Module 11

Plague: Vectors

At the end of the module, the participant will be able to understand:

- Disease Transmission
- Plague Vectors
- Biology
- Life Cycle
- Vector Surveillance
- Entomological Indicators
- Adult Vector Control
- Larval Vector Control

Plague is an infectious disease caused by the bacteria *Yersinia pestis*, a zoonotic bacteria, usually found in small mammals and their fleas. It is transmitted between animals through fleas. Humans can be infected through: the bite of infected vector fleas, unprotected contact with infectious bodily fluids or contaminated materials, and the inhalation of respiratory droplets/small particles from a patient with pneumonic plague.

Plague is a very severe disease in people, particularly in its septicaemic (systemic infection caused by circulating bacteria in the bloodstream) and pneumonic forms, with a case-fatality ratio of 30% to 100% if left untreated. The pneumonic form is invariably fatal unless treated early. It is especially contagious and can trigger severe epidemics through person-to-person contact via droplets in the air.

From 2010 to 2015, there were 3248 cases reported worldwide, including 584 deaths. Plague epidemics have occurred in Africa, Asia, and South America; but since the 1990s, most human cases have occurred in Africa. The three most endemic countries are the Democratic Republic of Congo, Madagascar, and Peru. In Madagascar, cases of bubonic plague are reported nearly every year, during the epidemic season (between September and April). The 1994 plague in India was an outbreak of bubonic and pneumonic plague in south-central and southwestern India from 26 August to 18 October 1994. 693 suspected cases and 56 deaths were reported from the five affected Indian states as well as the Union Territory of New Delhi. These cases were from Maharashtra (488 cases), Gujarat (77 cases), Karnataka (46 cases), Uttar Pradesh (10 cases), Madhya Pradesh (4 cases) and New Delhi (68 cases). There are no reports of cases being imported to other countries.

Signs and Symptoms

There are three clinical types of plague:

- Bubonic plague: Swellings (buboes) filled with bacteria develop in the lymph nodes, especially in the armpits and groin. This form is normally transmitted to humans by infected fleas. If left untreated, it causes death in about 50% of cases.
- Pneumonic plague: This is a secondary form in which the lungs become affected. It is highly contagious, the plague bacillus easily spreading from person to person in sputum or droplets coughed up or sneezed by sick people. Pneumonic plague occurred in epidemics in past centuries, killing millions of people. If left untreated it very often results in death.
- Septicaemic plague: The bloodstream is invaded by the plague bacillus, resulting in death before one of the above two forms can develop.

Fleas

Fleas (order Siphonaptera) are small insects flattened from side to side, usually dark in colour, generally possessing many stiff bristles. Their legs are well developed and the hind legs are especially strong. Fleas are excellent jumpers and some species are able to jump 50 times their length. Flea eggs drop off the host and fall amongst debris on the ground. The active, immature stage is a maggot Iike, legless larvae (see Figures 1–15). The larvae do not live on the animal but in the nest of the host in the yard or wherever the host is found. In buildings with pets, flea larvae thrive particularly well in carpets where they feed on organic debris such as dried blood, cast skins, or excreta. Larvae undergo three moults during development, and the entire larval period is frequently as short as 2 to 3 weeks. The larva spins silken cocoons just prior to pupation and camouflages them with bits of debris. Here the pupae develop into adults during a resting period that varies in length from a few days to a year or more. Adults are normally long-lived and feed on a wide variety of animals including humans.

