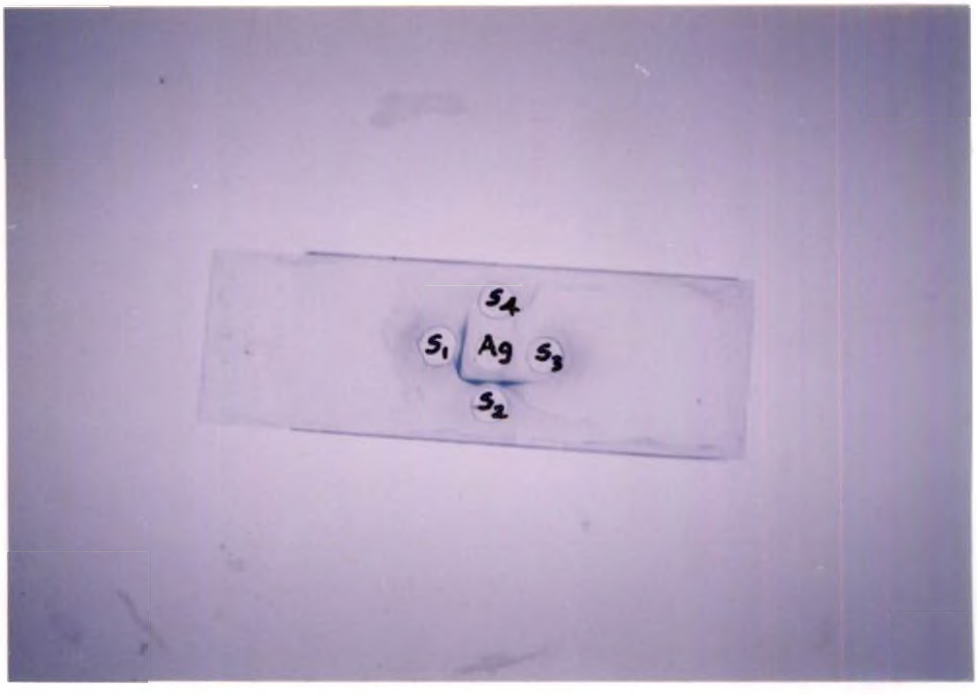
The immune serum showed a mean antibody titre of 256 on 14^{th} day after immunization and increased to 1024 on 21^{st} day and then remained the same on 28^{th} day of immunization. The preimmunization serum gave button at the first well indicating a negative reaction (Fig.15).

Table 2
Details of the *in vitro* study on biology of *S. calcitrans*

Serial number	Particulars	Values
1	Egg measurement	1.3 x 0.3 mm
2	Incubation period of egg	1-3 days
3	First larval instar (L_1) measurement	1.8x0.25 - 2.3x0.4 mm
4	Period of L_1	22-25 h
5	Second larval instar (L_2) measurement	2.5x0.5 - 4.0x0.7 mm
6	Period of L_2	26-29 h
7	Third larval instar (L_3)	4.2x0.8 - 12x1.3 mm
8	Period of L_3	9-13 days
9	Pupal measurement	5 x 1.5 mm
10	Period of pupa	5-8 days
11	Period from egg to egg stage	32 days
12	Male and female percentage of emergence	52:48
13	Time of mating after emergence	3-4 days
14	Preoviposition period	8-10 days
15	Longevity of fly in laboratory	35 days

Fig. 13. Agar gel precipitation test
(Ag – Thoracic muscle antigen of *S. calcitrans*
S_1 & S_2 serum on 21st day of immunization
S_3 & S_4 preimmunization serum)

Fig. 14. Counter immuno electrophoresis
(Ag – Thoracic muscle antigen of *S. calcitrans*
S_1 serum on 21st day of immunization
S_2 preimmunization serum)


S4
S1
Ag
S3
S2


Ag
S1
Ag
S2

Fig. 15. Passive haemagglutination assay

Row T (upto 12th column) – Serial dilution of serum from immunized rabbit (21st day of immunization)

