MANUAL

FOR THE CONTROL OF THE SCREWWORM FLY <u>Cochliomyia hominivorax,</u> Coquerel



FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

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PREFACE

The recent appearance of the New World screwworm fly, <u>Cochliomyia hominivorax</u>, Coquerel, on the African continent gives cause for great concern for the future of livestock and wildlife populations. This concern should perhaps also be extended to the animal population in the adjacent Mediterranean countries of Europe and the Near East.

This dipterous insect is an obligatory parasite of all warm blooded animals including humans, dependent on live flesh for development of the larval stages of the life cycle and thus a major cause of myiasis. In its previously restricted natural habitat in the tropical and sub-tropical areas of North, Central and South America, the New World screwworm is recognised as a considerable constraint to livestock production. In the State of Texas alone, annual losses were estimated at US 300 million dollars, thus justifying the initiation of a large-scale joint USA/Mexico eradication campaign.

There exist several species of diptera which, like the screwworm fly, are obligatory parasites responsible for myiasis. However, except perhaps for <u>Chrysomya</u> <u>bezziana</u>, the Old World screwworm, no others can be considered as parasites of such major economic importance to livestock production.

The recent introduction of <u>C</u>. hominivorax to Africa is currently confined to relatively limited territory in North Africa. If not contained within the present limits but eventually allowed to spread to the highly productive areas south of the Sahara Desert, the consequences to domestic livestock and wildlife could be disastrous. This would be particularly so for wildlife which does not benefit from the prevention and treatment available through human intervention. It can be expected that humans will also be directly affected by the disease. The present restricted distribution of the parasite in Africa offers the possibility for eradication provided immediate action is taken towards this objective. If, however, the fly is allowed to spread more widely, the possibilities of successful eradication will diminish and the continuous presence of the parasite will constitute an additional major burden on economic development throughout the continent.

The accidental introduction of this exotic pest to Africa demonstrates the necessity, in this ever shrinking world, for international cooperation in implementing measures for disease prevention and control. It is towards this implementation that FAO, as the responsible international technical agency, can play a major role both through dissemination of information and by coordinating national and international activities, vital to the control of agents of disease. The Organization has initiated action, in the North African area, in cooperation with national agencies, to develop regional and national programmes, for the prevention, control and eventual elimination of screwworm.

Within these programmes, training of national staff at all levels is regarded, by FAO, as of high priority. This manual has, therefore, primarily been produced to support the training activities being undertaken throughout the region. It is hoped that it will also serve as a useful guide to veterinary staff in other countries where screwworm myiasis is endemic and to those under risk due to their geographical location or through the importation of animals from infested countries.

FAO wishes to record its appreciation to the Mexico-United States Commission for the Eradication of Screwworm for supplying some of the information contained in the manual and to acknowledge the contributions of the principal authors, Dr. M. Vargas, Mexico-USA Screwworm Commission and Dr. M.J. Hall, British Museum (Natural History). Mr. B.S. Hursey, Animal Health Officer, Animal Health Service undertook the final editing.

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INTRODUCTION

The term myiasis is used to describe the infestation of live animals by various species of tissue-devouring insect larvae. The New World screwworm fly, scientifically known as <u>Cochliomyia</u> hominivorax (Coquerel), is one of the main <u>causative</u> agents of this parasitic disease being a voracious and obligatory parasite of all warm-blooded animals. Myiasis caused specifically by <u>C. hominivorax</u> is often termed "Cochliomyiasis" to differentiate it from other infestations.

In contrast to other diptera which cause myiasis but which also exist on dead tissue (facultative parasites), the New World screwworm (an obligatory parasite) is solely dependent on living flesh for the survival and development of the larval stages of its life cycle.

The New World screwworm is the larval stage of a fly, <u>Cochliomyia hominivorax</u> (Coquerel), which parasitises many warm blooded animals including humans. Until recently it was confined to the New World, its current range there extending from southern Mexico to Northern Argentina and Uruguay, and to a number of islands in the Caribbean. It was, however, collected in North Africa in 1988, near Tripoli, Libya, although the identification was not confirmed until 1989. Since then further cases have been reported from Libya but none, so far, September 1989, from the surrounding countries.

How the flies arrived in North Africa is unknown, but they probably came with a shipment of contaminated livestock from the naturally endemic areas. The increasing use of rapid transport in the latter half of this century has facilitated the spread of many species of insect pest. The New World screwworm has previously been introduced into Canada with infested livestock, but the population established was unable to overwinter. Even the transportation of <u>Calliphorid</u> flies from one continent to another is not unprecedented. Four species of Old World blowflies in the genus <u>Chrysomya</u>, which is analogous to the New World genus <u>Cochliomyia</u>, have become established in South America in the last ten years. Living larvae of <u>Cochliomyia</u> hominivorax have been accidentally imported into France from Brazil in the wounded ear of a dog. They were removed before reaching maturity and were bred to the adult stage in a laboratory near Paris.

The adult screwworm fly is essentially harmless, but the females lay eggs on the wounds of mammals. The maggots, or larvae, that emerge immediately begin feeding on the living flesh, burrowing into the wound which enlarges as the larvae feed. Screwworm infestations can seriously injure and disable the animal in which they occur and death is likely unless the wound is treated.

The screwworm has had a major impact on the livestock industry in the New World, particularly cattle. Wounds due to de-horning, castration, branding, tick bites and, in particular, calving are very attractive to egg-laying females. In much of the New World, <u>Cochliomyia hominivorax</u> is considered the major insect pest of livestock, coming a close second to ticks as the major arthropod pest. Economic losses caused by screwworm are high because, even though animals may not always die, their susceptibility to other diseases is increased, meat and milk production drops and the hide is damaged. In addition, the costs of labour to inspect and treat the livestock and the costs of the treatments themselves can be very large. The effect on wildlife is probably even more devastating as it is unmanageable and can not benefit from the treatment and protective measures available to domestic stock.

When favourable conditions of host and climate coincide, the potential for growth of the screwworm population is considerable. In addition to being transported great distances in the wounds of livestock, individual flies can migrate up to 290 Km in less than two weeks. Each female may lay several batches of eggs in her lifetime. The practices recommended in this manual for the diagnosis, treatment, control and possible eradication of the parasite and the disease are essentially drawn, therefore, from the many years of investigation and experience gained in the American region. Future scientific studies and field trials may, however, result in modifications of these techniques for their improved application under local conditions in new areas of infestation.

CHAPTER I

BIOLOGY

The New World screwworm belongs to the insect order Diptera, meaning two-winged. Its classification within that order is as follows:

Order: Diptera Sub-order: Cyclorrhapha Division: Schizophora Calyptratae Section: Superfamily: Oestroidea Family: Calliphoridae Subfamily: Chrysomyinae Tribe: Chrysomyini Cochlionyia Genus: Species: hominivorax (Coquerel)

The nomenclature of the New World screwworm has a history which has been somewhat confusing. Firstly, the genus <u>Cochliomyia</u> has also been known as <u>Callitroga</u>. Secondly, the actual species has also been referred to as <u>Lucilia hominivorax</u>, <u>Calliphora</u> <u>infesta</u>, <u>Calliphora</u> <u>anthropophaga</u>, <u>Somomyia</u> <u>fulvobarbata</u> and <u>Cochliomyia</u> americana.

There are just three other species in the genus <u>Cochliomyia</u>, namely, <u>minima</u>, <u>aldrichi</u> and <u>macellaria</u>. All are presently restricted to the New World. <u>Cochliomyia macellaria</u> is a carrion breeder but can become a facultative agent of myiasis. Its similarity to <u>Cochliomyia hominivorax</u> has led to many mistaken identifications.

1.1 GEOGRAPHIC DISTRIBUTION

C. <u>hominivorax</u> is endemic in the tropical and sub-tropical regions of the New World, (North, Central and South America and the Caribbean islands). Its distribution is largely determined by an inability to survive persistently cold weather. Warm, moist conditions are optimal, prolonged dry heat or cold sub-optimal. Activity decreases below 21°C and the fly cannot survive in areas where the mean temperature is below 9°C for 3 months, or 12°C for 5 consecutive months of the year.

The adults are powerful fliers and strongly dispersive. Individuals have been reported to travel up to 290 km in two weeks. In favourable conditions the fly can, therefore, spread rapidly far beyond its overwintering limits. This occurred annually in North America before the introduction of eradication techniques which have now pushed the northern limits of the fly south to the southern borders of Mexico. The southern limits of the fly are in Chile, Argentina and Uruguay.

The presence of the New World screwworm was first confirmed in North Africa in 1989.

1.2 THE LIFE-CYCLE

<u>Cochliomyia hominivorax</u> has been recorded from a wide range of livestock, wildlife and from man. The species was first described in the literature from a human case of myiasis in man in Cayenne, French Guiana, in 1858. Translated from the Latin, its name literally means "devourer of man". Man is particularly at risk from infestation when living in conditions of poor hygiene and in close proximity to infested livestock. If not treated rapidly, human infestations with screwworm can become very debilitating and lead to death, especially when the nasal and frontal sinuses, eyes, ears and mouth are involved. The screwworm is, however, primarily a veterinary problem as demonstrated during an epidemic in Texas in 1935 when there were approximately 230 000 cases in livestock compared to only 55 recorded in humans.

The list of animal hosts is very long since almost all warm blooded animals may be attacked.

Cattle, horses, sheep, goats, pigs and dogs are frequently reported as hosts. In North Africa the camel has become a host. The behaviour of some hosts and their physiological response to wounding can make them more resistant to screwworm attack. A wide variety of wildlife is also at risk, both in Zoos and in natural situations. The eradication of screwworm from North America and most of Mexico has led, in those areas, to an increase in the populations of larger wild mammals.

Gravid adult female screwworms are attracted to open wounds on warm blooded animals. Essentially all wounds are attractive including accidental laceration, for example on barbed wire, or wounding as a result of animal husbandry practices, such as shearing, castration, de-horning and branding. Many screwworm infestations are a result of natural wounding. Thus, in some affected areas, 90 percent of screwworm infestations of cattle start at the site of tick bites, and on new born animals the most frequently infested site is the unhealed umbilical scar. On deer farms in the southern USA, prior to eradication, up to 80 percent of fawns were lost each year due to such infestations.

While the majority of infestations initiate on skin wounds, larvae may also invade body orifices, such as the nostrils, eyes, mouth, ears and vagina. Invasion of the nasal fossae is the most frequent infestation of the natural cavities in humans. Screwworm larvae begin their development only on live animals. They may, however, complete their development on the host if it dies after infestation, providing they have reached the second larval instar stage and the body remains warm.

Egg laying behaviour on a host lasts on average 15 minutes (6 minutes before, 6 minutes during and 3 minutes after egg-laying), and may be preceded or followed by feeding at the wound. Before deposition of eggs, the abdomen is groomed. The ovipositor is then extended and used to probe the wound area for a suitable oviposition site. Once probing is initiated the fly is not easily distracted from ovipositing. A female lays from 10 to 490 eggs, average 200, in a flat, shingle-like mass at the dry edge of the wound. All the eggs are oriented in the same direction while the ovipositor is swept from side-to-side (Fig. 5).

