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Training Module on Ticks, Mites, and Flea Borne Diseases for Medical Entomologists

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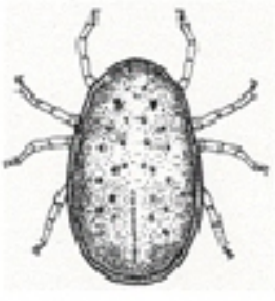
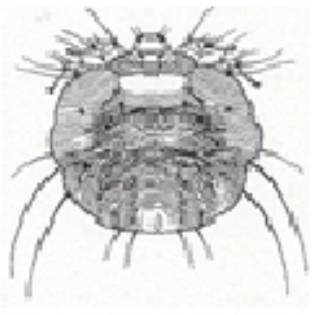


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Ticks-, Mites- & Flea-Borne Diseases

I. Introduction

Tick- and mite-borne diseases are significant global health concerns caused by various bacterial, viral, and protozoan pathogens. These diseases result in considerable illness and thousands of deaths worldwide each year. Ticks are the most versatile carriers of pathogens that infect humans and animals among arthropod vectors, second only to mosquitoes in their role in spreading diseases. Fleas also contribute as carriers of numerous pathogens, many of which remain poorly studied, except for *Yersinia pestis.* Flea-borne diseases have the potential to reappear as epidemics. This is evident in the evolving patterns of murine typhus, the detection of *Rickettsia* species in new hosts, and the occurrence of fleas in hosts or regions not previously documented. Additionally, the geographic range of these vectors and the pathogens they carry continue to change and expand. Key diseases transmitted by ticks, mites, and fleas include tick-borne relapsing fever, Lyme disease (LD), babesiosis, anaplasmosis, Boutonneuse fever, Q fever, Rocky Mountain spotted fever, Crimean–Congo haemorrhagic fever, Forest disease, Kyasanur Forest Disease, scrub typhus, and plague along with diseases caused by *Rickettsia rickettsii* (associated with Rocky Mountain spotted fever), and various other flaviviruses within the tick-borne encephalitis complex.

Tick-borne flaviviruses rank among the most medically significant arboviruses in Europe and Asia, with tick-borne encephalitis alone causing 10,000 to 15,000 cases annually across both regions. In the United States, Lyme disease is the most frequently reported tick-borne illness. In 2017, the Centers for Disease Control and Prevention (CDC) documented over 42,743 confirmed cases and 382,700 probable cases of the disease. Crimean–Congo haemorrhagic fever (CCHF), the most widespread tick-borne viral infection affecting humans, is endemic in numerous regions. With an estimated 3 billion people at risk, approximately 10,000 to 15,000 cases and 500 deaths are reported each year, primarily from endemic areas such as Africa, Asia, and parts of Eastern and Southern Europe.

A few human diseases are associated with pathogens transmitted by mites, with scrub typhus being the most significant. This illness, caused by the bacterium *Orientia tsutsugamushi*, is transmitted by several species of the genus *Leptotrombidium* (family Trombiculidae) found in Southeast Asia, Australia, and the Pacific Islands. Each year, over one million cases of scrub typhus are reported, placing more than one billion people at risk. Although scrub typhus has been a historically prominent concern, recent findings indicate that *Leptotrombidium* mites may also carry and potentially transmit other pathogens, including Hantavirus and *Bartonella* species such as *B. tamiae*, which causes bartonellosis in humans. Flea-borne infections are increasingly emerging or re-emerging globally, with a notable rise in their incidence. Plague outbreaks have historically affected Africa, Asia, and South America; however, since the 1990s, the majority of human cases have been concentrated in Africa. Between 2010 and 2015, a total of 3,248 cases were reported worldwide, resulting in 584 fatalities.

Rickettsial diseases are increasingly recognised in India as some of the most elusive emerging and re-emerging infections. Fatal tick-borne viral diseases in the country, such as CCHF and Kyasanur Forest Disease (KFD), are transmitted by *Hyalomma anatolicum* and *Haemaphysalis spinigera* ticks, respectively. KFD, first identified in 1957 in the Shimoga district of Karnataka, has re-emerged in recent years. Its range has expanded to seven districts within Karnataka and has further spread to the neighbouring states, including Kerala, Goa, and Maharashtra. CCHF, an emerging disease, was first reported in Gujarat in 2011. In recent years, cases have also been documented in Rajasthan and Uttar Pradesh. Other tick-borne diseases, including relapsing fever, Lyme disease, Ganjam virus disease, and Q fever, have been sporadically reported from various regions across India. However, Lyme disease has only been reported in a limited number of cases. Scrub typhus has been observed in areas such as Rajasthan, Jammu & Kashmir, Vellore, Sikkim, Darjeeling, Nagaland, and Manipur. Indian tick typhus infection is widespread in the hilly forest areas throughout India, with sero-epidemiological studies confirming its presence in regions like Nagpur, Jabalpur, Jammu and Kashmir, Kanpur, Sagar, Pune, Lucknow, and Bangalore. A significant outbreak of plague occurred in 1994, with cases of bubonic plague in Maharashtra and pneumonic plague in Gujarat, resulting in 876 cases and 54 deaths.

Currently, states and union territories report data on diseases such as malaria, dengue, chikungunya, filaria, Japanese Encephalitis, and Kala-azar at the national level under the National Health Mission (NHM). Sporadic cases of Zika, scrub typhus, and KFD have been reported from various parts of the country. However, there is no structured national programme for these diseases due to the absence of consistent surveillance efforts. With international movement from endemic regions, there is a risk of yellow fever and Zika entering the country. Regular surveillance for plague and CCHF is essential in areas at a higher risk.

2. Disease Profile

Scrub Typhus

Scrub typhus, also referred to as bush typhus, is an infection caused by the bacterium *Orientia tsutsugamushi.* The disease is transmitted to humans through the bites of infected chiggers, which are the larval form of mites.

Global Scenario

Scrub typhus is geographically distributed across an area of approximately 13 million km², encompassing regions from Afghanistan and Pakistan to the west, Russia to the north, Korea and Japan to the northeast, extending to Indonesia, Papua New Guinea, and northern Australia to the south, along with some smaller islands in the western Pacific. Recently, rickettsioses, including scrub typhus, have emerged along the Thai-Myanmar border. Additionally, there have been reports of the disease emerging in the Maldives and Micronesia.