Row P (upto 12th column) – Serial dilution of preimmunization serum

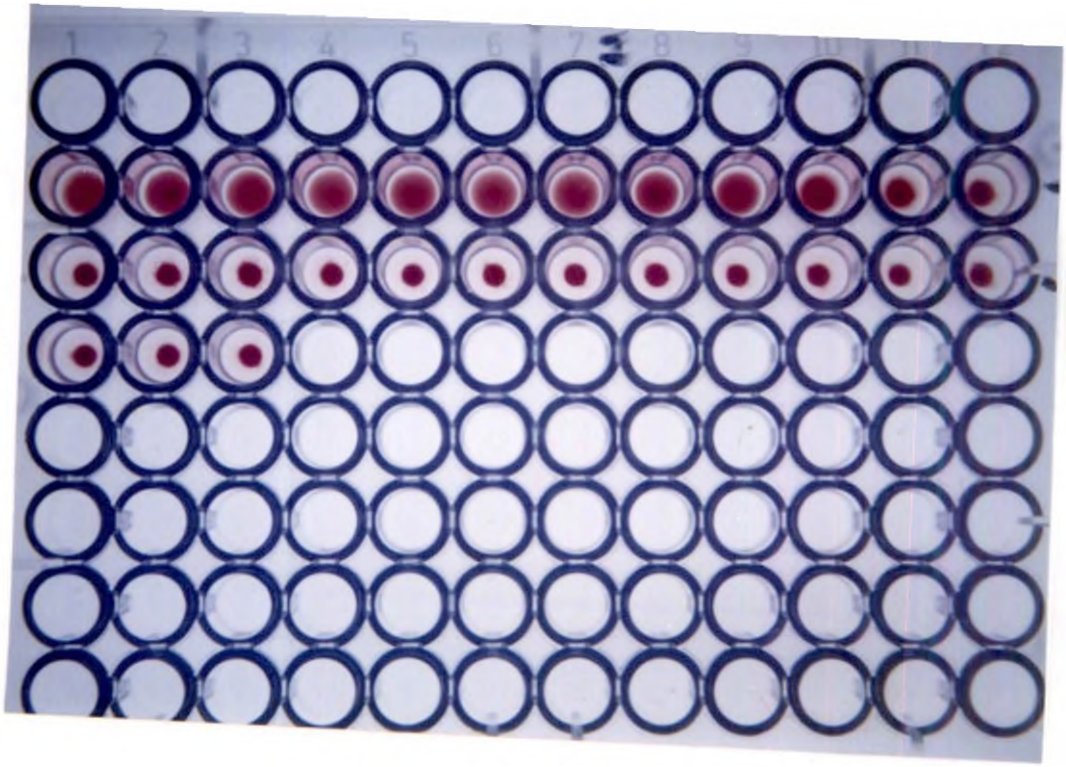
Row C - Control

Fig. 16. Wing paralysis in the *Stomoxys* fly fed with immunized rabbit blood

T

P

C



4.5.2 Feeding normal flies with immunized rabbit blood

Feeding the normal flies with blood from immunized rabbit produced higher mortality than in the control. Abnormal effect like wing paralysis was noted (Fig.16) in test group. The cumulative percentage of mortality was hundred per cent on 20th day in test group against 66.7 per cent in control group (Graph 2; Table 3).

The egg yield and viability data showed that the test group produced a lesser number of flies than the control group (Table 4).

4.6 Insecticidal Effect

The mean mortality percentage and the knockdown effect of different insecticides were evaluated and statistically analysed from the six replications during the 12 days observation period. It was found that the Deltamethrin (72.2±0.43 per cent mortality) was highly effective in controlling flies. The other insecticides in the descending order of effectivity were Diazinon (68.6 ± 1.00 per cent mean mortality), Carbaryl (67.2 ± 1.65 per cent mean mortality) and Ethofenprox (44.1 ± 1.71 per cent mean mortality). The day-wise mean mortality and mean knockdown percentage of various compounds tested are presented in Table 5.

Deltamethrin at 0.0025 per cent concentration produced greater than 90 per cent mean mortality and mean knockdown effect up to four days and two days post-treatment respectively and at 0.005 per cent concentration the same effects were extended up to six days and four days post-treatment respectively.