Frequently the egg clutches are divided into two or more masses, that may be laid several minutes apart at the same or a different wound. Females subsequently ovipositing on a wound already occupied by an egg mass will invariably deposit their eggs in direct contact with the older egg mass. One apparent mass could, therefore, originate from two or more females.

Oviposition is carried out at approximately three-day intervals. Under field conditions, a female fly has been recorded laying eight separate clutches of eggs over a 33 day period, but an average female will lay about four clutches.

First instar larvae hatch from the egg some 11-24 hours after deposition and immediately start feeding on the living tissues. With <u>Chrysomya</u> <u>bezziana</u> they share a characteristic, gregarious, screwworm method of feeding, burrowing head-downwards into the tissues with just the posterior tips protruding. Should a larva become immersed in wound fluids the posterior tip of the abdomen, containing the posterior spiracles is periodically extruded above the surface of the liquid for respiration. Otherwise, once embedded in the flesh the larvae do not move about the wound as do many secondary agents of myiasis. The large backwardly projecting spines prevent them from being easily removed.

The larvae feed, grow and moult from first to second and then third instar stages. As they feed, exudates are produced that promote secondary bacterial infections and prevent healing. Larger infested wounds discharge pus and blood and have a characteristic bad odour which is very attractive particularly to female flies. As more egg masses are laid and hatch, the wound is greatly enlarged and deepened, by the increasing number of growing larvae. Secondary myiasis causing species may also be attracted to the wound, but, unlike screwworm, their larvae are not generally found deep in the wound.

The rate of development of larvae is influenced by temperature and by the size and nature of the wound and the number of larvae present. There is a distinct feeding period lasting for about 4-8 days, after which larvae leave the animal and drop to the ground, usually during the early morning or after midday. Within a radius of about 45 cm of where they drop they burrow several centimetres below the soil surface. The depth of burrowing is affected by the type of soil and by the vegetative cover, larvae burrowing deeper in soils with little cover. texture and temperature of the soil are impo The important parameters for survival of the larva. The pH of the soil does not seem to influence the longevity of the pupal stage or the emergence of adults. On locating favourable conditions the larva becomes motionless; 60 percent of larvae remaining in a vertical position, 30 percent oblique and 10 percent horizontal. The cuticle hardens and darkens to a dark reddish coffee colour. This transformation is completed within 24 hours of leaving the wound.

The duration of the pupal period is very temperature dependent, ranging, as for example, in southern USA (Texas), from 7 days in the summer to 54 days in the winter. Under controlled laboratory conditions of 100 percent relative humidity, the pupal period varies from six days at 34.5°C to 32 days at 15°C. The life cycle is completed in about 21 days under favourable conditions and at a temperature of 22°C, but in cold conditions may be extended to two to three months.

Adult flies generally emerge in the early morning, between 04:00 and 07:00 hours, females earlier than males on average. To escape the puparium the adult inflates the ptilinum, an eversible sac at the front of the head, found in all Cyclorrhaphan Diptera. This can be expanded by forcing haemolymph into the head from the thorax and abdomen. It is retracted by a reverse procedure, muscles in the head forcing haemolymph back into the thorax. The pressure of the expanded ptilinum forces the cap off the puparium releasing the adult fly, which then burrows to the surface, again using the ptilinum to force its way through the soil. After emergence the cuticle of the fly is initially soft and pale, the wings wrinkled and folded. Upon reaching the soil surface the fly remains stationary for about 15-20 minutes, during which time the wings are gradually extended, being smoothed into place with the posterior legs. Within a few hours the abdomen loses its whitish appearance and the whole body becomes a metallic blue-green colour. Three longitudinal bands darken on the thorax, the middle one being shortest and thinnest. After hardening of the body, the ptilinum is no longer eversible, and the muscles associated with it degenerate.

In the first two days after emerging the flies disperse over a wide area. Flight is reduced, however, in winds greater than 8 Km/h. Flies are mainly found in low stature vegetation, not in dense foliage or in buildings.

Males are sexually mature within 24 hours and are polygamous, mating 5 to 6 times. Females usually only mate once, on about the second or third day after emergence. Mating occurs during daylight and averages about 1.6-3.8 minutes. The female is ready to lay her first egg batch about four days later. At that time she begins to search for a suitable host, virgin females are rarely found at a host. If none are available nearby, she is able to travel great distances to find one. Individual flies have been observed to travel up to 290 Km in less than two weeks, so the capacity for dispersal of the population is very great.

Female blowflies are autogenous for at least the first gonotrophic cycle, meaning that they do not require a protein feed before laying the first batch of eggs. They may require protein for subsequent batches. The protein needed for production of the first batch of eggs is taken in during the larval feeding stage. In addition to protein, adult flies require a source of carbohydrate and water. In the laboratory, pupal fat body is exhausted within three days of emergence and flies survive only 2-5 days without carbohydrate, even when offered dung, fresh meat, wound fluid or carrion. Honey is often used as a carbohydrate source for flies in culture. In the field, a major source of carbohydrate is the nectar of flowering plants and many species of plant are visited during the season. Flowering shrubs and trees are also used as sites for feeding, mating and resting. Males establish "waiting stations" from which they strike at any passing object in flight of about the same size as a female Cochliomyia hominivorax, even small pebbles tossed in the air. A study of nocturnal resting sites has shown that 90 percent of flies pass the night resting on leafless twigs, 1.2-1.5 metres above the ground in low-canopy trees, particularly along rivers where humidity is higher. Adult flies may live on average for 2-3 weeks in nature. In the laboratory they may live for up to two months.

The screwworm can be described as a species which is opportunistic. Its longevity, autogeny and ability to lay many egg batches, combined with its great ability to disperse, allows it to exploit intermittently favourable environments. This is well demonstrated by the regular summer advances made each year into the central USA from overwintering sites in Texas, Mexico and Florida, before the screwworm eradication programmes began.



Fig. 1 Life cycle of the New World screwworm fly

1.3 LABORATORY REARING TECHNIQUES

- (a) Incubation of eggs
 - An egg mass collected from a sentinel animal is placed on meat in a petri dish, lined with moist filter paper, and left to incubate, until larvae emerge, 8-12 hours.
 - Ensure good air circulation and maintain temperature and humidity at about 39°C and 70 percent RH respectively.
- (b) Initial incubation of larvae
 - Make up the following diet: 30 g dried blood; 15 g dried milk; 15 g dried chicken egg; 1.75 ml formol; 500 g ground meat; 1 l water, mixing in the meat last. Place 0.5 l of the medium on a metal tray.
 - Add the newly hatched larvae, from step (a) above, and maintain at 39°C and 70 percent RH for 24 hours.
- (c) Second incubation of larvae
 - Make up the following diet: 70 g dried blood; 30 g dried milk; 30 g dried chicken egg; 1.5 ml formol; 1 kg ground meat; 1 l water, mixing in the meat last. Place in a metal tray.
 - Introduce larvae from step (b) and maintain at 37.8°C and 70 percent RH for 24 hours.
- d) Final larval development
 - After the larvae have been developed through stages (b) and (c) for 48

hours, adjust culture (c) to 35°C and 70 percent RH and incubate until larvae crawl out of medium.

- Spent medium can be removed to reduce the volume.
- e) Pupation
 - Allow larvae to drop from medium (d) into sawdust and keep for 24 hours at 26.7°C and 50 percent RH. Separate pupae and keep exposed on a tray for about 5.5 days at 25.6° and 50 percent RH.
- f) Maintenance of adults
 - Place pupae from step (e) above into experimental holding cages.
 - For food, add a cup containing 1 part honey and 3 parts water together with a cotton wick to facilitate probing.
 - Maintain at 25.6°C and 50 percent RH.
 - Seven to nine days after the first emergence add an oviposition stimulating medium consisting of a warm ball of ground meat impregnated with residue from the larval medium. The ball may be warmed in hot water and placed in a plastic cup.
 - Eggs collected may be recycled through steps (a) to (f) above.

When the rearing and handling of live fertile screwworms is undertaken extreme care must be exercised to ensure that specimens do not escape into the wild and cause further infestation.

CHAPTER 2

IDENTIFICATION

2.1 THE ADULT

Adult flies have three basic body divisions, the head, the thorax and the abdomen. In general terms, the head contains most, but not all, of the apparatus for sensing the environment, the thorax contains the locomotory apparatus, wings and legs, and the abdomen contains the digestive and reproductive apparatus.

The free-flying adult screwworm is not immediately obvious to the observer and detection and identification of the insect is usually based on the more readily available larval stages taken from infested animals. Generally adult flies of the genus <u>Cochliomyia hominivorax may be distinguished by their</u> deep greenish-blue colour with three dark thoracic stripes visible on the dorsal surface (Fig. 2). Males and females may be distinguished by an examination of the eyes. In the males they almost touch in the mid-line, frons narrow, whilst those of the females are widely separated, (Fig. 3).



Fig. 2 Adult C. hominivorax illustrating three dark longitudinal stripes on thorax (f. femur; ta, tarsus; ti, tibia).



Fig. 3 <u>C. hominivorax</u> – adult head illustrating narrow frons in male and wide in female.

2.2 THE EGG

The discovery of eggs at the site of wounding will immediately discount members of the family <u>Sarcophagidae</u> which never lay eggs but deposit larvae directly onto the egg laying medium. The eggs of most other species hatch within a few hours of oviposition and are unlikely to be encountered, except on a wounded sentinel animal deliberately placed in the field to survey for females ready to oviposit. Whenever possible, eggs should be reared through to at least the third stage larva for a confirmatory diagnosis.

The egg comprises a tough outer shell, the chorion, enclosing an embryo which develops to a fully-formed first instar larva before hatching. The orientation of the egg is taken from its position while still in the mother. Thus, the anterior end of the egg points towards the head of the female and emerges last at oviposition. Similarly, the dorsal surface is uppermost in the mother's abdomen and thus uppermost when the egg is extruded through the backwards pointing ovipositor.

At the anterior end of the chorion is the micropyle, a tiny aperture through which sperm enter to fertilize the egg. To provide the respiratory

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needs of the embryo, there are minute holes through the chorion named aeropyles, which allow the exchange of gases. They may be scattered across the chorion or concentrated in a special area named the plastron. The hatching strip, or dorsal seam, is a line of weakness along which the egg ruptures at hatching. In <u>Calliphorids</u> it runs dorsally for most or all of the length of the egg. The surface between the hatching lines functions as the plastron.