Indian Scenario

In India, scrub typhus has been reported in various regions, including Rajasthan, Jammu & Kashmir, Vellore, Sikkim, Darjeeling, Nagaland, Manipur, Mizoram, Tamil Nadu, Maharashtra, Kerala, Himachal Pradesh, and Meghalaya.

Incubation Period : 1–3 weeks

Clinical Picture

A papule forms at the site of infection, which later ulcerates and develops into a black eschar as it heals. Common symptoms include a sudden high fever (> 40 °C or 104 °F) accompanied by relative bradycardia, severe headache, apathy, muscle pain (myalgia), generalised lymphadenopathy, photophobia, and a dry cough. About a week later, a rash appears, initially as spots, which then progresses to a maculopapular form. This rash first appears on the trunk and later spreads to the extremities, becoming blanchable within a few days.

Complications may include interstitial pneumonia (affecting 30–65% of cases), meningoencephalitis, and myocarditis. Typically, symptoms subside within two weeks, even without treatment. However, in severe cases with pneumonia and myocarditis, the mortality rate can rise to 30%.

Kyasanur Forest Disease

Kyasanur Forest Disease (KFD) is an illness caused by the Kyasanur Forest Disease Virus (KFDV), which belongs to the Flaviviridae family of viruses. The virus was first identified in 1957 when it was isolated from a sick monkey in the Kyasanur Forest of Karnataka (formerly Mysore) state, India. Since its discovery, approximately 400–500 human cases are reported annually, with a fatality rate ranging from 3% to 10%. The primary source of the virus is the hard tick *Haemaphysalis spinigera*, which remains infected for life once it carries the virus. Rodents, shrews, and monkeys frequently serve as hosts for KFDV after being bitten by an infected tick. The virus can also lead to widespread outbreaks, causing significant mortality in primates.

Transmission

Humans can contract KFD through a tick bite or contact with an infected animal, particularly a sick or recently deceased monkey. Person-to-person transmission has not been reported. While large animals such as goats, cows, and sheep can become infected with KFDV, they play a minimal role in the disease's transmission. These animals provide blood meals for ticks, and it is possible for infected animals with viraemia to pass the virus to other ticks. However, transmission of KFDV to humans from these larger animals is exceedingly rare. Additionally, there is no evidence suggesting that the disease can be transmitted through the unpasteurised milk of these animals.

Signs and Symptoms

Following an incubation period of 3 to 8 days, KFD manifests abruptly with symptoms such as chills, fever, and headache. Within 3 to 4 days of symptom onset, individuals may develop intense muscle pain, nausea accompanied by vomiting, digestive issues, and bleeding disorders. Some patients may also exhibit dangerously low blood pressure and reduced counts of platelets, red blood cells, and white blood cells. While a number of individuals recover fully within 1 to 2 weeks without any complications, the disease follows a biphasic course in 10–20% of cases. In these instances, a second

phase of symptoms begins during the third week, marked by recurrent fever and neurological problems, including severe headaches, altered mental states, tremors, and vision impairments. The fatality rate for KFD is estimated to be between 3% and 5%.

Risk of Exposure

KFD was traditionally confined to the western and central districts of Karnataka state, India. However, in November 2012, human and monkey samples from the southernmost district of Karnataka, bordering Tamil Nadu and Kerala states, tested positive for KFDV, suggesting a broader geographic spread of the virus. The disease has also been detected in Maharashtra, Goa, and Gujarat.

Individuals engaged in outdoor or rural activities, such as hunters, herders, forest workers, and farmers, are at risk of infection through contact with infected ticks. Additionally, the disease exhibits seasonal patterns, with most cases occurring during the dry months from November to June.

Crimean–Congo Haemorrhagic Fever

Crimean–Congo haemorrhagic fever (CCHF) arises from an infection caused by a tick-borne virus known as Nairovirus, which belongs to the Bunyaviridae family. The illness was first identified in Crimea in 1944, where it was named Crimean haemorrhagic fever. In 1969, the same virus was linked to an outbreak in the Congo, leading to the adoption of its current name. The disease has a wide geographic distribution, occurring in regions such as Eastern Europe (notably in the former Soviet Union), the Mediterranean, northwestern China, central Asia, southern Europe, Africa, the Middle East, and the Indian subcontinent.

Cases of CCHF reported in India between 2011 and 2019 are as follows:

January 2011: Nosocomial (infections caught in hospitals) outbreak in Gujarat, Ahmedabad

2012–2015: Several outbreaks and cases of CCHF were reported in the states of Gujarat and Rajasthan. Cases were documented from 6 districts of Gujarat (Ahmedabad, Amreli, Patan, Surendranagar, Kutch and Aravalli), 3 districts of Rajasthan (Sirohi, Jodhpur and Jaisalmer), and the state of Uttar Pradesh.

2019: Rajasthan (3 cases) and Gujarat (17 cases) (Bhavnagar, Botad, Amreli, Kheda, Jamnagar, Rajkot, Surendranagar, Morbi, and Jodhpur)

Transmission

Ixodid (hard) ticks, particularly those from the *Hyalomma* genus, act as both reservoirs and vectors for the CCHF virus. Various wild and domesticated animals, including cattle, goats, sheep, and hares, function as amplifying hosts for the virus. Humans can become infected through contact with blood from these animals or bites from infected ticks. The virus can also spread among people through exposure to infected blood or bodily fluids. Additionally, outbreaks of CCHF have been documented in healthcare settings due to inadequate sterilisation of medical instruments, reuse of needles, and contamination of medical supplies.

Signs and Symptoms

CCHF begins abruptly, presenting with symptoms such as severe headache, high fever, backache, joint pain, abdominal discomfort, and vomiting. Common visible signs include redness in the eyes, a flushed face, a reddened throat, and petechiae (small red spots) on the palate. Additional symptoms may include jaundice and, in severe cases, altered mood or sensory perception. As the disease advances, extensive bruising, heavy nosebleeds, and uncontrolled bleeding at injection sites typically develop around the fourth day and can persist for up to two weeks. Fatality rates among hospitalised patients during outbreaks have ranged from 9% to 50%. While long-term effects in survivors are not well understood due to limited research, recovery from the illness tends to be slow.