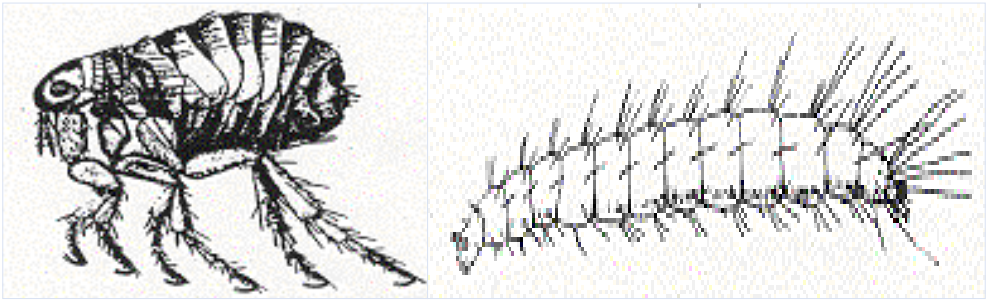


Figure 34.Adult Flea & Flea Larva

Fleas are active biters and proven vectors of human disease. The oriental rat flea, Xenopsylla cheopis, is the most important vector of bubonic plague and murine typhus. Other species of fleas associated with rodent hosts may also be vectors of plague in humans but are not considered as important as Xenopsylla cheopis. There are approximately 1,500 species of fleas. Besides being vectors of disease, fleas can be very annoying and their bite may produce extreme itching and dermatitis in sensitive individuals.

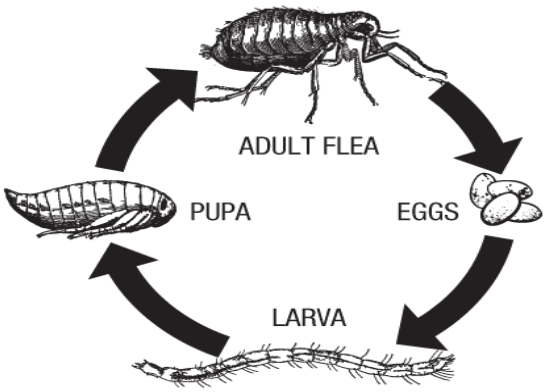


Figure 35.Life Cycle of Flea

There are four stages in the life cycle of a flea: egg, *larva*, pupa, and adult. Depending on the environmental temperature and humidity levels, the total life cycle will take anywhere from a couple of weeks to many months. Optimal conditions for fleas are between 70-85°F and 70% humidity. Fleas like to live in an environment that is warm, where they can live up to a year.

- Eggs are laid 24-36 hours after the first blood meal.
- Larvae have three stages and a life span of 5 to 12 days.
- Pupae: best protected and resistant life stage.

- Preemergent adults: The waiting stage, emergence of adults upon stimuli (pressure, heat)

Fleas

Fleas are normally associated with rodents in the wild and can be recovered from rodent burrows by swabbing. The burrow swab should be inserted into the rodent hole and then removed slowly while rotating the handle (Figure 4). Fleas in the rodent hole will be briefly trapped in the folds and fibres of the cloth. They can be removed with forceps and placed in a vial of alcohol for subsequent identification.

An easier method is to take many 4X4-inch squares of cloth to the field and place each piece of cloth positive for fleas in an individual ziplock bag for removal after the fleas have been refrigerated or frozen to incapacitate them.

Fleas also can be removed from the bodies of trapped rodents. However, this is a complex and potentially dangerous task and should not be attempted without employing stringent safety measures. This technique is addressed further in the section on rodent surveillance.

Figure 36.Proper Use of a Burrow Trap to Sample Fleas

Preservation and Mounting

General

The kinds of arthropods that may be mounted on slides are immature Diptera such as mosquito larvae and pupae; small adult Diptera such as Phlebotomines and Culicoides; and adult ectoparasites such as fleas, mites, and lice. Either temporary or permanent slide mounts can be made. For reference collections, permanent mounts are preferable; but for rapid survey work, temporary mounts are frequently prepared. Berlese's medium, Hoyer's medium, and methylcellulose are examples of temporary mounting media. Permanent mounting media include Canada balsam, euparal, clarite, piccolyte, and permount.