The egg of <u>C</u>. <u>hominivorax</u> (Fig. 4) is approximately 1.04 mm long and 0.22 mm wide. It is a glistening, slightly creamy white colour. It is not noticeably curved, being cylindrical and equally rounded at both ends, although somewhat flattened around the micropyle. The dorsal seam extends from the micropyle almost to the posterior end. It is virtually parallel sided but diverges at both ends, particularly anteriorly where it is divided to almost completely surround the micropyle. Under high magnification, the chorion of <u>C</u>. <u>hominivorax</u> has a reticulated or lacework appearance. The eggs are deposited in a characteristic mass, all oriented in the same direction (Fig. 5).

The eggs of <u>Cochliomyia</u> <u>macellaria</u> may be distinguished from those of <u>Cochliomyia</u> <u>hominivorax</u> by having a dorsal seam that is narrower and only extends less than halfway around the micropyle.



Fig. 4 <u>C. hominivorax;</u> egg showing dorsal surface and anterior end (m. micropyle; d. dorsal seam).



Fig. 5 <u>C. hominivorax</u> showing typical orientation of eggs in an egg mass.

2.3 THE LARVA

The larval stage is that most often encountered in cases of myiasis because the egg stage is of short duration, causes no damage to the host and is, therefore, usually not detected. There are three larval instars and, generally, the first two instars are very difficult to identify to species level except by association with other mature larvae or subsequently reared adults. A description of each instar of <u>C. hominivorax</u> is given below with notes on the characteristic features. This is followed by keys for positive identification based on the morphology of the third instar larva and adults.

The main features of typical Calliphorid larvae are that they are soft bodied with no clear distinction between the thorax and the abdomen. The head capsule is hardly distinguishable except for the presence of small antennae and palpi and for the mouthparts. The mouthparts consist of a pair of mouth-hooks and associated sclerites, for attachment of muscles, collectively known as the cephalopharyngeal skeleton (Fig 7).

The body of the larva comprises 12 segments, a small head segment, incompletely divided from a prothoracic segment, followed by a mesothoracic, a metathoracic and eight abdominal segments. The head segment is divided by a ventral furrow into left and right cephalic lobes, with the mouth opening at the base of the furrow. The head bears two pairs of peg-like sensory organs. These dorsal and ventral organs are often referred to, respectively, as the antennae and the maxillary palps. The larvae do not have true segmental appendages and are thus technically legless (apodous).

Respiration is achieved through spiracles which are simple openings connecting the outside air to the internal tracheal network. There are a pair of anterior spiracles on the prothoracic segment and a pair of caudal or posterior spiracles on the 12th segment. Both provide useful taxonomic characters. The anterior spiracles protrude through the body wall and divide into a fan-shaped series of finger-like lobes, each ending in a small aperture (Fig 10). The anterior spiracles are not visible in the first instar larvae.

The posterior spiracles generally comprise a pair of sclerotized plates set flat on the body cuticle of the last abodminal segment (Figs 12, 14). The sides of this cavity can close to seal off the spiracles and prevent their contamination when the larva submerges in noxious media. The outer rim of the spiracular plate is more heavily sclerotized than the rest of the plate and is known as the peritreme. It may form a complete circle or be incomplete. On the portion of the plate towards the midline of second and third instars is a structure called the button, which may or may not be clearly visible. This is the scar left from the spiracle of the previous instar after moulting. There are slits for gaseous exchange on the surface of spiracular plates, most clearly seen in second and third instars which have, respectively, two and three slits per plate. In first instars there are two small oval slits which touch closely at their inner lower edges, appearing fused and presenting a 'V' shape.

The posterior segment bears the anus surrounded by the anal plate, the cuticle of which is thinner than the rest of the body.

2.3.1 First instar larva

The first instar of <u>C</u>. hominivorax has a typical maggot shape (Fig. 6). On hatching, it

averages 1.2 mm long by 0.23 mm wide, and when fully developed measures approximately 3.6 mm by 0.57 mm. It should be remembered that there is great variation in the size of larvae depending on the quantity and quality of available food.



Fig. 6 <u>C. hominivorax</u> – First instar larva, dorsal aspect.

The anterior margins of segments bear spines about 20μ long, in the following pattern:

Seg.	2–9	-	completely encircled by a ring of
1221			spines;
Seg.	10	-	band interrupted for short space
			on dorsum;
Seg.	11	-	spines completely absent on
			dorsum; reduced on sides;
Seg.	12	—	spines on ventral/ventrolateral
			surfaces only.

On the ventral surfaces of segments 6-12, the anterior spine bands are wider and transversely divided by a narrow spineless area.

The posterior margins of the segments have no spines except for two or three rows of small spines on the ventral surfaces of segments 5-12. On the side of each of segments 5-10 is a small swollen area of spines, known as the lateral fusiform area.

The posterior spiracles are located near the upper end of the rear surface of the twelfth segment, in a shallow cavity. Each spiracle bears two small oval apertures, very closely approximated. The peritreme is not apparent. The anal protuberance on this last segment has two conical fleshy projections, the anal tubercules. A group of spines is found in front and behind on the anal protuberance and between the anal protuberance and the posterior cavity. The tubercules bordering the cavity are poorly defined. The cephalopharyngeal skeleton is as shown in Figure 7; note the small mouth hooklets which are not found in the later instars.



Fig. 7 <u>C. hominivorax</u> – Cephalopharyngeal skeleton, first instar larva. (h. mouth hooklets)

2.3.2 Second instar larva

The second instar grows from about 3.5 mm long and 0.6 mm wide at moult to about 6.3-7.4 mm long and 1.5 mm wide when fully developed (Fig. 8). The body is heavily armed with dark spines about 55μ long, which usually have one or two, but occasionally up to three points. The anterior spine banding is as follows:

Seg.	2–9	- completely encircled by a ring of
		<pre>spines; - band generally narrowly inter- rupted on dorsum;</pre>
Seg.	11	 band absent on dorsum; reduced on sides;
Seg.	12	- spines on ventral/ventrolateral

The lateral fusiform areas on segment 5-10 are as in the first instar larva. The posterior margin of segment 11 is encircled by a narrow band of smaller spines facing forwards. On the posterior margin of segment 10 are irregular ventral and lateral rows of spines and a few scattered spines extending dorsally, but not encircling the segment. On the posterior sides of segments 8-9 are a few scattered spines, but on segments 6-7 they are restricted to the ventral surface only. The spines on these posterior margins are much smaller than those on the anterior margins and the lateral fusiform areas.



Fig. 8 <u>C. hominivorax</u> - second instar larva; dorsal aspect.

The anterior spiracles are clearly visible, each having 7-9 branches. Each posterior spiracle has a definite, incomplete peritreme, with lighter pigmentation dorsally, that encloses 2 slits. The main tracheal trunks that lead from these slits are darkly pigmented for about half of their length in the last segment. This pigmentation is an important identifying character for second instar larvae.

The appearance of the posterior surface of the second instar larva is like the third instar (Fig. 12), but the fleshy processes are less well defined. The cephalopharyngeal skeleton is as in Figure 9.



2.2. -

Fig. 9 <u>C. hominivorax</u> - Cephalopharyngeal skeleton; second instar larva.

2.3.3 Third instar larva

The third instar larva is a robust maggot with a length of 6.4-17 mm and width from 1.6-3.5 mm. Fully mature third instar larvae average 15-16 mm in length (Fig. 10). When newly moulted they are a creamy white colour, but mature larvae gain a reddish tinge.



Fig. 10 <u>C. hominivorax</u> - Third instar larva, dorsal aspect. (a. anterior spiracles; m. mouthhook; l. lateral fusiform area; s. spines)

The spines are very prominent, about 130μ long, and arranged on the anterior margins of the segments as follows:

Seg. 2-9 - complete ring of spines, anterior ones largest; Seg. 10 - band narrower and generally interrupted dorsally; Seg. 11 - band interrupted dorsally and reduced on sides; Seg. 12 - spines only on ventral/ventrolateral surfaces.

The lateral fusiform areas are as in the first instar larva.

On the posterior margins of segment 11 is a band of 2-3 rows of small, forward curving spines. On segment 10 these spines may be present laterally and dorsolaterally, but always ventrally and ventrolaterally. On segments 7-9 the spines of the posterior margin are confined to the ventral surfaces in 1-2 rows.

The anterior spiracles have from 6-11 branches each, but usually 7-9. The branches are relatively long and well separated, (Fig. 10).



Fig. 11 <u>C. hominivorax</u> – Third instar larva; cephalopharyngeal skeleton.



Fig. 12 C. hominivorax - Posterior aspect of twelfth segment, third instar larva. (a, anus; ap, anal protuberance; at, anal tubercules; d, dorsal i/m/o turbecules; i, inner tubercule; m, median turbercule; o, outer tubercule; ps, posterior spiracle; v, ventral i/m/o tubercules.)

The posterior spiracles have a darkly pigmented incomplete peritreme, within which are three straight, oval shaped, slits which point towards the break in the peritreme (Fig. 12). The two main tracheal trunks are darkly pigmented, forwards from the spiracles to the tenth or ninth segment (Fig. 13). This feature is unique to <u>C</u>. hominivorax. The appearance of the posterior surface of the terminal segment is shown in Fig. 12 and the cephalopharyngeal skeleton in Fig. 11.

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Fig. 13 <u>C</u>. <u>hominivorax</u> – Third instar larva; representation of darkly pigmented tracheal trunks showing through posterior dorsal surface. Dissection of preserved larvae may be necessary to clear opaque fatty tissue.



Fig. 14 C. <u>hominivorax</u> – Third instar larva, posterior spiracles. (b, button; p, open or incomplete peritreme; s, spiracular slit.) The puparium of C. <u>hominivorax</u> is a cylindrical shape, rounded at both ends, approximately 10.2 mm long and 4.3 mm wide. The bands of large spines from the third instar larva can be clearly seen on the surface (Fig. 15). It has a dark brown colour.



Fig. 15 <u>C. hominivorax</u>, puparium. (s, sclerotized spine bands of third instar larva.).

2.5 IDENTIFICATION KEYS

The main objective of this identification section is to enable confirmation of a case of wound myiasis as either positive or negative for <u>C</u>. <u>hominivorax</u>. The identification of negatives is not given emphasis and, therefore, keys for third instars and adults are mostly to the level of genus only.

2.5.1 Key to the identification of third instar larvae

- 1. Larva with obvious fleshy projections on dorsal and lateral surface of body (Fig. 16).....Chrysomya <u>albiceps/rufifacies</u>

- Slits in posterior spiracles are straight and more or less parallel (Fig. 17).....4
- 4. Pigmented accessory oral sclerite present (Fig. 19).....Calliphora spp.
- Pigmented accessory oral sclerite absent.....Lucilia spp.
- 5. Posterior spiracles sunk in a deep cavity which can close over to conceal them (Fig. 20)...... Sarcophaga or Wohlfartia spp.