Risk of Exposure

Animal herders, livestock handlers, and slaughterhouse workers in regions where CCHF is endemic face a heightened risk of infection. Healthcare workers in these areas are also at risk, particularly when they come into contact with infectious blood or bodily fluids without proper protective measures. Additionally, individuals and international travellers who have direct exposure to livestock in endemic regions may be vulnerable to the virus.

Plague

Plague is a zoonotic disease caused by the bacterium *Yersinia pestis*, which is commonly found in small mammals and their fleas. Transmission occurs among animals via fleas, while humans can become infected in several ways: bites from infected fleas, direct contact with infectious bodily fluids or contaminated materials, or inhaling respiratory droplets from individuals with pneumonic plague.

Plague is highly dangerous to humans, particularly in its septicaemic form, where bacteria spread through the bloodstream, and its pneumonic form, which affects the lungs. If untreated, the case-fatality rate ranges from 30% to 100%. The pneumonic form is invariably fatal without prompt treatment and is highly contagious, capable of causing severe epidemics through the spread of airborne droplets during person-to-person contact.

Between 2010 and 2015, there were 3,248 reported cases of plague globally, resulting in 584 deaths. Plague epidemics have been recorded in Africa, Asia, and South America, though since the 1990s, the majority of human cases have occurred in Africa. The Democratic Republic of Congo, Madagascar, and Peru are the three most endemic countries. In Madagascar, cases of bubonic plague are reported nearly every year during the epidemic season, which lasts from September to April. The 1994 plague outbreak in India involved both bubonic and pneumonic forms, occurring from August 26 to October 18, 1994, in the south-central and southwestern regions. A total of 693 suspected cases and 56 deaths were reported across five Indian states and the Union Territory of New Delhi. The affected states were Maharashtra (488 cases), Gujarat (77 cases), Karnataka (46 cases), Uttar Pradesh (10 cases), Madhya Pradesh (4 cases), and New Delhi (68 cases). No cases were reported as imported from other countries.

Signs and Symptoms

Plague manifests in three clinical forms:

- **Bubonic plague:** This form is characterised by the development of swollen, painful lymph nodes, or buboes, particularly in the armpits and groin. It is typically transmitted to humans via infected fleas. If untreated, the disease can lead to death in approximately 50% of cases.
- **Pneumonic plague:** This form occurs when the lungs are affected, often as a secondary complication. It is highly contagious, with the plague bacteria easily spreading through respiratory droplets or sputum from coughing or sneezing. Pneumonic plague has caused major epidemics in history, killing millions. If untreated, it often leads to death.
- **Septicaemic plague:** In this form, the plague bacteria invade the bloodstream, often leading to death before either of the other two forms can develop.

3. Arachnida

Phylum - Arthropoda

Class - Arachnida

Order - Acarina

Super Family - Ixodoidea

Family - Ixodidae, genus - *Ixodes, Haemaphysalis, Amblyomma, Hyalomma, Dermacentor*, *Rhipicephalus* and *Nosomma* (7 genera) (Hard ticks – jungle ticks)

Family - Argasidae, genus - *Ornithodorus, Argas* and *Otobius* (3 genera) (Soft ticks – domestic ticks)

Both ticks and mites are members of the Arachnida class of organisms, which includes spiders and scorpions. Before we get into the differences between ticks and mites, let's look at what they have in common.

Commonalities

In their larval stage, both ticks and mites have six legs, but as they progress to the nymph and adult stages, they develop two additional legs, giving them eight legs in total. Unlike some other arthropods, ticks and mites do not have distinct heads and bodies; instead, their bodies are a single mass. The part of their body that contains the feeding structures is called the capitulum (Figure 1).

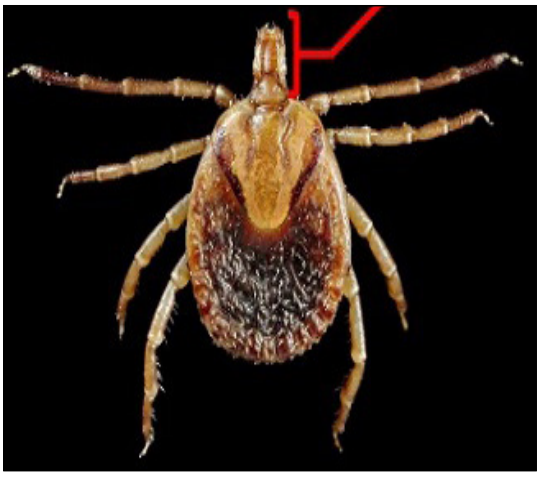
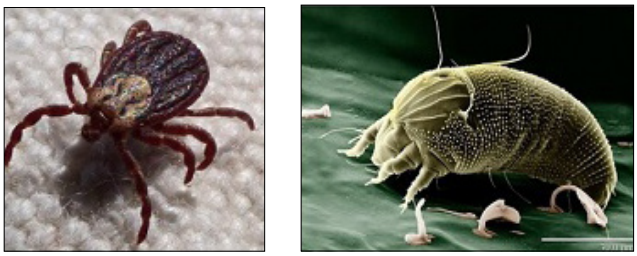


Figure 1.Capitulum of a Tick

Differences

Size

The most noticeable difference between ticks and mites is their size. Ticks are visible to the naked eye and typically measure around 1 millimetre in length, though they can grow up to 3 centimetres after feeding (Figure 2). In contrast, mites are microscopic and usually smaller than a millimetre, making them difficult to see without a microscope.



(a) Figure 2(a).Tick (b).Mite (b)

Body Hair and Hypostome

Ticks have short or no hair on their bodies, whereas mites typically have longer hair covering their bodies. Both ticks and mites possess a structure called the hypostome on their capitulum, which allows them to attach to their host. In ticks, the hypostome is visible and features backwards-facing barbs that help anchor the tick firmly to the host (Figure 3). This adaptation makes it challenging to remove a tick during feeding, often resulting in the separation of the capitulum from the rest of the tick's body.