Temporary Slide Mounts

Thin, translucent arthropods too small to be studied without the aid of a microscope should be mounted on slides. Such specimens include small larvae, adult fleas, lice, mites, bedbugs, ticks, and male genitalia of mosquitoes. The temporary slide mount is easy and efficient. It is important that the arthropod be killed properly; most soft-bodied, immature arthropods are killed in hot water (65º C). Larvae should never be boiled since this procedure introduces air bubbles into the body of the larva and destroys delicate structures necessary for identification. Most arthropods should be killed and preserved in alcohol until ready for slide mounting (but see para 4-7a for mosquito larvae). When mounting such specimens as fleas, ticks, lice, and mites, it is necessary to decolour or dissolve nonchitinous tissue in order to observe internal or obscured structures. The decolouring procedure may be accomplished by mechanical or chemical means. Some mosquito larvae may be adequately studied without decolouring.

The Mechanical Procedure. This procedure can be accomplished by puncturing the body wall of the specimen with a fine needle. The membrane punctures should be made in the membranous areas between the segments. The body fluids

and contents are then “pumped out” by slight intermittent pressure with a brush or a small blunt probe. Unremoved fragments are especially annoying if the specimen is to be stained since specimens often stain intensely. The “pumping out” process is best accomplished in a shallow dish of water under a dissecting microscope. Care should be taken to avoid destruction of internal taxonomic structures used in identification.

The Chemical Procedure. This procedure is accomplished by soaking the specimen in a mild caustic solution. Either sodium hydroxide (NaOH) or potassium hydroxide (KOH) (1 KOH pellet per 5 ml water) can be used in concentrations of 5 to 10%. Bleaching agents such as sodium hypochlorite (bleach) may also be used. High concentrations of any clearing agent should be avoided as they will result in damage to the specimen. The specimens may be left in the solution for several hours at room temperature. Clearing may be accelerated by heating the solution. Care should be taken to preclude specimens from becoming too pale. In any case, observation should be clearing time will vary greatly from specimen to specimen. On occasion air bubbles may get into the specimen being treated; therefore, the specimen should be removed. This is best accomplished under a microscope in the same manner used for “pumping out” the body contents. Unless the caustic solution is neutralised, the tissues will continue to deteriorate after the specimens are mounted. Therefore, after the specimens have been sufficiently cleared or depigmented, the next step should be to transfer them to a small dish of distilled water that has been acidified with a drop or two of glacial acetic acid or 15% hydrochloric acid.

Mounting Procedures: Once the decolouring procedure has been done, the specimen is ready to be mounted. First of all, the centre of the slide should be determined. This is done by laying a blank glass slide on a sheet of paper and drawing an outline of the slide on the paper. Then the slide is removed and diagonal lines are drawn from corner to corner of the slide. Where these lines cross, is the exact centre of the slide. (See Figures 4–6 for an example of how to centre a glass slide). After these preliminary procedures are completed (that is, the decolouring procedure and the determination of the centre of the slide), a temporary mounting medium is chosen. There are three types in general use: Hoyer’s medium, Berlese’s medium, and methylcellulose. Of the three, Hoyer’s is preferred as it will last one to three years. Hoyer’s Medium can be prepared by adding the following ingredients as shown below:

Distilled Water - 50 ml
Gum Arabic (crystalline) - 30 gm
Chloral hydrate - 200 gm
Glycerine - 20 ml

The arthropod specimen is mounted in the following manner:

1. Place several drops of mounting medium on the centre of the slide.
2. Position the arthropod on the slide being sure to center it properly.
3. Add enough media to cover the specimen as well as the area that the glass slip will cover.
4. Paint one edge of the cover glass with medium. This allows increased surface cohesion between the medium on the cover glass and the slide.
5. Gently lower the coverslip on the slide. (If air bubbles occur in the medium, they may be removed by gently tapping the cover slip or by heating the slide over a bunsen burner. Be careful not to crush the specimen when tapping the glass).
6. Dry the slide in an incubator at 40º C for four days and air dry for an additional two weeks.
7. If the media shrinks, add more media around the coverslip edge, and it will be drawn under the glass. The cover glass must be ringed with shellac or fingernail polish to prevent drying and cracking of the medium.