Diazinon (0.3 per cent) and Carbaryl (0.1 per cent) failed to produce greater than 90 per cent mean mortality after one day post-treatment and their mean knockdown percentage were low.

Table 3

Data on feeding of *S. calcitrans* with immunized and control rabbit blood

Days	Test group			Control group		
	Live	Dead	Cumulative percentage of mortality	Live	Dead	Cumulative percentage of mortality
1	30	-	-	30	-	-
2	29	1	3.3	30	-	-
3	28	1	6.7	30	-	-
4	27	1	10.0	29	1	3.3
5	25	2	16.7	29	-	3.3
6	24	1	20.0	28	1	6.7
7	22	2	26.7	28	-	6.7
8	20	2	33.3	27	1	10.0
9	18	2	40.0	25	2	16.7
10	17	1	43.3	24	1	20.0
11	15	2	50.0	22	2	26.7
12	14	1	53.3	21	1	30.0
13	12	2	60.0	19	2	36.7
14	10	2	66.7	17	2	43.3
15	8	2	73.3	16	1	46.7
16	7	1	76.7	15	1	50.0
17	5	2	83.3	13	2	56.7
18	3	2	90.0	12	1	60.0
19	1	2	96.7	12	-	60.0
20	-	1	100.0	10	2	66.7

Graph 2. Cumulative percentage of daily mortality in *S. calcitrans* fed with immunized rabbit blood compared with cumulative mortality in control group