The hypostome of mites is not visible externally and lacks barbs, which means that mites can be more easily removed from their hosts compared to ticks.

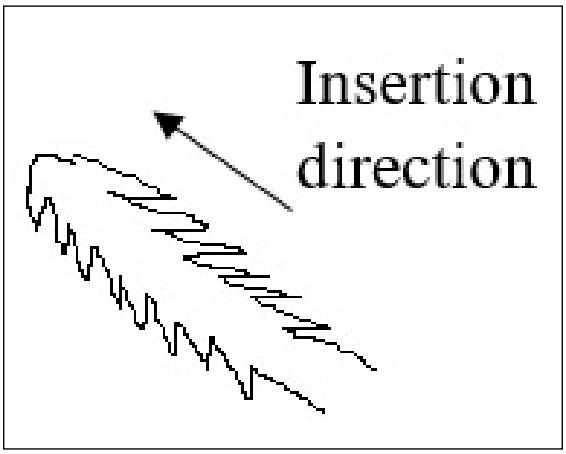


Figure 3.Barbed Hypostome of a Tick

4. Ticks

Ticks are found worldwide, in both tropical and temperate regions, and are classified into two main groups: the soft ticks of the family Argasidae (comprising about 100 species) and the hard ticks of the family Ixodidae (including around 700 species). Hard ticks are known to transmit various diseases, such as tularemia, Rocky Mountain spotted fever, Lyme disease, Q fever, and tick-borne encephalitis. They can also cause tick paralysis through direct injury. On the other hand, soft ticks can transmit spirochetes responsible for tick-borne relapsing fever. These spirochetes are acquired by ticks when they ingest the blood of infected animals. The spirochetes multiply within the tick's body, invading its tissues and body cavity. Notably, the spirochetes can be passed on to the tick's offspring through transovarian transmission, even reaching the third generation.

a. Hard Ticks (Family Ixodidae)

Globally, the family Ixodidae is represented by 13 genera and 650 species, of which 7 genera and 88 species are recorded in India.

The mouthparts of hard ticks are visible from above, and the back of the tick is covered by a shield (Figure 4). Hard ticks undergo a type of gradual metamorphosis, with four life stages: egg, larva (which is not worm-like), nymph, and adult (Figure 5). The entire life cycle can take anywhere from 6 weeks to 2 years to complete. All stages, except for the egg, feed on the blood of vertebrates, mainly mammals. During feeding, the female tick becomes greatly enlarged, a process that usually takes about 5–10 days. Copulation occurs while the female is feeding on the host. After mating, the female continues to feed, then drops to the ground, finds a sheltered spot, and lays a gelatinous mass of eggs, often numbering in thousands. This egg-laying process may take several days, after which the female dies. Under favourable conditions, the eggs of hard ticks typically hatch in about a month, though in colder weather, hatching may be delayed for several months. A few days after hatching, the six-legged larvae, also known as "seed ticks", climb plants or walk along the ground in search of a suitable host, such as a small mammal. Once they find a host, they feed on its blood, then drop to the ground to molt into the nymph stage. The nymph then seeks out another host to feed on, drops to the ground after feeding, and molts into an adult. The adult then continues the cycle by repeating the process.

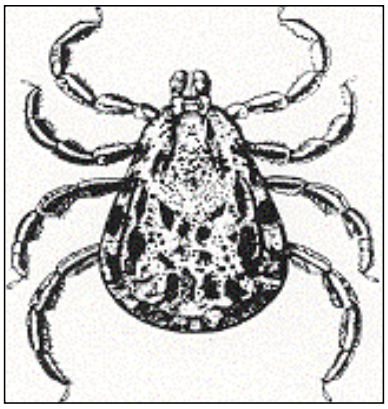


Figure 4.Adult Hard Tick

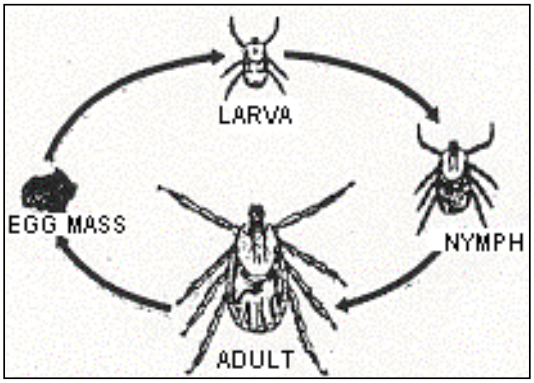


Figure 5.Life Cycle of a Tick (Note: The larval stage has six legs.)

b. Soft Ticks (Family Argasidae)

Soft ticks are typically round or oval in shape and do not have a shield on their back. Their skin is leathery, wrinkled, and tough. In adult soft ticks, the mouthparts are not visible from above (Figure 6). Their life cycle is similar to that of hard ticks, though soft ticks may have two or more larval and nymphal stages. They are secretive feeders, typically feeding at night and hiding during the day in crevices or cracks near their host's nest or roost. The female soft tick alternates between feeding and laying eggs over an extended period. As a result, a single soft tick may feed on multiple hosts throughout its life, which increases its potential to transmit diseases. While many soft ticks feed on birds and reptiles, others prefer mammals as their hosts.

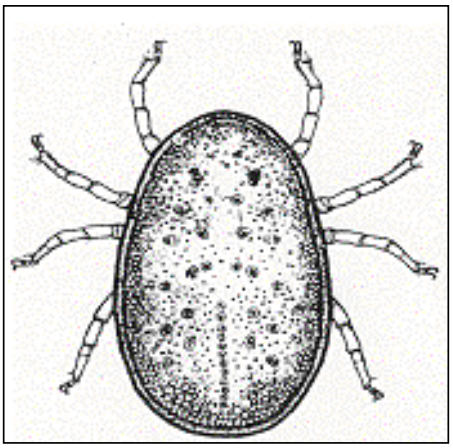


Figure 6.Soft Tick

Transmitted Diseases

Bovine piroplasmosis is a disease caused by protozoa of the *Babesia* species. Horse tick fever is caused by two species of *Babesia*. Theileriasis is a disease affecting goats and sheep, caused by the protozoan *Theileria parva*. *Anaplasmosis* is another protozoan disease, caused by *Anaplasma marginale*. Indian tick typhus is a rickettsial disease caused by *Rickettsia conorii*, transmitted by ixodid ticks. Lyme disease is also transmitted by ticks. Additionally, KFD is a viral disease transmitted by ticks.