Permanent Slide Mounts

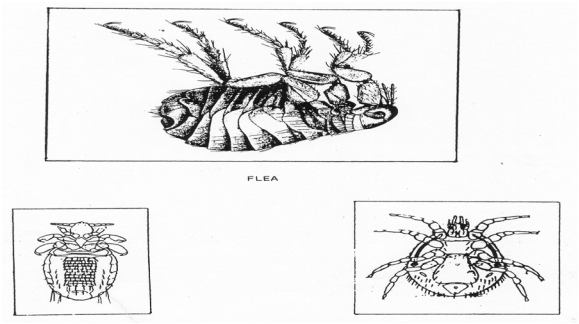
In making permanent slide mounts, the killing and decolouring procedures described for temporary mounts may be used. However, unlike temporary media, permanent mounting is not soluble in water; so, specimens must be dehydrated by placing them in ascending series of ethyl alcohol concentrations of 70%, 90%, and 95%. The choice of alcohol concentrations and the sequence of dehydration are dependent on the delicacy of the specimen. For example, specimens with soft-body walls will not collapse if processed gradually through the entire alcohol series in ascending order of concentration. Specimens with hard integuments can be dehydrated by placing them directly into high alcohol concentrations without causing any damage to the specimens. Following the dehydration series, the specimen should be rinsed in xylene that is miscible (capable of being mixed) in permanent mounting media.

Preliminary Procedures. Preliminary procedures for permanent mounts are the same as those described for temporary mounts. There are several permanent mounting media available. Canada balsam is probably the most widely used. After the desired media has been selected, the mounting procedures are conducted in the same manner as described earlier; however, permanent mounts should be dried at 50 ºC for two weeks.

Correct Mounting Positions. For easy examination, specimens mounted on slides must be properly oriented.

(1) Fleas are placed ventral side up with the head to the right. Lice, bedbugs, and mites are placed ventral side up, with the head pointing down, toward the mounter. If desired, two specimens of the same species may be mounted on one slide-one with dorsal side up, and the other with ventral side up. The correct mounting position for a flea, a louse, and a mite are shown below.

Louse Mite



Correct Mounting Positions for a Flea, a Louse, and a Mite

Mounting Mites in Methylcellulose

- Kill the specimen in 70% alcohol. Immerse the specimen in a solution of 10% lactic acid for 10 to 30 minutes depending upon the size of the specimen. As many as ten specimens can be handled at one time conveniently.
- Remove the specimen to the methylcellulose medium and heat, being careful not to boil. The heating procedure may be repeated a number of times until the desired clearing, relaxing, and straightening of the legs have been obtained.
- Transfer the specimen to a slide containing methylcellulose. Arrange the specimen ventral side up, and head down, and apply the cover slip. It is desirable that only one specimen be mounted per slide.

Rodents

Most rodent surveillance is accomplished to determine rodent presence and infestation levels in warehouses, dwellings and similar structures. Surveillance, in this case, is usually done by a visual survey for faeces, damage, rub marks and sightings of dead or live rodents, or sometimes with live traps, snap traps or glue boards. Commensal rodents usually do not cause problems in the field as they normally do at permanent installations, but other wild rodents may become nuisances or serve as reservoirs of disease. Rodents, as well as their ectoparasites, occasionally must be collected to determine the presence of known, or perhaps new, vector-reservoir systems.

Trapping Rodents

Snap traps can be used, but must be emptied almost immediately after the animal is captured. Snap traps work better if the triggers are “expanded” with hardware cloth, thin metal, etc. At sunrise or sunset, set 50 to 100 traps in a line in areas where rodents are active. Areas such as fence lines, along paths, where a wooded area meets a grassy area, etc., are ideal. Bait the traps with chewed oatmeal, peanut butter, or other useful bait, and place the traps five to ten feet apart as rapidly as possible. As soon as the last trap is set go back to the first trap and start picking up the traps. If rodents are caught, put each rodent and the trap that caught it in a separate ziplock bag to make sure parasites remain associated with their hosts. Speed is essential because fleas will leave a dead host as soon as its body temperature drops two or three degrees.