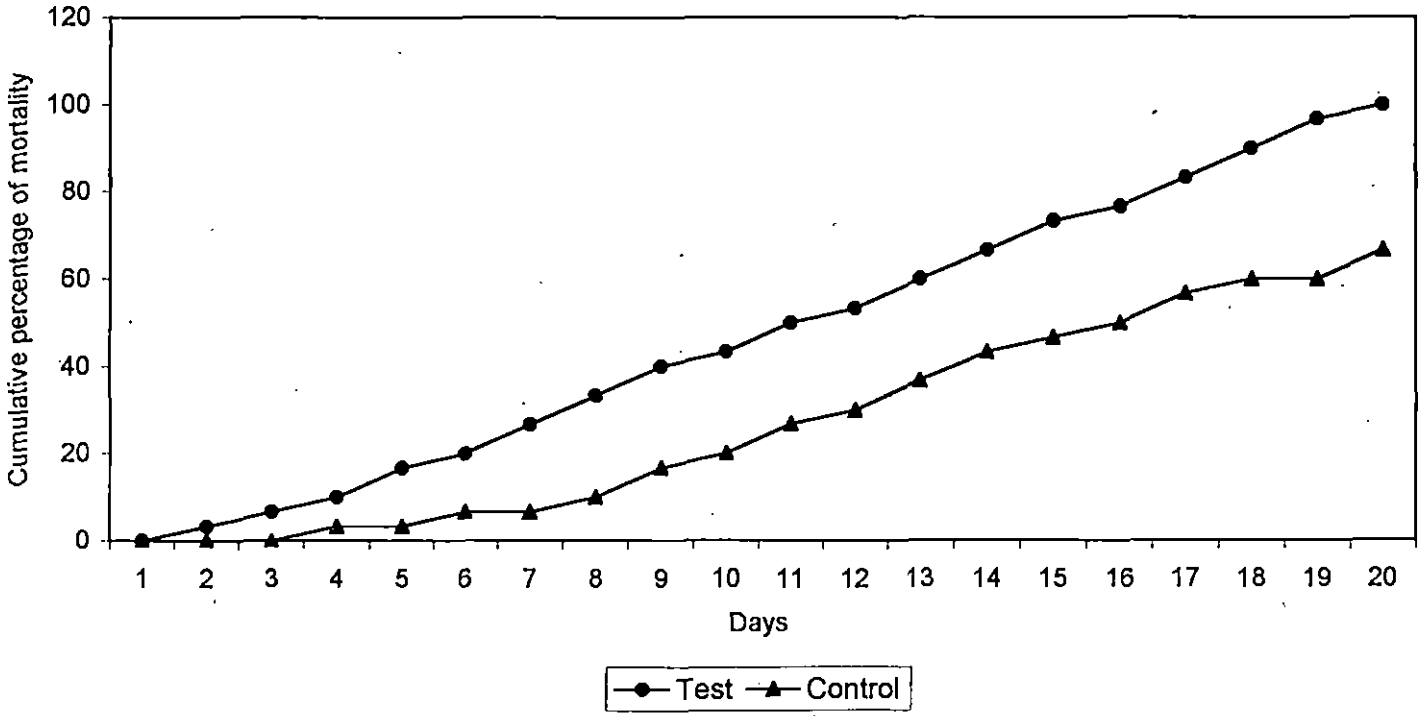


Table 4
Egg yield and viability data from feeding of *S. calcitrans* with immunized and control rabbit blood

Parameters	Test group * (No.)	Control group * (No.)
Eggs	676	1643
Larvae	536	1346
Pupae	347	863
Adult flies	235	564

* Values represent the number of eggs, larvae, pupae and adult flies obtained during the observation period of 20 days

Table 5

Day-wise mean mortality and knockdown per cent of different insecticides

Days	Mean mortality (%)						Mean knockdown effect (%)					
	A	B	C	D	E	F	A	B	C	D	E	F
1	100	100	100	91.7	60.8	2.5	100	100	40.8	56.7	-	-
2	100	100	75	73.3	27.5	4.2	100	97.5	18.3	23.3	-	-
3	100	98.3	30.8	36.7	-	4.2	95.8	89.2	-	-	-	-
4	97.5	92.5	-	-	-	2.5	90	71.7	-	-	-	-
5	93.3	88.3	-	-	-	5.0	71.7	51.7	-	-	-	-
6	91.7	75	-	-	-	3.3	47.5	26.7	-	-	-	-
7	88.3	55	-	-	-	2.5	25	5.8	-	-	-	-
8	85	30	-	-	-	3.3	1.7	-	-	-	-	-
9	66.7	10.8	-	-	-	2.5	-	-	-	-	-	-
10	36.7	-	-	-	-	2.5	-	-	-	-	-	-
11	17.5	-	-	-	-	2.5	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-

A - Deltamethrin 0.005 per cent (Butox 0.4%)
B - Deltamethrin 0.0025 per cent (Butox 0.2%)
C - Diazinon 0.3 per cent (Neocidol 1.5%)
D - Carbaryl 0.1 per cent (Sevin 0.2%)
E - Ethofenprox 0.002 per cent (Trebon 0.02%)
F - Control

Ethofenprox (0.002 per cent) failed to produce greater than 50 per cent mean mortality after one day post-treatment and it lacked the knockdown property.

Statistical analysis of the data showed a significant difference between the mean mortality percentage of various insecticides tested (except between Diazinon and carbaryl) and between the insecticides tested and the control group. There was also a significant difference between the mean knockdown percentage of various insecticides tested (Table 6).

Table 6

Statistical analysis of mortality and knockdown per cent of various insecticides

Insecticide	Mortality per cent Mean ± SE	Knockdown per cent Mean ± SE
Deltamethrin 0.005%	80.4 ± 1.37 [a]	75.70 ± 1.16 [a]
Deltamethrin 0.0025%	72.2 ± 0.43 [b]	63.20 ± 0.94 [b]
Diazinon 0.3%	68.6 ± 1.00 [c]	28.75 ± 1.25 [d]
Carbaryl 0.1%	67.2 ± 1.65 [c]	40.40 ± 1.36 [c]
Ethofenprox 0.002%	44.1 ± 1.71 [d]	-
Control	3.9 ± 0.26 [e]	-

Means within a column with different superscript differ significantly at 1% level
Means within a column with same superscript does not differ significantly

DISCUSSION

5. DISCUSSION

5.1 Prevalence

The study on the prevalence of *Stomoxys* fly infestation in cattle from June 1999 to May 2000 showed a higher (>90 per cent) incidence during the months of June, July, August and September. The prevalence rate was highest (100 per cent) in the month of July and lowest (61.6 per cent) in February. The prevalence of the fly was inversely correlated with mean atmospheric temperature and directly correlated with humidity and rainfall.