Figure 7.Soft Tick

The clinical symptoms of the disease include fever, headache, and malaise, lasting for 12–14 days, with a low mortality rate. A maculopapular rash typically appears between the second and fifth day of fever.

Ticks go through four stages in their life cycle: egg, larva, nymph, and adult. Ixodid ticks have three hosts and typically require at least a year to complete their life cycle. Argasid ticks can have up to seven nymphal stages (instars), with each stage requiring a blood meal. Due to their blood-feeding behaviour, ticks are vectors for at least twelve diseases that affect both humans and animals.

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The following ixodid ticks have been reported to be vectors of tick typhus:

1. *Rhipicephalus sanguineus*
2. *Ixodes ricinus*
3. *Haemaphysalis indica*
4. *H. kinneari*
5. *H. turturis*
6. *H. spinigera*

Rodents and dogs serve as hosts for potential tick vectors and play a key role in maintaining the reservoir of the disease in nature. Among the various tick species, *Rhipicephalus* sanguineus (commonly known as the brown dog tick) is the most widespread in India.

$$\text{Prevalence rate of } \textit{Hyalomma} \text{ spp.} = \frac{\text{Tick species collected}}{\text{Total number of ticks collected}} \times 100$$

$$\text{Infestation rate} = \frac{\text{Total positive livestock for ticks}}{\text{Total livestock surveyed/ investigated}} \times 100$$

$$\text{Tick index} = \frac{\text{Number of ticks collected}}{\text{Total number of hosts examined}} \times 100$$

5. Mites

Mites are tiny creatures, with most species being nearly invisible to the naked eye. They are found globally, with over 29,000 species identified, though only a small number affect humans. Mites are medically significant because some species transmit scrub typhus in regions like the southwest Pacific and the Orient. When they attack humans, different mite species can cause conditions ranging from mild irritation to more severe forms of dermatitis.

Human Itch Mite *Sarcoptes scabiei*

The mite responsible for scabies (Figure 8), also known as the "seven-year itch," has a life cycle that includes the egg, larva, nymph, and adult stages, with the female undergoing two nymphal stages. The mature female burrows into the skin, particularly in areas like the hands, wrists, and other body parts with skin folds or creases. As she burrows, she lays about two eggs per day, continuing this process for 4 to 5 weeks, with a total of 40 to 50 eggs. The eggs typically hatch within 3 to 4 days, and the larvae leave the maternal burrow to create new burrows nearby. After about 3 days, the larvae molt into nymphs in these burrows. The complete development for females takes about 14 to 17 days. Once mature, mating occurs either in the burrows of virgin females or on the skin's surface, after which the fertilised female repeats the burrowing and egg-laying cycle.

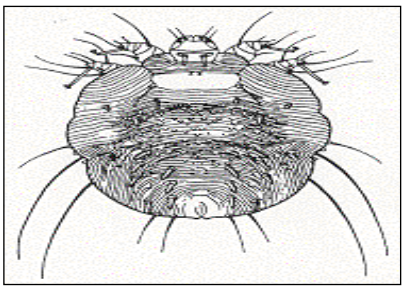


Figure 8.Adult Human Itch Mite

Chiggers (Trombiculid Mites)

The common chigger, also called the "red bug", is known for its bite, which causes intense itching, and scratching the bites can lead to infection. In the Asiatic-Pacific regions, some species of this mite act as vectors for scrub typhus. The life cycle of the trombiculid mite is complex. Female mites lay eggs one at a time in the soil. After about two weeks, the six-legged larvae hatch and search for a host, crawling through nearby vegetation or along the ground. These larvae feed on a wide range of vertebrate hosts, including reptiles, rodents, large mammals, and humans. They feed by attaching to the surface of the host's skin, typically taking 1 to 3 days or more to become engorged. Once fully fed, the larvae detach, burrow into the soil, and molt into eight-legged nymphs.

Mites are tiny parasites that infect various animals, including humans, horses, goats, sheep, cattle, and dogs. They are usually about 0.1 mm in size, oval-shaped, and pale to greyish in colour. Adult mites have eight stubby legs, while larvae have six. They lack eyes, and their mouthparts are adapted for piercing and sucking. Some mite species burrow into the skin, creating galleries or wounds that cause intense itching. Over time, fluid-filled pimples form and become scabbed over, containing large numbers of mites, which are the primary source of infection through contact. Infected hosts may also experience hair loss.

Mites are typically very small, ranging from 0.5 to 2.0 mm in length, with thousands of species, many of which live on animals. Like ticks, they have eight legs and a body with little or no segmentation. Mites undergo an egg, larval, nymphal, and adult life cycle, with immature stages resembling the adults but in smaller forms.

Distribution

Mites are distributed in a patchy manner over small areas due to their specific environmental needs. Nymphs and adults require particular soil conditions to survive and develop, while the larvae depend on host animals such as wild rats, small rodents, and birds. Suitable habitats for mites include grassy fields, shrubby regions, forests, abandoned rice fields, and cleared forests. They are also found in parks, gardens, lawns, and moist areas near lakes and streams.

The larvae typically wait on leaves or dry grass stems for an animal or a human to pass by. Humans often become infested by walking or standing in mite-infested areas. In tropical and subtropical regions, bamboo bushes are a preferred habitat for mites.