Live capture traps of several varieties are effective in trapping rodents for ectoparasite surveys. These do not kill the rodent so immediate pickup is not as essential. They may be set in the evening and collected the next morning.

If Sherman or similar solid-wall traps are used, they must be picked up very early in the morning or the sun will raise the temperature within the trap to levels lethal to the rodent, and ectoparasites will leave. As with snap traps, live capture traps with their contained rodent should be placed in individual ziplock bags so ectoparasites will not be separated from their hosts. When returned to the laboratory, rodents must be euthanised (if living) and their ectoparasites removed. If identification of rodents in the field is impossible or impractical, the rodent should be prepared so it can be identified by an authority on rodents.

When the rodent is dead, remove it from the trap and check the trap and killing chamber for ectoparasites, which will also be dead. Place any parasites found in a vial of alcohol. Then the rodents must be processed in one of two ways to remove their parasites. Use a nit comb or small, stiff-bristled brush to vigorously brush the rodent, against the grain of the hair, into a white enamel cake pan or similar container. The ectoparasites will be brushed out of the hair and into the cake pan. Remove them and store them in a vial of alcohol, along with any ectoparasites that were removed from the ziplock bag, trap and killing chamber.

a

b

c

Figure 37.Images of Different Types of Traps Used in Rodent Survey as A. Snap Trap, B. Sherman Traps, C. Live Trap

Cattle and Buffaloes are known to be among the principal hosts of tick vectors of KFD. Small mammals such as various species of rodents and insectivores including *Rattus rattus wroughtoni, Rattus rattus rufescens, Rattus blanfordi, Funambulus tristriatus, Ratufa indica, Petaurista philippensis, Suscus murinus* etc. are important host particularly for immature stages. Small mammals can be held in a wire cage mesh cage placed over a tray of water so that the fully engorged ticks drop into the water from where they can be picked up without damage. The engorged ticks can be held until they moult into the next instar. The later instars are easier to identify as compared with the earlier ones.

The density of tick infestation on a host species is evaluated in terms of tick index, which represents the average number of ticks per host individual examined and may be calculated by the following formula.

$$\text{Tick Index} = \frac{\text{Total Number of ticks collected}}{\text{Total number of hosts examined}}$$

Prevention and Control of Tick-borne and Mite-borne Diseases

Community Awareness

- Awareness should be provided among the community, especially in endemic areas regarding tick and mite-borne diseases. A variety of agencies can be contacted to obtain information or speakers for the training of medical, pest management, or other personnel. Additional educational methods include: making brochures, pamphlets and fact sheets available for in-processing personnel; publishing periodic notices in the installation newspaper or plan of the day, particularly warm months and the fall hunting season; and posting warning signs in tick-infested woods or other areas frequented by troops, hunters or hikers.

Personal Protection

- Proper clothing should be used to limit access of ticks to the skin, thereby helping to prevent bites.
- Pants should be tucked into the boots or socks, and the shirt should be tucked into the pants.
- Avoid sitting or lying on scrubby areas.
- Avoid hanging clothes on scrubs or trees.
- Disinfesting your pets regularly.
- Long sleeves will help, and a hat will be useful if crouching or crawling in bushes or undergrowth. Light-colored clothing should be used to make ticks much easier to detect.
- Repellents like Deet 33% (N,N-diethyl-m-toluamide or N,N-diethyl-3-methylbenzamide), and permethrin should be used to prevent tick bites. These repellents provide protection for up to 12 hours, depending on environmental conditions. It should be applied in a thin film over exposed skin surfaces, according to label directions.
- Permethrin should be applied to the lower pant legs, crotch, waistband, shirt sleeves, collar, front placket and lower edge of the shirt, never to the skin.
- Permethrin aerosol contains 0.5% permethrin and should be sprayed liberally, to the point of dampness, over the entire outside surface of the uniform.