The present findings were in agreement with those of Dipelou (1975) who observed that the stable fly population was more during rainy season. Higher incidence in the months of June to September was in accordance with Miranpuri and Lahkar (1980) who observed a higher incidence of Diptera in cattle shed in the same months.

There are only two seasons in Kerala namely rainy (June to December) and summer (January to May) seasons and the present observation showed a higher fly population during rainy season. This gives an inference that the rainy season (low atmospheric temperature and high humidity) is the suitable climate for the breeding of stable flies.

5.2 Speciation of the fly

The species of *Stomoxys* fly encountered in Thrissur was *Stomoxys calcitrans* and this was in concurrence with Soulsby (1982) who stated that the stable fly was distributed world wide.

Stomoxys calcitrans identified during the present study revealed the similar morphological features as reported by Sen and Fletcher (1962), Soulsby (1982), Patton and Cragg (1984) and Kettle (1995).

5.3 Morphology of *Stomoxys calcitrans*

Studies on the morphology of various stages of *S. calcitrans* was made in the present work. Many workers have described the morphology and characters of identification of the different stages of stable fly.

The character of the egg studied is in conformity with the description of Sen and Fletcher (1962) and Patton and Cragg (1984). The observations on the morphological characters of larva and pupa are also in agreement with the findings of the above authors.

As regard to the morphology of the fly studied, it was broadly in conformity with Sen and Fletcher (1962), Soulsby (1982), Patton and Cragg (1984) and Kettle (1995).

5.4 Biology of *Stomoxys calcitrans*

Feeding adult flies with citrated bovine blood was safe compared to EDTA added blood which was found to be toxic. Information regarding this toxic effect is totally lacking in literature and the present work is a primary one.

The use of horse dung as a medium for oviposition and larval development was found to be satisfactory which was in agreement with the suggestions of Glasser (1924) and Sen and Fletcher (1962). This was in difference with the findings of Dotty (1937) who prepared a medium with wheat bran, alfalfa meal and yeast suspension for oviposition and larval development.

The life cycle parameters noted were similar to those observed by Glasser (1924), Dotty (1937), Sen and Fletcher (1962), Williams *et al.* (1981), Soulsby (1982) and Kettle (1995). The development period of egg and pupa observed in the present study was in contrary to the finding of Patton and Cragg (1984).

5.5 Immune response

5.5.1 Preparation of antigen

The protein concentration of the prepared thoracic muscle antigen was 4 mg/ml and the concentration was found to be sufficient for the serological tests which gave good results.

5.5.2 Agar gel precipitation test (AGPT) and Counter immuno electrophoresis (CIE)

A single clear precipitation line was produced both in AGPT and CIE, which shows that there was a good immune response to the given antigen. This was in concurrence with Alger and Cabrera (1972) who used double diffusion test to show the response of rabbit to mosquito antigen and obtained good results. This was also in agreement with Ravindran *et al.* (2000) who successfully detected the immune response of rabbit against *Psoroptes* mite using AGPT and CIE. The single precipitation line showed that there was only one antigenic fragment in the prepared antigen. The advantage of CIE over AGPT was, it facilitated quick reading.

5.5.3 Passive Haemagglutination Assay

The assay revealed an antibody titre of 256 on 14^{th} day after immunization and there after it increased to 1024 on 21^{st} day and remained the same till the observation period of 28 days. Schlein and Lewis (1976) determined

the titre using capillary precipitin test to make certain that the precipitating antibodies were present in serum of immunized rabbit but have not mentioned the titre they obtained. The present finding was in accordance with the observation of Ravindran *et al.* (2000) who used PHA to assess the antibody titre in rabbit immunized with *Psoroptes* mite antigen and obtained good results. From the test it can be concluded that the thoracic muscle antigen prepared was able to evoke a good immune response.