6. Fleas

Fleas (order Siphonaptera) are small, laterally flattened insects that are typically dark in colour and often covered with stiff bristles (Figure 9). Their legs are well-developed, with the hind legs being particularly strong, allowing them to jump remarkably high. Some species can leap up to 50 times their body length. Flea eggs fall off the host and land in the surrounding debris on the ground. The immature, active stage of the flea is a maggot-like, legless larva (Figure 10). These larvae do not live on the host but instead reside in the host's nest. In households with pets, flea larvae thrive on carpets, feeding on organic material such as dried blood, cast-off skin, and excrement. During their development, larvae undergo three molts, and the entire larval phase often lasts just 2 to 3 weeks. Before pupating, the larvae spin silk cocoons, which they camouflage with bits of debris. The pupae develop into adults during a resting phase, which can last anywhere from a few days to over a year. Adult fleas are long-lived and can feed on a wide range of animals, including humans.

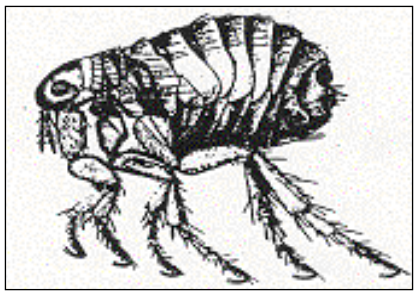


Figure 9.Adult Flea

Figure 10.Flea Larva

Fleas are active biters and are known to be important vectors of human diseases. The Oriental rat flea *(Xenopsylla cheopis*) is the primary vector responsible for transmitting bubonic plague and murine typhus. Other flea species, particularly those associated with rodent hosts, may also carry and transmit plague to humans, but they are not considered as significant as *Xenopsylla cheopis*. There are approximately 1,500 known species of fleas. In addition to their role as disease vectors, fleas are a nuisance to humans and animals, with their bites often causing severe itching and dermatitis in individuals who are sensitive to them.

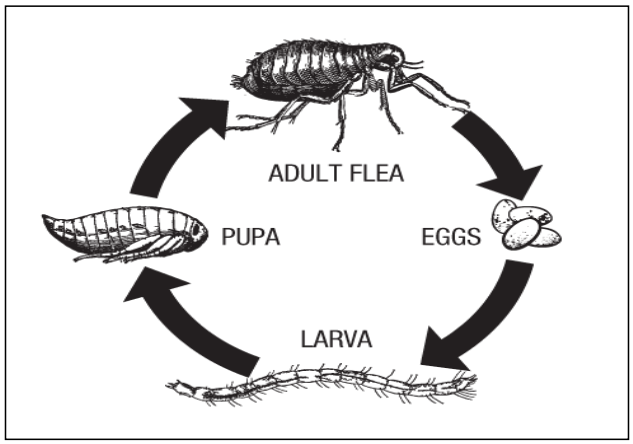


Figure 11.Life Cycle of A Flea

The flea life cycle consists of four stages: egg, larva, pupa, and adult (Figure 11). The duration of the complete life cycle varies, ranging from a few weeks to several months, depending on environmental factors like temperature and humidity. Fleas thrive in warm environments, with optimal conditions being between 70 and 85 °F and 70% humidity. Under these conditions, fleas can live for up to a year.

- Eggs are laid 24–36 hours after the first blood meal.
- Larvae have three stages, with a life span of 5 to 12 days.
- Pupae is the best protected and resistant life stage.
- Preemergent adult is the waiting stage, with the emergence of adults upon stimuli (pressure, heat).

7. Vector Surveillance

Vector surveillance is essential for identifying actual or potential breeding populations of vectors and pests, enabling the development of effective strategies for their control or eradication. This process involves several activities, such as operating light traps, locating and mapping breeding sites, conducting landing and biting counts, estimating population sizes, performing sanitary inspections, and collecting specimens from resting areas. These efforts provide valuable data to inform decisions on vector management and disease prevention.

Requirement of Equipment and Tools

Most of the equipment needed for an arthropod survey is standard and needs to be available at the time of surveys. The following list is considered as a minimum for most vector surveys:

- Mosquito light trap
- Flytrap
- Animal cage trap
- Roach sticky trap
- Black plates (10”X10”), one dozen
- Cloth tick drag or clothing lint roller (adhesive)
- White drop cloth or bed sheet
- Insect sweep net
- Entomological dipper
- Insect aspirator (battery-operated)
- Shallow pan
- Flashlight with optional coloured lenses
- Hard rubber comb
- Forceps
- Pocket knife
- Pipet medicine dropper
- Small artist’s camel’s hair brush
- Blood syringe or kitchen baster
- Laboratory dissecting needle
- Folding magnifier
- Hand-held counter
- Chloroform killing tube
- Chloroform or ethyl acetate, one pint
- Alcohol, 70%, one pint
- Assorted pillboxes
- Assorted vials
- Plastic storage bags
- Notebook and pencil
- Insect repellent
- Aerosol pyrethrin insecticide
- Gloves
- Cotton
- Facial tissues
- Masking tape or adhesive tape
- Rubber tubing, 3/8”

Tick Surveys

a. Tick surveys are essential for determining several key factors, including the species of ticks present in an area, the boundaries of infested regions, the need for control measures, and the effectiveness of existing control efforts. Ticks are typically found in brushy, wooded areas where wild or domestic animals serve as hosts for feeding. These areas often include training and manoeuvre sites.

b. Conducting a tick survey is not a simple task and requires practice and experience to refine techniques. One of the most effective methods used in tick surveys is the "tick drag." This involves a one-yard square piece of white flannel cloth, which is reinforced at both ends by rods or sticks. A string is attached to each end of one rod, allowing the cloth to be dragged behind the surveyor. There are three main methods for using the tick drag to collect ticks.

1. One method for conducting a tick survey is to pull the drag over a predetermined distance, typically 50 yards or 50 steps. After reaching the set distance, stop and collect any ticks that have attached themselves to the cloth. Ticks can be gathered using forceps or by rolling the cloth with a disposable lint roller.
2. Another technique involves placing the drag on the ground and sitting on it for 5 to 10 minutes. Afterwards, the ticks that have crawled onto the cloth can be collected. It is important to thoroughly examine clothing for ticks and roll it with a lint roller to remove them. Additionally, when removing clothing, inspect the body for ticks, especially on the legs and the nape of the neck. Ticks attached to the skin should be carefully removed using forceps, grasping the tick as close to the skin as possible and applying slow, steady traction to detach the mouthparts from the bite wound.
3. A third method involves placing the drag on the ground and positioning a block of dry ice (2 to 3 inches on each side) on an inverted, disposable pie tin at the centre of the drag. The ticks can be collected as they crawl across the cloth or near the dry ice. It is important to wrap the dry ice in paper to prevent damage to the tick specimens.