-	Posterior spiracles not in a cavity (Fig.12)6
6.	Tracheal trunks leading from the posterior spiracles with striking dark pigmentation extending anteriorly to the 10th or 9th segment (Fig. 13: dissection may be necessary in a preserved specimen)Cochliomyia hominivorax
-	Tracheal trunks lacking dark pigmentation7
7.	Posterior margin of segment 11 without dorsal spinesCochliomya macellaria
-	Posterior margin of segment 11 with dorsal spines
8.	Button (Fig. 14) of posterior spiracles indistinct (tropics and subtropics of Old and New World)Chrysomya
-	Button (Fig. 14) of posterior spiracles distinct (Holartic, approximately north of the Tropic of Cancer)Phormia or Protophormia
	A diagrammatic summary of this key is given in Figure 21.

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Fig. 16 Third instar larva of <u>Chrysomya albiceps</u>. A. dorsal aspect. B. Detail of fleshy process.



Fig. 17 Posterior spiracle of third instar larva of Lucilia sericata, illustrating closed or complete peritreme and straight slits.



Fig. 18 Posterior spiracle of third instar larva of <u>Musca domestica</u>, illustrating complete peritreme and sinuous slits.



Fig. 19 Lateral aspect of generalized cephalopharyngeal skeleton of Diptera. (a, accessory oral sclerite; d, dorsal bridge; da, dorsal apodeme; do, dorsal cornu; ds, dental sclerite; i, intermediate sclerite; m, mouthhook; ow, open window; p, parastomal bar; ps, pharyngeal sclerite; ve, ventral cornu; vp, ventral pharyngeal ridges; w, closed window).



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Fig. 20 Third instar larva of <u>Sarcophaga</u> <u>haemorrhoidalis</u>. A. Posterior <u>spiracles</u> with incomplete peritreme. B. Posterior view of terminal segment illustrating posterior spiracles just visible at the bottom of a deep cavity.



Fig. 21 Diagrammatic key to third instar larvae found in traumatic wound myiasis.

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2.5.2 Key to identification of adults

1.	Hypopleuron (Fig. 22) bare or with only soft hairs; thorax grey with four black longitudinal stripes; abdomen light brown with dark longitudinal stripe, more prominent in males; small species, 6-7 mmMusca domestica
-	Hypopleuron (Fig. 22) with a row of distinct bristles; other characters not exactly as above
2.	Grey flies with three black longitudinal stripes on thorax and patterned abdomen (Fig. 23)3
-	Metallic blue, blue-black or green flies4
3.	Abdomen with black spots (Fig. 23B); antennal arista bare or with very short hairs, length less than or equal to the greatest width of the arista; (Fig. 24C)
-	Abdomen chequered (Fig. 23A); antennal arista with long hairs above and below, length 2-3 times more than the greatest width of the arista; (Fig. 24B)Sarcophaga <u>spp</u> .
4.	Base of stem vein (Fig. 25) with a row of bristle like hairs above5
-	Base of stem vein (Fig. 25) bare above9
5.	Thoracic or lower squamae (Fig. 25) entirely covered with fine hairs above <u>Chrysomya spp</u> .
-	Thoracic squamae (Fig. 25) hairless above, except near base
6.	Three prominent black longitudinal stripes on the thorax7

.

-	No stripes on the thorax8
7.	Fronto-orbital plates of head (Fig. 26) with black hairs; postgenal setae (Fig. 26) golden yellow; fifth tergite without lateral dusted spots; female basicosta dark brown; body normally deep bluish-black with partial green or purple lustre, 8-10 mm
-	Fronto-orbital plates of head (Fig. 26) with pale hairs; postgenal setae (Fig. 26) pale yellow not golden yellow; fifth tergite with a pair of distinct lateral silvery-dusted spots; female basicosta yellow; body normally deep metallic green; 6-9 mm <u>Cochliomyia macellaria</u>
8.	Anterior (prothoracic) spiracle (Fig. 22) with bright orange hairPhormia
-	Anterior spiracle (Fig. 22) dark-haired Protophormia
9.	Wholly bright metallic green or bluish-coppery greenLucilia spp.
-	Deep metallic blue to blue-blackCalliphora spp.



Fig. 22 Taxonomic characters of adult fly thorax (<u>Calliphora</u>) in side view (as, anterior spiracle or prostigma; f, fore coxa; h, hind coxa; ha, haltere; hp, hypopleuron; hpb, hypopleural bristles, note group of eight; m, mid coxa; po, post scutellum; pp, pteropleuron; ps, posterior spiracle or poststigma; psb, prostigmatic bristle; s, scutellum; sp, sternopleuron; su, transverse suture; ts, thoracic squama; wb, wing base).



Fig. 23 Outline of <u>Sarcophagidae</u> with abdomen and thorax completed to show body patterns of, A. <u>Sarcophaga carnaria</u> and, B. <u>Wohlfahrtia</u> magnifica.



Fig. 24 A. Outline of antenna of typical Sarcophagid fly (a, arista; f, first flagellomere; p, pedicel; s, scape). B. Arista of <u>Sarcophaga</u> species. C. Arista of <u>Wohlfahrtia</u> species.



Fig. 25 Wing of <u>Calliphora vicina</u> illustrating taxonomic characters (an, anal vein 6; as, alar squama; ax, axillary vein 7; b, basicosta; c, costa; s, sub-costa; sv, stem vein; ts, thoracic squama). The small inserts at the stem vein and the thoracic squama illustrate the appearance of these characters in <u>Cochliomyia hominivorax</u>, where the stem veins have bristles dorsally and the thoracic squamae are hairless except for fine hairs at the base.



Fig. 26 Head of typical <u>Calliphorid</u> fly (f, fronto-orbital plate; p, postgena).

2.5.3 Summary of characters used to separate Cochliomyia from other genera:

- a) LUCILIA
 - LARVA: Lucilia Peritremal ring closed (Fig. 18) Cochliomyia - Peritremal ring open (Fig.
 - liomyia Peritremal ring open (Fig. 14)
 - ADULT: <u>Lucilia</u> Base of stem vein bare above (Fig. 25) <u>Cochliomyia</u> - Base of stem vein bristly above (Fig. 25)

b) CALLIPHORA

LARVA: <u>Calliphora</u> - Peritremal ring closed (Fig. 18) <u>Cochliomyia</u> - Peritremal ring open (Fig.

14)

- ADULT: <u>Calliphora</u> Base of stem vein bare above (Fig. 25)
 - <u>Cochliomyia</u> Base of stem vein bristly above (Fig. 25)

c) CHRYSOMYA

- LARVA: <u>Chrysomya</u> Tracheal trunks not pigmented <u>Cochliomyia</u> - Tracheal trunks darkly pigmented (Fig. 13)
- ADULT: Chrysomya Thoracic squamae with fine hairs above (Fig. 25)
 - <u>Cochliomyia</u> Thoracic squamae hairless above (Fig. 25)

d) <u>SARCOPHAGIDAE</u>

LARVA: <u>Sarcophagidae</u> - Posterior spiracles in cavity (Fig. 20) <u>Cochliomyia</u> - Posterior spiracles not in cavity (Fig. 12) - 41 -

ADULT: <u>Sarcophagidae</u> - Grey flies with patterned abdomen (Fig. 23 <u>Cochliomyia</u> - Metallic blue without abdominal pattern

2.6 LABORATORY TECHNIQUES FOR THE EXAMINATION OF SPECIMENS

2.6.1 General

Material may be prepared for preservation using the techniques described below. Care should be taken in handling the chemicals mentioned, to avoid the obvious dangers from inhalation or inflammation. In addition to preparing some of the material from a case of myiasis for preservation, where possible some of the eggs or larvae should be kept alive and reared to the adult stage for a confirmatory diagnosis. Laboratory rearing techiques for the various stages in the insects life history are described in section 1.3.

Immature specimens recovered from cases of myiasis should be cultured in containers that permit a transfer of gases with the outside air, e.g. the top being covered by gauze or tissue paper, secured in place by rubber bands. This will prevent asphyxiation and discourage the growth of mould. The main cause of mortality in open containers is desiccation and care must be taken to keep the larvae and their food source moist by dropping in clean water from a pipette. Mould is inhibited to some degree by the alkaline conditions created by the excretions of the feeding larvae. If mould does become a recurrent problem it may be countered by using a 0.24 percent formalin solution to moisten the medium, instead of plain water.

Larvae will develop well on a diet of minced meat, but meat may be mixed with other ingredients. Ideally, the rearing medium should be provided in a small, open-topped container placed inside a larger, covered, container. Dry sand or sawdust should be placed on the floor of the large container. When the larvae have finished feeding they will crawl out of the smaller container and pupate in the medium in the bottom of the larger container.

Pupae should be transferred to a cage with netted sides in which the adults can be examined as they emerge. A simple wire frame covered by a sleeve of muslin or mosquito netting is suitable. Before killing the adults, they should be allowed to inflate the wings and fully harden the cuticle. During this period their mature colouring will also develop.

If the adults are to be cultured and bred to produce a reference collection of eggs and larvae, they will require water and carbohydrate for survival. Water can be supplied from a well-soaked wad of cotton wool placed in a small container to prevent spillage. Honey, or a solution of sugar in water, will provide the necessary carbohydrate. A small amount of fresh meat should be placed in the cage daily for oviposition and to provide females with protein.

The importance of labelling cannot be overemphasized. A specimen is of little scientific value if it is without a label giving details of where and when it was captured. If larvae were recovered from a wound, then details should be given of the host and of the location and nature of the wound.

2.6.2 The egg

For general examination of eggs it is adequate to put them straight into 80 percent ethanol, for both fixation and storage. With eggs containing well formed and active larvae it may be necessary to fix the egg in KAA or in Kahle's (=Pampel's) fluid for 24 hours first, then wash and store in 80 percent ethanol. Formalin is not recommended as a preservative on its own.

KAA	Kerosene Ethanol (95 percent) Glacial acetic acid	1 part 10 parts 2 parts
Kahle's	Formalin (35 percent) Ethanol (95 percent) Glacial acetic acid Distilled water	6 parts 15 parts 2 parts 30 parts

When fresh eggs are being examined, in order to better define the structures on the eggs, put one drop of safranin directly onto the egg masses and wait for two minutes.

2.6.3 The larva

Larvae are best examined in a fully extended condition, but live larvae placed directly into ethanol usually die contracted. To obtain relaxed specimens there are two simple methods. Firstly, larvae can be placed live into KAA or Kahle's fluid for up to 24 hours, then rinsed and stored in 80 percent ethanol. This method improves the preservation of the internal structures. The use of acetic alcohol (3 parts 90 percent ethanol: 1 part glacial acetic acid) also overcomes the problems of contraction using alcohol alone and fixes the larvae in a suitable state for dissection. Alternatively, the living larvae can be dropped into hot water, just below boiling point, and then transferred to 80 percent ethanol for storage. If the water is actually boiling the larvae will tend to rupture.