Environmental Management

- Where acceptable, clearing of edge habitats by leaf litter removal, mechanical brush control, and mowing or burning vegetation are effective means of tick and mite control in residential areas and certain recreational areas.
- Removal of low-growing vegetation and brush eliminates the structural support that ticks need to contact hosts, thereby reducing the incidence of tick attachment.
- Removing leaf litter and underbrush also eliminates tick habitats and reduces the density of small mammal hosts, like deer mice and meadow voles.
- Mowing lawns and other grassy areas to less than 6 inches (16 cm) greatly reduces the potential for human-tick contact.
- When environmentally acceptable, controlled burning has been shown to reduce tick abundance for six months to one year.
- Another habitat modification technique is to thin early successional shrubs and grasses in early to mid-fall, stressing

- the overwintering tick population and reducing survivability. This should be done late enough in the season that regrowth does not occur.

Chemical Control

- The use of an acaricide, formulation (e.g., dust, granule, emulsifiable concentrate) is one of the primary considerations in selecting the type of equipment that will be used to apply an acaricide. This method is not feasible in controlling ticks.
- It is more efficient to allow livestock to collect ticks and then kill the parasites on the host, rather than to apply acaricides to pastures.

Flea Control

Dusting of Runways and Burrows: The most effective and rapid method of reducing flea population is the dusting of insecticides on the rodent runways and entrance of rodent burrows and residual spray in selected houses. Studies have shown that fleas are resistant to DDT. Malathion may be used for anti-flea measures.

Dust Formulation: 5% malathion powder (1 part of 25% wettable powder (wp) Malathion and 4 parts of chalk powder or inert material)

Residual Insecticidal Spray

Malathion 25% wp

Malathion suspension is applied @20 gm/sq.mt of active ingredient. To get 5% suspension, 2 kg of 25% of wp is mixed in 10 litres of water.

Deltamethrin 2.5% wp

The suspension is applied @35.0 mg/sqm t of active ingredient. To obtain 0.125% suspension, 500 gm of 2.5% deltamethrin is mixed in 10 litres of water. Two rounds of spray may be undertaken annually. It may be used only in case of an emergency.

Susceptibility Test for Insecticide Resistance

Limited studies have been carried out on the techniques to determine the susceptibility of ticks to insecticides. The existing methodology consists of tropical application of a known volume of insecticide in aqueous or alcoholic solution in a series of two-fold dilutions and to observe mortality after an incubation period of 24 hrs. Thus, LC 50 or LC 90 value can be determined. In the KFD area, it has been shown that species of the genus *Haemaphysalis* show higher susceptibility to BHC (now banned since 1997) as compared to other conventional acaricides.

In selecting a suitable laboratory test for acaricide resistance, the following requirements must be satisfied.

- The test should be sensitive enough to identify resistance early in its emergence. It should also cover the full range of chemical groups that are in use, including the most recently developed active ingredients.
- The diagnostic test should be simple and inexpensive.
- It should provide a rapid and reliable result, and be suitable for standardisation among laboratories in many countries.
- The most widely used *in vitro* tests are bioassays applied to larvae and engorged female ticks.
- None of them meet all of the above requirements, and improvement of protocols for diagnosis of acaricide resistance should be a continuing goal.

Resistant strains of ticks can be diagnosed without having internationally recognised standardised test protocols and reporting methods. However, to facilitate global monitoring and provide a basis for comparison of test results, standardised diagnostic methods should be adopted. In view of this and following the advice of experts since 1975, FAO has promoted the use of the Larval Packet Test (LPT) for field investigations of acaricide resistance.