Mites

Mites can be surveyed using the "black plate" method, which involves placing dark-coloured objects, such as approximately 12-inch square construction paper, paper plates, or rigid plastic plates, on the ground in mite habitats (Figure 12). These habitats typically include grassy or brushy areas with high rodent populations, as mites are primarily rodent parasites. When not feeding on rodents, the mites crawl around these areas. The plates should be placed directly on the ground or ground cover. While the plates do not attract mites specifically, the dark surface provides a clear contrast, allowing mites to be seen as they crawl randomly on the plates.

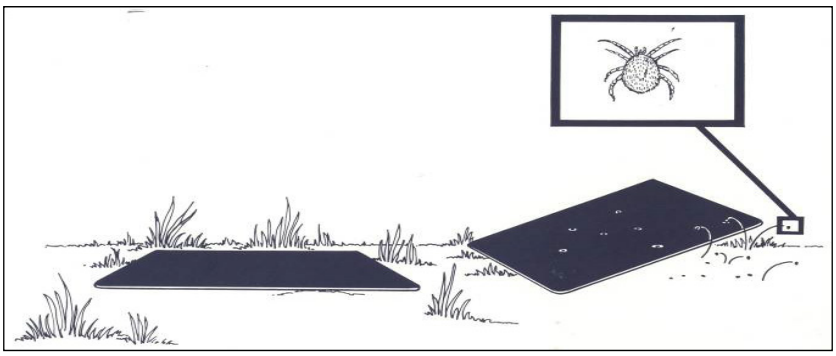


Figure 12.An Illustration of the Black Plate Method of Sampling Mites

The "black plate" method for surveying mites involves deploying the plates for no more than one hour. Once retrieved, both sides of the plates should be examined for small, rapidly moving white, yellow, orange, or red spots that are typically smaller than a pinhead. A hand lens or magnifying glass can be helpful in observing these mites. They can be removed using a small camelhair brush (or a similar type of brush) and placed in alcohol for later identification.

If the plates are made of a rigid material, mites can be removed by wetting a small paintbrush in alcohol, touching it to the mite, and then dipping the brush into a vial of alcohol. The mite will float free. If the plates are flexible, they can be rolled into a cone, with the small end placed over the vial. A sharp tap will cause the mites to fall into the vial.

Another effective method for sampling chiggers is to trap the rodent hosts that carry them and examine these animals for the presence of mites. Chiggers, which are typically yellowish or orange, tend to be concentrated in the ears or groin area of their hosts.

The tick index is used to evaluate the density of tick chiggers on a host species. This index represents the average number of ticks per host and can be calculated using the following formula:

$$\text{Chigger Index} = \frac{\text{Number of chigger (larval) mites collected}}{\text{Total number of rodents examined}}$$

The chigger index is relevant for the analysis of the presence of vector trombiculid mite i.e. *Leptotrombidium delicense* gp.

$$\text{Chigger Infestation Rate (CIR)} = \frac{\text{Number of mites collected}}{\text{Number of rodents found positive}}$$

Critical Chigger Index = 0.69 for *Suncus murinus*/month and 0.68 for *Rattus rattus*/month

An index above this level is a signal for an outbreak/ epidemic in a locality. Regular sero-surveillance should be carried out for the detection and isolation of *Rickettsia* antibodies. Susceptibility tests against vector mites should also be done regularly.

Fleas

Fleas are typically found in association with rodents in the wild, and they can be collected from rodent burrows using a swabbing technique. To do this, a burrow swab is inserted into the rodent hole and slowly removed while rotating the handle. Fleas in the rodent burrow will briefly become trapped in the folds and fibres of the cloth, where they can be removed with forceps and placed in a vial of alcohol for identification.

An alternative method involves using multiple 4x4-inch cloth squares in the field. Each cloth square that is positive for fleas is then placed in an individual ziplock bag. After the fleas are refrigerated or frozen to incapacitate them, they can be removed for further examination.

Fleas can also be collected from the bodies of trapped rodents. However, this process is more complex and carries potential risks, requiring careful safety precautions. Further details on this technique can be found in the rodent surveillance section.

Figure 13.Proper Use of a Burrow Trap to Sample Fleas

Flea Indices

Flea indices are important indicators of plague outbreaks. A few widely used flea indices are:

$$\text{Absolute Flea Index} = \frac{\text{Total number of fleas}}{\text{Total number of rodents}}$$

$$\text{Specific Flea Index} = \frac{\text{Number of flea species}}{\text{Number of rodents}}$$

Critical Index: Equal to or more than 1

Rodents

Rodent surveillance is primarily conducted to identify the presence and infestation levels of rodents in locations such as warehouses, residential areas, and similar structures. This typically involves visual inspections for signs like droppings, damage, rub marks, and sightings of live or dead rodents. Additionally, traps, such as live traps, snap traps, or glue boards, may be employed.

In the field, commensal rodents generally do not cause the same issues as they do in permanent installations. However, wild rodents can become problematic, either as nuisances or as reservoirs of disease. Occasionally, both rodents and their ectoparasites must be collected to assess the presence of known or potentially new vector-reservoir systems.

Trapping Rodents

Snap traps are effective for rodent capture but require prompt attention for the removal of the captured animal. Their efficiency improves if the triggers are modified or "expanded" with materials like hardware cloth or thin metal. For optimal results, 50 to 100 traps should be set in areas with noticeable rodent activity, such as along fence lines, pathways, or where wooded areas transition to grassy zones. This is best done at sunrise or sunset.