For the preparation of material for slides, or of preserved material for detailed examination of the mouthparts and spiracles, the material should first be 'cleared'. This is achieved by macerating the specimen in a 10 percent aqueous solution of potassium hydroxide (KOH) at room temperature for at least 15 minutes. Specimens that have been in alcohol for six months or more may need a longer period in KOH, up to 12 hours at room temperature or a shorter time in warmed KOH. Small larvae should be put into the solution whole, with punctures to allow penetration of the solution; larger larvae may be dissected first and only the required parts macerated. As the muscles soften they can be teased away with fine forceps or sharp needles. In order to avoid destroying the sclerites, care must be taken with dissecting instruments and with KOH.

When the muscle and fat body has been cleared away, the specimens should be placed in glacial acetic acid for at least 15 minutes to neutralise any residual KOH. They should then be rinsed well with 80 percent ethanol and are then ready for examination, mounting or storage.

There are many methods of mounting slides. One is to dehydrate the samples in absolute alcohol, transfer them to clove oil to clear, then mount in Canada balsam; alternatively, from absolute alcohol they may be mounted directly into Euparal or into Berlese mountant.

Slides can only be viewed in one plane and this should be taken into account when mounting specimens. The cephalopharyngeal skeleton can be mounted as one preparation allowing a lateral view. Anterior spiracles can be mounted simply, detached or with a portion of the surrounding cuticle. Posterior spiracles should be mounted as a pair to retain the relationship between them. The flat area of the posterior spiracular disc may be torn off and mounted, taking care to note the orientation of the spiracles, their ventral and dorsal sides. With careful dissection, the posterior spiracles may be mounted together with the dorsal tracheal trunks that lead from them.

The darkened dorsal tracheal trunks of C. hominivorax are usually very obvious in living larvae. Where there is doubt, confirmation of the degree of pigmentation of these trunks can be made by placing the specimen between a pair of glass microscope slides and exerting gentle compression. The slight flattening of the larva renders the trunks more visible.

2.6.4 The puparium

Puparia are best preserved dry, although they may be preserved in alcohol. A puparium from which an adult fly has emerged should, where possible, be mounted on the same pin as the adult. The pin may be passed directly through the puparium, but to avoid damage, it is better to place the puparium in a clear capsule of gelatine or plastic. When adult Diptera of the families considered here emerge, dorsal and ventral flaps at the anterior end of the puparium open. These flaps should be retained with the rest of the puparium as they contain relics of the third instar larva useful for identification. Inside the lower flap lie the sclerites of the third instar cephalopharyngeal skeleton. This skeleton does not lie in the same orientation as in the larva due to flattening against the puparial wall and it may be covered by membranes. It can, however, be cleared by KOH, as for larvae, and used to give an indication of the larval identity.

The larval anterior spiracles can be found on the upper flap of the puparium, while the larval posterior spiracles can be studied at the posterior end of the puparium. Again, clearing with KOH may be helpful since these spiracles often become very heavily sclerotized in the puparium, making observation of the slits difficult.

2.6.5 The adult

Adults may be killed by dropping them into a stoppered tube or jar containing a cotton wool ball soaked in a suitable killing agent. Ethyl acetate is recommended, but chloroform, carbon tetrachloride and other alternatives may be used. Dead specimens can be transferred to 70-80 percent ethanol for storage. However, for the best examination of characters used in identification, specimens should be pinned through the thorax, to one side of the mid line. Pinned specimens should be individually labelled and protected from mould and insectivorous insects.

CHAPTER 3

MYIASIS

3.1 DIAGNOSIS

Wounds infested with screwworms, <u>Cochliomyia</u> <u>hominivorax</u>, and <u>Chrysomya</u> <u>bezzania</u> are very characteristic. They are usually circular and very deep, 5-10 cm or more, resulting in extensive tissue destruction. They have a characteristic odour resembling that of putrefaction. The larvae of these species are found in the deepest parts of the wound unlike secondary species which stay near the surface.

3.1.1 Agents of wound myiasis

Myiasis has been defined as the infestation of live vertebrate animals with dipterous larvae, which, at least for a certain period, feed on the host's dead or living tissue, liquid body substances, or ingested food. Such larvae belong to one of two groups depending on their reliance on the host: the obligate parasites and the facultative parasites.

Obligate parasites normally develop exclusively on or in the tissues of living vertebrates. They include the Oestrinae (nasal bot flies), the Hypoderminae (warble flies), the Gasterophilinae (equid and rhino bots) and bloodsucking larvae of the genus Auchmeromyia. None of these is likely to be found in cases of wound myiasis. The three major species of facultative parasites encountered in wound myiasis are the New World screwworm, Cochliomyia hominivorax; the Old World screwworm, Chrysomya bezziana and Wohlfahrt's wound myiasis fly, Wohlfahrtia magnifica.

The two species of <u>Cochliomyia</u> encountered in wound myiasis are <u>C. hominivorax</u> and <u>C.</u> <u>macellaria</u>. <u>C. macellaria</u> has often been implicated in myiasis due to incorrect identification of C. hominivorax. The larvae of C. macellaria may be very abundant on carrion. When they are involved in myiasis they are only secondary invaders, feeding on the edge or surface of the wound and not producing the pocket-like lesions characteristic of primary The adults are common in slaughterhouses screwworms. and outdoor markets. Females lay up to 1 000 eggs in batches of 40-250, and they often deposit together producing masses of several thousand eggs. These may hatch in 4 hours and the larvae reach maturity in The total development time is in the 6-20 davs. range 9-39 days, depending on temperature humidity, and adults live from 2-6 weeks. and

Facultative parasites are normally free living and develop in decaying organic matter, including carrion. Under certain circumstances they may develop on living tissues, for example in the soiled dressings of bed-ridden patients who are unable to clean, or otherwise fend, for themselves.

It is vital to any control campaign to be able to identify the pest insect correctly. In screwworm eradication programmes, this ability becomes increasingly important as the campaign progresses. A measure used to estimate the success of control is the ratio of screwworm cases to non-screwworm cases. There are a large number of non-screwworm species that can be involved in myiasis and clearly it is important to be able to distinguish screwworm from them. Correct identification is also important in planning the eradication strategy, example, where to release sterile flies. for Great savings in time and money can be made if releases of sterile flies are directed against confirmed cases of screwworm, rather than against unconfirmed cases of myiasis which may be due to other, less serious, pests.

3.2 PATHOLOGY

The pathological effects of screwworm infestations on the parasitised host may be subdivided into four major components.

- a) A <u>traumatic effect</u> caused by the larvae tearing the body tissues, using the hook shaped mouth parts.
- b) An <u>irritating effect</u> through the constant boring movement of the larvae within the wound.
- c) <u>Secondary infection</u> of exuding wounds by other contaminating organisms such as bacteria, viruses, protozoa or fungi.
- d) <u>Toxic effect</u> through larval excretion of waste products.

An infested animal may survive for only a few days if the infestation is severe and not promptly treated. Even with treatment, particularly if delayed, secondary infections can spread through the bloodstream causing arthritis, enteritis and septicaemia. In screwworm infested regions up to 90 percent of newborn animals can be killed by the disease if treatment of the unhealed umbilicus is neglected.

The screwworms excretory products result in necrosis of the infested tissue. This, through smell, attracts other species of Diptera that infest the external area while the screwworm continue to enlarge and deepen the wound. Under these severe conditions, and without proper treatment, the animal will soon die. Contributory factors to death are the secondary infections that occur through bacteria or other micro-organisms, toxaemia and loss of liquids. These secondary infections are almost always present, but, in areas or low fly density wounds may not be subjected to continual reinfestation and may heal of their own accord once abandoned by the larvae.

The wound, although continuously exuding, remains without pus or scabbing while screwworm are present. Secondary infections complicate disease diagnosis.

In areas of low screwworm challenge the prognosis for recovery and survival of infested animals is favourable. However, under high risk conditions and particulary if infestations are prolonged for two weeks or more without treatment, then death is probable. Wounds treated within four days of infestation usually heal within one month, with an improvement being evident within 10 days following treatment. The prognosis for the recovery of newborn animals is considerably less favourable than for adults.

Bovines are generally fairly resistant to complications, and injuries normally heal quickly with good treatment, but ovines, caprines and equines frequently develop secondary infections. Infectious polyarthritis, caused by chlamydia, is frequently observed in bull calves that survive.

A superficial examination of infested wounds shows that larvae start to leave the wound immediately after the death of the animal, within a maximum of one hour. Second instar larvae may continue to develop to the pupae stage; more immature forms fall to the ground or may pupate in the wound, but the probability of adult emergence is minimal.

- 3.3 TREATMENT PRINCIPLES
- 3.3.1 Insecticides

Various insecticidal products are available that will kill screwworm larvae in the wound and prevent reinfestation.

The major points to be considered in the selection of an insecticide, and its formulation, for the treatment of myiasis are as follows:

- low mammalian toxicity, to ensure optimum safety to handlers and the host animal;
- high larvicidal/insecticidal activity to ensure effectiveness of treatment;

- retention of residues in the host and rate and mode of excretion;
- persistence at toxic levels, when applied in mass treatment, for prevention of infestation in wounded and susceptible animals;
- ability of formulations to penetrate deep wounds particularly in severe cases of myiasis;
- risk of environmental contamination and possible effects on non-target species.

Ideally the insecticide of choice would have high toxicity to screwworm at all stages of its life history whilst combining low toxicity to mammals with long larvicidal persistence. It should also have the ability to penetrate muscle tissue without being transformed into non-toxic breakdown products in the short term but with eventual transformation into non-polluting elements.

Specific products have been developed and proven for use against <u>C</u>. <u>hominivorax</u> within its original distribution in the <u>Americas</u>. In the case of new infestations in other regions, where environmental and climatic conditions may vary, these procedures for control and containment of the parasite may or may not be as effective. However due to the urgent need to implement control measures, at least initially, the experience gained in other successful control programmes should form the basis for immediate action in new outbreaks.

In the following section relating to treatment, prevention and control specific reference will be made to the organophosphate insecticide commonly known as Coumaphos as the compound of proven efficiency and national environmental acceptability in all American countries with eradication programmes. It is, however, appreciated that equally effective alternative compounds may exist, and be considered for use, provided they are nationally registered and/or approved and used in accordance with the manufacturers recommendations.

3.3.2 Application techniques

Insecticides are manufactured and mixed with other carrier compounds in various ways to produce formulations suitable for application under differing circumstances and by a variety of spraying techniques. For screwworm control we are only concerned with two methods of application. These are:

- direct or topical application of insecticide to a specific wound to clear or prevent an infestation and;
- mass treatment for protection of flocks or herds of animals considered to be at risk, or for the purpose of quarantine control.