5.5.4 Feeding of the normal flies with immunized rabbit blood

Feeding stable flies with blood from rabbit immunized with thoracic muscle antigen produced higher mortality compared to the flies fed with blood from control rabbit. The cumulative percentage of mortality was 100 per cent on day 20 in test group whereas only 67.3 per cent mortality was obtained in the control group. This observation was similar to the reports of Schlein and Lewis (1976), who recorded 100 per cent mortality on 20^{th} day in the stable flies that were fed on rabbit immunized with thoracic muscle antigen.

Abnormal effects like wing paralysis was noted in the test group and may be due to the binding of antibodies to the thoracic muscles, as opined by Schlein *et al.* (1976) who explained that immunoglobulins can enter the haemolymph without destruction and binds specifically to muscle tissues in *Sarcophaga* fly.

The egg yield and viability data showed that the test group produced lesser number of flies than control group. This is in accordance with Webster *et al.* (1992) who showed that the stable flies fed with blood from rabbit immunized with gut antigen of same fly produced low percentage of viable eggs. The reason for low egg yield in the present study may be attributed to high mortality in test group.

The result justifies further research on immunization using thoracic muscle of the *Stomoxys* fly as antigen. However, factors like degree of contact between antibody and target tissue, level of tissue vulnerability and concentration of specific antibodies have to be considered and it should be possible to purify antigens by fractionation and purification to increase the protective effects obtained.

5.6 Insecticidal study

The comparative study on the efficacy of different insecticides on stable flies showed that Deltamethrin (0.0025 per cent) was most effective among them. The other compounds in their descending order of efficacy were Diazinon (0.3 per cent), Carbaryl (0.1 per cent) and Ethofenprox (0.002 per cent) but there was no significant difference between Diazinon and Carbaryl. The present finding slightly differs from the observation of Dukes and Hallmon (1984) who reported that synthetic pyrethroid was most toxic followed by carbamates and organophosphates. The difference in the findings may be due to the difference in concentration of the compounds used.

Deltamethrin at 0.0025 per cent concentration was effective (>90 per cent) for four days and this may be compared with the finding of Bertel and Robijns (1985) who obtained six week protection against *Haematobia irritans* at 2 per cent concentration as pour-on. The variation in the results may be due to difference in concentration used. As such no reports were available regarding the use of Deltamethrin against stable fly as topical application and hence the present work may be considered as a primary one.

As Deltamethrin gave good results at 0.0025 per cent concentration, the compound was again tested at 0.005 per cent concentration and an increase in residual effect (up to 6 days) and knockdown effects (up to 4 days) was obtained.

Diazinon at 0.3 per cent concentration produced greater than 90 per cent mortality on the day of application, after which the effect drastically reduced. This is in partial agreement with Dahm and Raun (1955) who evaluated the residual effect of diazinon on stable fly at one per cent concentration on fly resting places and obtained good initial reduction in fly population and effective fly control for 7 to 35 days post treatment. The higher residual effect obtained by them may be due to higher concentration of the drug used and difference in the place of application.

Carbaryl at 0.1 per cent concentration failed to produce greater than 90 per cent mortality after one day post-treatment. The same result was obtained by Blume *et al.* (1973) at 0.5 per cent concentration.

Ethofenprox, a newer insecticide tried at 0.002 per cent concentration failed to produce greater than 50 per cent mortality after one day post treatment. As such no report was available regarding the effect of Ethofenprox on stable flies and the present work is a primary one. Further study at higher concentration is needed to evaluate its effect on stable flies.

In field level, the flies visit the host only to feed and they are exposed to the compound for a short period of time. So the compound with better knockdown effect like Deltamethrin is better suited for application on animals. The present study also gives a fair indication of the residual effects of the different insecticides tested.

SUMMARY

6. SUMMARY

The prevalence, biology, immune response and control of *Stomoxys spp.* of flies were studied in detail.

The prevalence study on *Stomoxys* fly infestation in cattle of Thrissur during June 1999 to May 2000 showed comparatively a higher incidence in the months of June (94.4 per cent), July (100 per cent), August (91 per cent) and September (92.8 per cent) than in other months. The mean month wise prevalence rate of infestation varied between a maximum of 10.9 flies/animal in July 1999 to a minimum 0.7 fly/animal in February 2000. The effect of seasonal and climatic variation on fly infestation in cattle indicated a higher incidence when the atmospheric temperature decreased and humidity and rainfall increased. Higher prevalence was noted in rainy season, which denotes the suitability of the environment for the flies to breed. The flies collected from cattle and animal dwellings were speciated and were found to be *Stomoxys calcitrans*.