For topical application to wounds it is recommended that in the case of organophosphates a wettable powder (W.P.) formulation be used. This can be applied directly by dusting on to the wound a 5 g packet of 5 percent Coumaphos as supplied in individual treatment packs, or the contents of the packet may be mixed in a vegetable oil by using 15 x 5 g packets mixed in 500 ml of oil to form a thin paste which is then applied directly to the wound, using a paint brush of approx 2-3 cm width. Careful application is necessary in deep wounds to ensure that the paste reaches all the pockets formed by the burrowing maggots. A thin layer, between 5-7 cm wide, should also be applied to the surrounding skin. (Fig. 27). For very moist, suppurating and exuding wounds the dry insecticide powder should be applied direct and mixed with the wound exudate, using rubber gloves for protection. (Fig. 28).

If necessary treatment should be repeated at three day intervals until the wound has healed and the danger of infestation or reinfestation has passed.

Care must be taken to ensure that any insecticides used are not contra-indicated for the species of animal to be treated.



Fig. 27 Topical treatment of infested wound with insecticide paste.





- (1) READ THE INSTRUCTIONS CAREFULLY
- (2) TEAR OPEN ONE CORNER OF THE PACKET





- SPRINKLE THE POWDER IN AND ON THE EDGE OF THE WOUND BE SURE THAT MOST OF IT COES INTO THE WOUND
- USING A CLOVE, RUB THE HEALING POWDER WELL INTO THE WOUND MIXING IT WITH THE WOUNDS SERUM

5 REPEAT APPLICATION AFTER THREE DAYS IF WOUND HAS NOT HEALED

Fig. 28 Topical treatment of exuding wound.

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The insecticide treatment of whole herds or is usually flocks of animals undertaken for prevention of infestation in times of increased vulnerability, such as newly shorn sheep which may have been cut during shearing, or as a quarantine measure to prevent the spread of the parasite, through carriage, on infested migratory and transported animals. The method of treatment may be through the immersion of the animal in the insecticide by dipping or spraying each individual animal to ensure a thorough wetting, penetrating to the skin. Of the two dipping is considered the most efficient and economical method although it should not be used for very young animals or for those species susceptible to insecticide. Efficient treatment by spraying cannot always be assured as it is dependent on the diligence of the spray operators.

Some examples of the calculations required to determine the dilution factors for mixing of insecticides are given below. These are based on starting with a 50 percent wettable powder formulation supplied in 1 kg packs, to be applied at a 0.25 percent concentration, as would normally be the case when using Coumaphos.

> Motor driven sprayer of 750 litre (1) capacity:

> > to fill a 750 l spray tank with 0.25 percent spray mixture requires 0.25 grammes active ingredient (g.a.i) insecticide per 100 ml or 2.5 g a.i. per litre of water.

> > Therefore 750 l requires 750 x 2.5 g a.i. = 1 875 g

1 kg of 50 percent wettable powder contains 500 g a.i.

To make 750 l of a 0.25 percent of spray mixture requires $\frac{1\ 875}{500} = \frac{3.75}{500}$ kg of insecticide.

ii) To fill knapsack sprayers of 10 1 capacity;

1 kg of 50 percent insecticide will dilute to 200 1 of 0.25 percent suspension.

Therefore a 1 kg pack mixed correctly will give sufficient liquid to fill 20 machines of 10 l capacity - or one machine twenty times.

- iii) For treatment by dipping; the cubic capacity of the dip tank must be calculated and 1 kg of 50 percent insecticide added for every 200 1 of water.
- 3.3.3 Handling and use of insecticides
 - Store all insecticides in a secure place, under lock and key, away from food stuffs and at a safe distance from human and animal habitation. Protect from weather and ensure all containers are in good condition with no leakages.
 - 2) <u>Neutralize all spilt insecticides</u> immediately according to the manufacturers recommendations.
 - 3) <u>Handle insecticides with care</u>, wear the appropriate protective clothing and avoid spraying into the wind. Avoid direct contact.
 - 4) Do not spray near human habitation or open water.
 - 5) Dispose of empty containers safely, do not re-use for other purposes.
 - 6) Wash clothing and spray application equipment thoroughly after use and repair any leaking parts immediately.

- 7) Keep casual observers, especially children, away from spraying areas. Remember the toxicity of insecticides is often related to body weight and small children are most vulnerable.
- 8) Dispose of spray waste and washing water wisely, preferably through a well constructed soakage pit, fenced and gated to prevent unauthorised access.
- 9) Dipping areas should be constructed so that excess insecticide dripping off treated animals flows back into the dip and does not pollute the surrounding area.
- 10) Remember that insecticides are poisons and at all times handle, store and use them accordingly.

3.4 DISEASE CONTROL AND PREVENTION

The objective of these activities is reduce the number of wounds on potential host animals and minimise their attractiveness to screwworm attack. However the total elimination of attractive open wounds is, in most circumstances, unattainable. The inspection and treatment of animals, as recommended below, will ensure that the number of open wounds susceptible to screwworm attack is considerably reduced. Consequently these activities will result in a suppression in the population density of the parasite and a corresponding decrease in disease incidence.

For successful implementation livestock owners must be informed of the procedures and encouraged to participate in implementation. The degree of success achieved will depend on the number of livestock owners collaborating. If only one person allows his animals to become infested and remain untreated the increase in the screwworm population will place neighbouring animals at greater risk.

- 3.4.1 Control measures
 - Examine all animals, including pets, thoroughly and frequently.
 - <u>Search all wounds</u> for egg masses and larvae and if found, collect and preserve in 10 percent formalin or 70 percent methyl- or ethyl- alcohol. Record details of collection on the form provided with the sample kit or, if not available, on a separate sheet of paper. Larva situated deep in the wound may be extracted using forceps. Care should be taken to ensure that all larvae remaining in the wound are destroyed and that none are dropped on the ground and allowed to survive.
 - Treat infested wounds immediately with a recommended insecticide and if secondary infection is suspected administer a systemic high spectrum antibiotic.
 - Despatch the larval samples collected, to the official agency responsible for identification of specimens, by the quickest possible route and without delay.
 - Report the suspected infestation to an official of the Veterinary services by telephone or any other rapid means of communication that may be available.
 - Do not move animals from or into the infested area unless given official permission to do so.
 - When buying or selling animals make sure they are free of screwworm infestation.
 - All livestock in, or adjacent to, high risk areas should ideally be regularly treated with insecticide, by dipping or spraying, to reduce the risk of infection. The frequency of treatment will depend on

the effective persistence, at toxic levels to screwworm, of the insecticide used.

- 3.4.2 Prevention measures, livestock owners and handlers
 - Physically examine all animals, including pets, thoroughly and daily, treating all wounds with a recommended persistent insecticide preparation to prevent infestation.
 - Minimise the risks of animals being wounded by checking for protruding nails, wire and other sharp objects in their enclosure and by avoiding dense and thorny vegetation during free grazing. Identify all other possible causes of wounding within the local situation and take measures for their elimination.
 - If possible avoid the undertaking of veterinary and animal husbandry practices, which result in wounding, such as calving, branding, dehorning, castrating and shearing, during the periods of seasonal high risk.
 - <u>Separate aggressive animals</u> to avoid the risk of injury through fighting.
 - During periods of high seasonal risk, such as the rainy season, <u>minimise animal/fly</u> <u>contact</u> as much as possible by grazing away from areas of high screwworm density and by utilising hours when flies are less active, such as at night.
 - Ensure the regular and systematic control of other livestock ectoparasites, particularly those that wound the skin and promote attraction to screwworm.

The above measures are recommended to reduce the incidence of infestation amongst local livestock. If carried out diligently by all owners they can be expected to result in a reduction of the screwworm population through interruption of the life cycle. They would not, however, eliminate the parasite and therefore require systematic implementation on a continuous, long term basis.

3.4.3 Prevention and control at national level

For maximum effectiveness in the control, prevention and eventual eradication of the disease, measures must be taken at a national, or even regional level, to achieve a concerted effort over the total area of infestation and to prevent the spread of the parasite to adjacent uninfested areas.

The important steps in any control campaign can be classified as:

- determination of the seasonal distribution limits and density of <u>C</u>. <u>hominivorax</u> within the infested area (surveys);
- prevention of spread of the parasite into non-infested areas (quarantine and livestock movement control);
- conducting of a sustained and effective public awareness campaign to ensure cooperation and participation of all sectors of the community;
- implementation of préventative measures to avoid and minimise the infestation of livestock held at risk;
- initiation of control techniques to reduce parasite populations, thus reducing the incidence of infestation and the risk of expansion of the parasite's distribution;
- constant evaluation of the effectiveness of the operation to allow for modification as may be required;
- evaluation of the feasibility of introducing large-scale eradication;

 applied research in identified areas to support and increase the efficiency of field operations.

3.5 DISEASE CONTAINMENT

Following confirmation that an area has become infested with <u>C</u>. hominivorax it is essential that procedures be <u>implemented immediately</u> not only to control the disease but to also prevent its spread <u>to new areas</u>. Any action undertaken to place stress on the insect and so reduce the population density or to retard reproduction will minimise the risk of spread of the adult insects which have been recorded as capable of travelling up to 290 km in less than two weeks, in search of favourable habitatand hosts.

It is, therefore, imperative that the measures described for protection and control at livestock owner and national levels, sections 3.4.2 and 3.4.3, be implemented as soon as an outbreak is confirmed. These actions must also be continued for as long as the parasite persists, regardless of the level of infestation.

3.5.1 Animal movement and quarantine

The major risk to the infestation of other areas is through the transportation of the live egg/larval stages on infested domestic livestock or wild animals.

This movement can be effected in various ways such as:

- localised movement of animals on the limits of infestation which gradually extend the boundaries of the infection;
- migratory movement of wild animals and the seasonal transhumance of livestock owning pastoralists, often involving the crossing of national borders;

- use of pack animals for transportation of commercial and agricultural products from areas of production to market centres and vice versa;
- long and short distance vehicular haulage by road or rail, of animals, usually for sale at markets;
- intercontinental transfer of live animals by aircraft/boat which could result in global screwworm infestation.

It is, therefore, essential that animal movement from infested, and even suspected infested areas, be monitored and efficiently controlled at all levels ranging from local livestock movements to international import/export for commercial purposes.

In all cases the technical procedures for control are essentially the same and as a general rule the following practices should be adopted:

- all patterns of animal movement within the infested area should be closely examined and those presenting a possible risk for the carriage of screwworm to other areas identified and recorded;
- <u>control posts should be positioned at</u> <u>strategic points and in the numbers</u> required, based on the information obtained from the animal movement study;
- the person in charge of animals presented at the control stations should be issued with written certification of inspection including details of the result and any treatment provided;
- all animals entering or leaving the infested area should only do so by passage through veterinary control/quarantine posts established for the control of screwworm and must be inspected and

certified to be free of infestation. Movement should be restriced as much as possible;

- animals transported by vehicle must be unloaded to facilitate close physical examination of each individual;
- the inspection of animals must be carried out diligently by examination of the entire body surface for wounds. All wounds must be closely inspected bearing in mind that screwworm larvae may be buried relatively deep in the tissue and may not be apparent on casual inspection;
- all animals presented at the control post and in which screwworm is not detected and which seem free of wounds should be treated with a recommended insecticide formulation either by thorough spraying of the entire animal or by immersion in a dip;
- all vehicles transporting animals and passing through the control point <u>must be</u> <u>sprayed</u> to kill any larvae that may have dropped off the animal on to the floor of the vehicle. This should be done even if there is no evidence of infestation;
- wounded animals which are not infested should receive topical treatment of the wound, after having received the full body spray or dip;
- animals having infested wounds suspected of being C. hominivorax must receive curative treatment and specimens collected for confirmation of the parasite. They must be held in quarantine until the identification is made and if positive, must not be allowed to proceed on their journey until the infestation has been eliminated by daily curative treatment, at least three days. Prior to leaving the

control point such animals must also receive the full body spray or be subjected to dipping;

- it is advisable that all animals showing <u>myiasis</u> infestations <u>be</u> <u>subjected</u> to <u>quarantine</u> and treatment until the infestation is cleared, usually at least three days. This precaution will eliminate the risk of incorrect identification of the parasite;
- animals, with uninfested wounds, intending to enter infested areas should be subjected to preventative treatment at the control point. The person responsible for such animals should be instructed to continue such treatment until wounds are healed and the danger of infestation eliminated;
- measures should be devised to ensure that owners and handlers are not availed of the opportunity to pass clean animals through the inspection point and then change them for possibly infested animals once a certificate has been issued. To this end it may be necessary to officially seal the transporting vehicle or to individually mark inspected animals for later identification. Such action. with details, should be recorded on the certificate of inspection;
- lines of communication, to ensure the rapid flow of information between control stations and coordinating offices, should be established so that the progress of transported animals can be checked. This will deter unauthorised deviation;
- each station should be equipped for and capable of, the accurate identification of screwworm larval stages and eggs;

- for efficient organisation, the functioning of the control/quarantine system should have the support of appropriate and enforceable legislation.
 Penalties should be determined and imposed for the non-observance of such regulations;
- countries importing live animals from those with screwworm should ensure the quarantine, inspection and treatment of such animals to avoid the introduction of the disease. Likewise screwworm infested countries should take the action necessary to ensure animals are parasite free prior to export.

3.6 SCREWWORM ERADICATION

The eradication of <u>C</u>. hominivorax from areas of optimal environmental conditions has not been proved feasible through the intensive use of control and prevention techniques. Under such circumstances these activities result in a noticeable population reduction but the species survives through completion of the life cycle in wild animal hosts and untreated livestock. To achieve eradication it has been necessary to integrate control and prevention measures with the sterile insect technique, (SIT).

The SIT requires the weekly rearing of hundreds of millions of insects in specially constructed plants. These insects are sterilized, normally by controlled radiation, and aerially released over infested areas. Matings between wild females and steriles males produce no offspring and thus interrupt the life cycle and progressively reduce wild populations. Supported by other control measures, as described in this manual, SIT has been used successfully for the eradication of <u>C</u>. hominivorax from the islands of Curacao and Puerto Rico, and also from the Florida Peninsula, the southern United States of America and Mexico.
The distribution limits of screwworm must be accurately defined so that when treated with SIT no infested area remains to provide a source for re-invasion.

For success adequate numbers of laboratory reared sterile insects should be released to compete with the wild males in mating with females. Efficiency of the technique, therefore, relies on integration with other prevention and control methods, to achieve an initial reduction of the population and should also be undertaken during the period of natural seasonal decline, (dry season). It is estimated that, theoretically, 10 sterile males should be released for each wild male present per unit area. In the Mexico/America campaign an average of between 700 to 2 500 sterile flies per km² are released weekly.

Operations are monitored mainly through recording the incidence of infestation in livestock and also by surveillance for sterile and fertile egg masses on sentinel animals and microscopic examination of trapped females to determine sterility. Releases of sterile insects are continued until these surveys confirm that the wild population has been eliminated.

Prior to operations the sexual compatibility and competitiveness of the laboratory reared with wild screwworm populations must be ascertained. The effect of seasonal climatic changes on population density of wild flies must also be determined to assist in calculating the number of competing sterile insects to be released.

The mass rearing of screwworm under factory conditions is expensive and logistically and

technologically demanding. It is estimated that the time required to construct such a facility and to build up production levels to those required for eradication would be in the order of two years. As an example of the costs involved; in the Mexico eradication campaign the rearing plant cost US 40 million dollars to construct and equip over 3 years, at 1977 currency values. A budget of 50 million dollars was allocated annually to cover al1 activities including field operations. Eradication may be justified by considering the extent of infestation, the effect on livestock and wild life and through the predicted consequences of the spread of the parasite to uninfested areas.

To avoid the establishment costs required for mass production, consideration can be given to long distance transportation of sterile insects from existing rearing units, providing compatibility of the stains is confirmed.

3.7 DISEASE NOTIFICATION

<u>C. hominivorax</u> is an efficient parasite well adapted to its environment. The potential for the spread of the the disease to non-infested areas is very high. The economic consequences, on livestock production and wildlife populations, of introduced infestations can be disastrous. It is, therefore, essential that should screwworm be recorded in new areas <u>immediate action is taken to prevent its spread</u> and to initiate activities for control and eradication.

It is strongly recommended that any new outbreaks be immediately notified to FAO and to adjacent countries so that necessary action can be taken without delay. The format suggested for disease reporting is shown in Fig. 29. The address for immediate reporting to FAO is as follows;

> FAO Animal Health Service (AGAH) Via delle Terme de Caracalla 00100 Rome, Italy Tel: 5797-4106 Telex: 610180 Facsimile: 625852/625853

	Disease code Nº Year		
1,	Country	_	
2.	Name and designation of sender		
3.	Telex number or telegraphic addr	ess	of sender
4.	Date of transmission of message	5.	Date of initial detection
6.	Estimated date of first infection	7.	Number of separate outbreaks identified so far

8. Geographical location of the outbreak(s)

9. Details of outbreak(s)

	N° of animals in outbreaks (affected herds) (b)	Number of		
Species Infested (a)		Cases (c)	Deaths (d)	Animals (e)
	lii_			

10. Comments concerning affected population

11. Comments to date concerning epidemiology of the disease

12. Control measures taken to date

Fig. 29 Format for notification of new outbreaks of C. hominivorax.

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3.8 RESEARCH NEEDS

The biology of <u>C. hominivorax</u>, under the natural environmental conditions of its previously restricted distribution on the American continent, is well documented. This information serves as a useful guide to its anticipated development and behaviour in new areas of infestation. However, as environmental conditions will not always be the same and due to the ability of insects to adapt to changing circumstances, it is essential that certain basic information be confirmed in any new outbreak.

- The influence of climatic factors on the life cycle and determination of extremes that may limit distribution;
- Confirmation of screwworm behaviour in relation to available habitat and influence of vegetation and other biological factors on distribution;
- Identification of survey techniques and odour attractants of optimum efficiency;
- Evaluation of insecticides available for prevention, treatment and control;
- Studies to ascertain the effect of other wound causing diseases on screwworm infestation levels and measures identified for their control.

CHAPTER 4

SURVEYS

4.1 SURVEYS

Screwworm surveys can be based on the detection of any of the four stages in the parasites life cycle, egg, larva, pupa or adult. However, the larval stage is most suitable because it is easier to detect, collect, preserve, transport and identify. The other stages require special entomological training, experience in identification and are more difficult to locate and preserve. The points to be considered in the implementation of surveys are listed below.

4.2 LARVAL SURVEYS

All livestock owners and handlers must be motivated to carry out frequent physical examination of their animals for the detection of infested wounds. The areas to be sampled must include all areas of suspected infestation <u>plus</u> a large fringing barrier where flies and the disease may exist in very low density and their presence easily overlooked. The survey area, if large, should be divided into sectors with a veterinary officer in charge of each.

The organisation of such a large-scale operation, depending on the willing participation of many livestock owners, will rely for success on the support of a well organised veterinary field extension service and an efficient and sustained public awareness campaign. This campaign should place emphasis on the importance of constant diagnosis, surveillance and intensive follow-up on all suspected screwworm cases and reports.

All those involved with livestock must be instructed in the recognition of suspected myiasis

infested wounds, in the collection and preservation of larval samples and in the recording of the disease.

A national coordinating unit will be required for the receipt of suspect samples, their entomological identification and the recording of the location of collection. The objective of this unit will be to produce and maintain an up-to-date record on the distribution and density of screwworm as indicated by the samples received. This will also include information from surveys for the detection of adult and egg stages.

To encourage and assist farmers to cooperate they should be supplied with packs of insecticide for treatment of wounded animals, collection and reporting instructions, written in the local language, and sample tubes containing a suitable preservative, such as 70 percent methyl- or ethylalcohol, for collection of larvae together with details on method of transmission to the appropriate coordinating unit for identification and recording. Tubes and reporting sheets should be numbered to avoid error.

The collection tubes and insecticide can be made up into kit form containing a packet of insecticide powder, a specimen tube with preservative and an instruction and reporting leaflet all in a single pack, often referred to as a "treatment and sampling kit", Figures 30 & 31.

It must be stressed that any weakness in the efficiency of such an intensive and sustained survey programme could result in erroneous plotting of the parasite's distribution and seriously jeopardise control and eradication operations. Limits of distribution and the density of parasite populations within those limits must be accurately determined.









Suggested questionnaire for inclusion in the treatment, sampling kit.

- 1. Collection location and date
- 2. Collectors name and address
- 3. Date of larval collection
- 4. Species of animal infested
- 5. Approximate age of animal
- 6. Suspected initial cause of infested wound
- Number of infested animals recorded over last seven days
- Details of any animals introduced into the area over the last 12 days.
 - a) Number.....
 - b) Species.....
 - c) Origin.....
- 9. Details of treatment given

For official use:

Inspector	Date of investigation
Sample N°	Result of identification
Location	Follow-up action

4.3 EGG MASS SURVEYS

Locating of eggs is not recommended when the objective is the determination of disease distribution and incidence as eggs are short-lived, difficult to detect by untrained personnel and not easily identified.

Surveys for the detection of egg masses are mainly used to evaluate levels of sterility and estimate density in wild screwworm populations being subject to eradication through the sequential release of sterile, laboratory reared, flies. However, in order to complete the section on survey techniques the method will be described here.

Usually three sentinel adult animals are chosen and confined in a pen, sited under optimum conditions for the attraction of adult screwworm. Water and food are provided to ensure the health and well-being of the animals.

In order to attract and stimulate oviposition of mature wild female flies one animal is deliberately wounded surgically by making a cross-cut incision some 5-7 cm long, usually in the muscular area of the shoulder or rump. The wound should be prevented from healing. Each animal should be utilised for one week only then alternated with the other two. The animal is inspected daily, early morning and late afternoon, for the presence of egg clusters. Any found may be left in the wound or recovered to the laboratory for incubation and rearing. In either case the ability to develop from egg to larva is noted to determine wild female fertility. The ratio of sterile to fertile egg masses will give an accurate indication as to the efficiency of the sterile males in competing with wild insects.