The life cycle of the fly was studied in the laboratory. The flies were fed with citrated bovine blood which was safe whereas EDTA added blood was found to be toxic. Horse dung was used as a medium for oviposition and larval development and was found to be good. The developmental period of various intermediary stages, time of mating after emergence, preoviposition period and longivity of fly were observed and noted.

The thoracic muscle antigen prepared from the pharate adult fly had a protein concentration of 4 mg/ml and was used to immunize the rabbit to assess the immune response. Agar gel precipitation test and counter immuno electrophoresis revealed a single clear precipitation line which showed that there was only one antigenic fragment in the prepared antigen. The passive haemagglutination assay

done using tanned sheep RBC's revealed a serum titre of 256 on 14th day and 1024 on 21st and 28th days of immunization.

Feeding of the normal flies with blood from immunized rabbit produced a cumulative mortality of 100 per cent on day 20 whereas it was 66.7 per cent in group fed with control rabbit blood. Abnormal effects like wing paralysis was noted in the test group. Egg yield and viability data showed that the test group of flies produced lesser number of flies than control group which may be attributed to high mortality of the flies in the test group.

The mortality and knockdown effects of insecticides like Deltamethrin (0.0025 per cent), Diazinon (0.3 per cent), Carbaryl (0.1 per cent) and Ethofenprox (0.002 per cent) were evaluated against stable fly infestation. Deltamethrin gave better protection (up to 4 days) than the other compounds and its effect was more at 0.005 per cent concentration (up to 6 days). Diazinon and Carbaryl failed to give good protection (>90 per cent mortality) after one day post treatment but their knockdown effect was low. Ethofenprox was least effective (<50 per cent mortality after one day post treatment) and its knockdown effect was nil. Statistical analysis showed that there was a significant difference between the mean mortality percentage of different insecticides tested (except between diazinon and carbaryl) and between the insecticides tested and control group. There was also a significant difference between the mean knockdown percentage of different insecticides tested.

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PREVALENCE, IMMUNE RESPONSE AND CONTROL OF *Stomoxys spp.* OF FLIES

By

N. BASKAR

ABSTRACT OF THESIS

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Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

DEPARTMENT OF VETERINARY PARASITOLOGY
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR-680 651
KERALA

2000

ABSTRACT

A detailed study on the prevalence, biology, immune response and control of *Stomoxys spp.* of flies was conducted. The prevalence study on *Stomoxys* fly infestation on cattle of Thrissur, during June 1999 to May 2000 showed a higher incidence (>90 per cent) during the months of June, July, August and September than in other months. The prevalence increased as temperature decreased and humidity and rainfall increased. The fly infesting cattle was identified as *Stomoxys calcitrans*.

The biology of the fly was studied in laboratory and were fed with citrated bovine blood. The use of horse dung as oviposition and larval development medium was found to be satisfactory. The life cycle parameters of the fly were also noted.

The thoracic muscle antigen prepared from pharate adult fly was used for immunization. Agar gel precipitation test and counter immuno electrophoresis revealed a single clear precipitation line. The passive haemagglutination assay revealed an antibody titre of 1024, on days 21st and 28th of immunization.

Feeding the normal flies with blood from immunized rabbit produced a cumulative mortality of 100 per cent on day 20 against 66.7 per cent mortality in flies fed with control rabbit blood. Abnormal effects like wing paralysis was noted in test group.

The insecticidal property of Deltamethrin, Diazinon, Carbaryl and Ethofenprox were evaluated against stable fly infestation and was found that Deltamethrin (0.0025 per cent) was highly effective in controlling flies. At 0.005 per cent concentration Deltamethrin gave better protection. Diazinon (0.3 per cent) and Carbaryl (0.1 per cent) failed to provide good protection after one day post treatment. Ethofenprox (0.002 per cent) was found to be least effective.