If the eggs are allowed to develop on the wounded animal they should be removed at the second larval instar, after approximately two days, for identification. After removal of a sufficient number of larvae (at least 12) the remaining larve in the wound can be killed by pushing a cotton-wool plug soaked in chloroform into the lesion. The sentinel animal should be treated daily with an antibiotic to prevent secondary infection.

Daily records should be maintained indicating the exact location of the sentinel herd, its reference number, number of sterile and fertile egg masses recorded and climatic conditions such as temperature, rainfall and wind direction.

If the sterile insect release operation is successful there will be a progressive increase in the ratio of sterile egg masses recorded until complete sterility is reached.

4.4 ADULT FLY SURVEYS

Due to the relative ease with which larval stages of screwworm may be detected in animals, surveys for adult flies are not normally conducted when the objective is the determination of disease distribution and incidence. As in the case for egg mass detection they are, however, useful when the purpose is to evaluate the progress of eradication using the sterile insect technique and to provide an estimate of the population density of sterile and wild flies.

4.4.1 Traps

Two designs of adult fly traps have been developed and modified for current use in the Mexico-USA Joint Screwworm eradication programme and have been found to be the most efficient. They are described below. Both rely on enhancing attraction through the addition of an odour bait, resembling the smell of putrid flesh, which is particularly attractive to gravid females. Males are also captured in reasonable numbers as they are attracted to the smell in response to a mating instinct.

a) The bishop trap (modified)

The trap is baited with 500 g of raw bovine liver cut into small pieces, placed in an equal volume of water and allowed to decompose for one week whilst protected from flies. The liver is then placed in the plastic tray of the trap and the water replenished as required. The liver should be replaced every week. New traps increase in efficiency, reaching a maximum after about two weeks.

The handling of flies caught by the traps should follow the following steps:

- Traps should be inspected and emptied on a daily basis. Repairs should be carried out as necessary and without delay;
- Remove plastic tray and place the upper portion of the trap, containing the flies, into a plastic bag containing a wad of cotton wool soaked in ethyl acetate or chloroform;
- Empty the flies from the trap into the plastic bag making sure that none escape;
- Once flies have been immobilised by the chemical transfer them to a paper bag, or other suitable container, recording the date, location and trap number. Seal the container;
- Despatch for identification as soon as possible, if there is any delay specimens should be stored under refrigerated conditions.



Fig. 32 Bishop trap (modified).

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b) The wind orientated trap (WOT)

In the America/Mexico situation, following considerable field investigation, the wind operated trap WOT is considered preferable to the Bishop trap for two main reasons. It is more selective in attraction to Screwworm, thus eliminating the collection of large numbers of other species, and is also more efficient. It relies, however, on a synthetic olfactory bait which although more convenient to handle than raw liver depends on the availability of a variety of chemical compounds for production.

The bait, commonly known as Swormlure 4 (SL-4) is more specific to screwworm than liver and less attractive to other species of blow fly. The chemical components of SL-4 are as follows:

Swormlure-4 ingredient	<pre>% (Volume)</pre>	Amount per	litre
SEC-BUTYL ALCOHOL	18.7	187	ml
ISO-BUTYL ALCOHOL	18.7	187	ml
DIMETHYL DISULPHIDE	18.7	187	ml
ACETIC ACID	18.7	187	ml
BUTYRIC ACID	6.2	62	ml
VALERIC ACID	6.2	62	ml
PHENOL	5.0	50	ml.
P-CRESOL	5.0	50	ml
BENZOIC ACID	1.2	12	g
INDOLE	1.2	12	ğ

To prepare, mix ingredients in a container that can be capped and efficiently sealed.

First weigh the solid compounds, Benzoic acid and Indole, into the container.

Then measure out and add the liquid compounds, in any order, but ensuring that <u>DIMETHYL</u> DISUSLPHIDE is added last.

Close the container after the addition of each chemical and shake lightly to mix.





Store under refrigerated conditions allowing the temperature of the mixture to rise to air temperature before use.

Maximum storage life is one month.

Most of the chemicals used, and the mixture itself, is caustic. Care must therefore be taken in handling and preparation. <u>Metal containers must not</u> be used and the eyes and skin must be protected. The strong and unpleasant odour permeates most materials including clothing and persists for weeks.

The Swormlure-4 attractant is placed in two uncapped glass bottles of 40 ml each at the front of the trap, Fig. 33. The bottles are fitted with a wick consisting of a wire mesh cylinder packed with an absorbent material, such as cotton wool, which is placed in the neck ensuring that the lower end is always in contact with the liquid contents. The trap orientates to the wind direction by means of the vanes. A funnel of fine mesh wire, with a 0.8 cm hole to allow the entrance of flies, is inserted into the wire cone at the end of the plastic bucket. Upwind flies are attracted by the odour plume, orientate towards the trap and eventually enter. Once captured they may survive for a few hours up to a few days, depending on climatic conditions. The efficiency of the trap is greatest during low humidity which increases the evaporation of the odour.

Traps should be inspected daily, repaired and replenished as required, and the flies removed. Preferably during the morning.

The technique for emptying the trap and recording captures is essentially the same as for the Bishop trap.

The trap should be cleaned as frequently as required using water only, soaps, detergents or other solvents should not be used.

Used wicks must be burned on replacement to avoid the possibility of competing odour plumes.

Traps should be sited in locations favourable for the presence of screwworm, as indicated by the incidence of myiasis. They should be suspended approximately 1.60 m above ground level and if possible protected from strong winds and heavy rains. They should be sited so that there are no obstructions in the vicinity that might interfere in the dispersion of the odour plume.

If no captures are recorded over four days traps should be relocated to new sites. Full details must be recorded of any captures made including date, exact location, number of captures (male and female) and if part of an SIT campaign, whether wild or sterile, temperature, rainfall and wind direction. Negative captures must also be recorded to assist in the determination of fly distribution density.

CHAPTER 5

ECONOMIC CONSIDERATIONS

5.1 ECONOMICS

In estimating the economic consequences of screwworm infestation in domestic livestock it is necessary to draw on the information available from endemic areas in the Americas.

Prior to eradication from the USA the costs to the livestock industry, though production losses, are quoted as exceeding US 140 million dollars per annum, based on the 1958 situation when the seasonal northward migration of the insect, during the summer months, distributed the disease over some 3 million km^2 .

In the Caribbean Islands the annual estimated costs of surveillance and medication ranged from US 4.82 dollars to US 10.71 dollars per animal. Nationally these losses amounted to 0.30 million dollars in Surinam, 1.02 million dollars in Trinidad and Tobago, 4.33 million dollars in Guyana and three million dollars in Jamaica. Except for Jamaica these estimates do not include productivity losses such as reduced weight gain, low milk production and hide injury which are all considerable. Neither is consideration given to wildlife where losses may be even greater due to the non-availability of treatment through human intervention.

In Australia the predicted annual economic losses, assuming the introduction and nation-wide establishment of C. bezziana, the Old World screwworm fly, based on estimated overwintering and summer ranges is calculated at 55 to 65 million dollars (US). These figures, assume that only wound treatment is carried out as required, and no action taken to reduce fly distribution.

In Papua New Guinea, a study, of 600 cattle recorded an annual rate of 82 percent C. bezziana

infestation whilst on an adjoining property some 30 percent of the newborn calf population died annually through infestation of the unhealed navel.

In Malaysia a group of 3 200 cattle recorded yearly strike rates, due to C. bezziana, in excess of 90 percent with 15 000 individual treatments being recorded annually, illustrating the multiple infestation being experienced by individual animals.

It is clear, from these examples, that the introduction of screwworm to countries where either livestock, wildlife or both play an important role in the economy would result in massive losses.

The costs of maintaining animals under continuous risk of infestation are estimated at US 5.50 dollars per animal per year. This figure is calculated on inspecting each animal two to three times per week, treating wounds with insecticide, to prevent attack, and to cure infestations. The cost for insecticide is calculated at US 1.50 dollars per animal/year and is included in the total above.

As mentioned previously in this manual, costs of prevention and treatment are recurrent for as long as the disease situation exists. It is, therefore, evident from the figures quoted above that even very high cost techniques would be justified for eradication providing the ultimate objective can be guaranteed over an acceptable period of time. In the American programme, which included Mexico, the application of the sterile insect technique achieved eradication at an estimated annual expenditure of US 50 million dollars.

When consideration is being given to the implementation of a large scale campaign it is necessary, for economic justification, to take into account the total fly distribution, whether confined to a single country or affecting several. If such an operation is not continued to natural limits then the danger of reinvasion will enforce relatively high annual expenditure on the maintenance of a protective barrier, the efficiency of which cannot be guaranteed. In such situations the extension of eradication to distribution limits could prove more economical even if considerable sections were not suitable for wildlife or livestock based economies but merely being treated to protect productive cleared areas.

Following the eradication of C. hominivorax from 90 percent of the Republic of Mexico in 1984, a study was conducted to quantify the benefits and the economic impact of screwworm eradication. The Annual benefits to Mexico were estimated at US 130 million, and showed a net profit return of between 2 to 4.5 dollars for each dollar invested. This benefit was mainly achieved through reduced production costs to livestock owners through decreases in the purchase of medicines and insecticides, reduction in labour inputs required for animal movement control, inspection and treatment, veterinary services and equipment. The greatest benefit, per head of livestock, was to swine producers, but, overall, cattle producers received the greatest financial benefit due to the large numbers of cattle involved.

The economic benefits and constraints, in any new outbreaks, will vary according to the local situation. The calculation of these should not only give consideration to the direct effects on local livestock production but, must also include effects on wildlife, sociological implications and the consequences of the spread of the disease to new potential limits of infestation. As in the examples given above, there is no doubt that economically the ultimate objective of eradication will invariably be strongly justified.

ACKNOWLEDGEMENTS

The following sources, for various figures shown in the text, are hereby acknowledged:

Fig. 15; Laake, E.W. et al (1936)

- Fig. 16, 17, 18, 20, 22, 23 and 25; Smith, K.G.V. (1986)
- Fig. 23; Zumpt, F. (1965) and
- Fig. 19; Ferrar, P. (1987)

This list of specialised textbooks and scientific papers has been included for those workers who desire a deeper knowledge of some aspects of screwworm biology and control which have been mentioned in the manual.

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Photo 1 <u>C. hominivorax</u>: adult flies showing broad frons between eyes in female (right) and narrow in male (left).



Photo 2 Surveillance using sentinel sheep; note deliberate wound on rear leg of animal nearest camera.



Photo 3 Typical myiasis infested wound in ear of animal.



Photo 4 Assembled wind oriented trap.

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