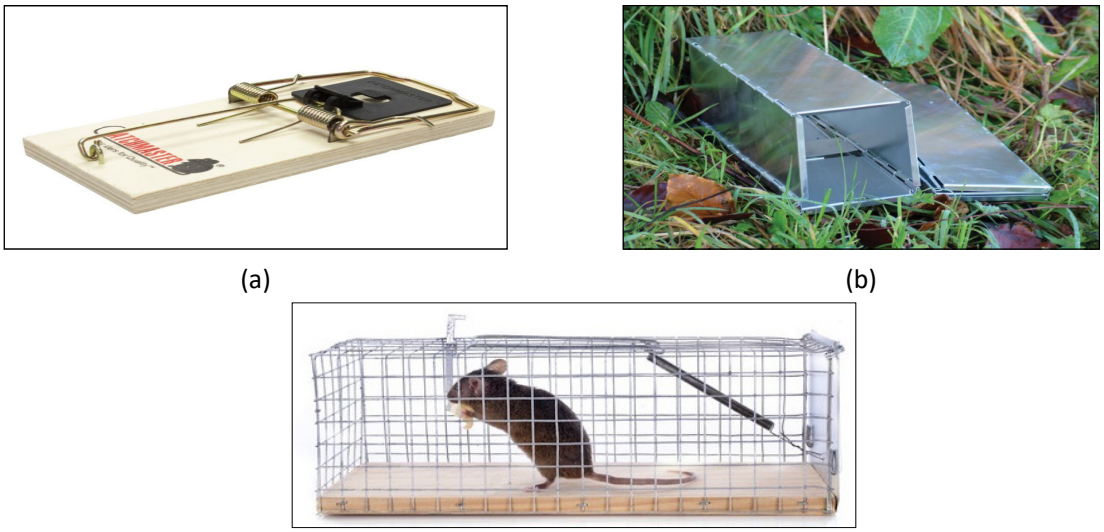
The traps should be baited with appealing options like chewed oatmeal or peanut butter and must be set quickly with a spacing of about five to ten feet between each trap. Once the last trap is placed, the first trap should be visited immediately to begin collecting them. If rodents are captured, each rodent must be placed, along with its respective trap, in a separate ziplock bag to ensure that the parasites remain associated with their host. Swift handling is critical, as fleas tend to leave a dead host once its body temperature drops by two to three degrees.

Live capture traps, available in various designs, are effective tools for capturing rodents for ectoparasite surveys. These traps keep the rodents alive, reducing the urgency for immediate collection. Traps can be set in the evening and retrieved the following morning.

If using Sherman or similar solid-wall traps, it is crucial to collect them early in the morning. Prolonged exposure to sunlight can cause the trap's interior temperature to rise to levels lethal for the rodent, leading to the dispersal of ectoparasites. As with snap traps, each live trap containing a rodent should be sealed in an individual ziplock bag to prevent ectoparasites from detaching from their host. Upon returning to the laboratory, living rodents must be euthanised humanely, and their ectoparasites should be collected for analysis. If rodent identification cannot be performed in the field, the rodent should be preserved appropriately and referred to a rodent identification expert.

Once the rodent has died, it should be taken out of the trap and both the trap and the killing chamber must be inspected for ectoparasites, which would also be deceased. Any parasites found should be collected and placed in a vial containing alcohol. To remove parasites from the rodent, one of the two methods must be used. Either the rodent's fur must be combed against the direction of hair growth with a nit comb or with a stiff-bristled brush, doing so over a white enamel cake pan or a similar container. This will dislodge the ectoparasites into the pan. The dislodged parasites must be gathered and stored in a vial of alcohol, along with those retrieved from the ziplock bag, trap, and killing chamber.

Cattle and buffaloes are among the primary hosts for tick vectors of KFD. Additionally, small mammals, including various rodent and insectivorous species such as *Rattus rattus wroughtoni, Rattus rattus rufescens, Rattus blanfordi, Funambulus tristriatus, Ratufa indica, Petaurista philippensis,* and *Suncus murinus*, serve as significant hosts, particularly for the immature stages of ticks.



(a) (b)

(c)

Figure 14.Different Types of Traps Used in Rodent Surveys (a).Snap Trap (b).Sherman Trap (c).Live Trap

Small mammals can be confined in wire mesh cages placed over trays of water, allowing engorged ticks to drop into the water for easy collection without damage. These engorged ticks can then be kept until they molt into the next developmental stage, which is easier to identify than earlier instars.

Tick infestation on host species is assessed using the tick index, calculated as the average number of ticks per individual host examined.

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

8. Preservation & Mounting

General

Arthropods suitable for slide mounting include immature Diptera such as mosquito larvae and pupae, small adult Diptera like Phlebotomines and *Culicoides,* and adult ectoparasites such as fleas, mites, and lice. Slide mounts can be either temporary or permanent. Permanent mounts are ideal for reference collections, while temporary mounts are commonly used for quick surveys. Examples of temporary mounting media are Berlese's medium, Hoyer's medium, and methylcellulose. Permanent mounting media include Canada balsam, euparal, clarite, piccolyte, and permount.

Temporary Slide Mounts

Small, translucent arthropods that require microscopic examination, such as larvae, adult fleas, lice, mites, bedbugs, ticks, and mosquito male genitalia, should be mounted on slides for study. Temporary slide mounts are a practical and straightforward method. It is essential to kill the arthropods correctly; most soft-bodied immature specimens are killed in hot water at 65 °C. However, boiling should be avoided as it can introduce air bubbles and damage delicate structures needed for identification. After being killed, most arthropods should be stored in alcohol until they are ready to be mounted (with exceptions like mosquito larvae). For specimens like fleas, ticks, lice, and mites, non-chitinous tissues must be decoloured or dissolved to expose hidden or internal structures. This process can be performed mechanically or chemically, though some mosquito larvae can be examined without decolouring.

a. **Mechanical Procedure:** This procedure involves puncturing the specimen's body wall with a fine needle, targeting the membranous areas between segments. The body fluids and contents are then expelled by applying light and intermittent pressure using a brush or small blunt probe. Any remaining fragments can interfere with staining, as they often absorb the stain excessively. The "pumping out" process is best performed in a shallow dish of water under a dissecting microscope. Caution is required to preserve internal taxonomic structures critical for identification.
b. **Chemical Procedure:** This process involves soaking the specimen in a mild caustic solution, such as sodium hydroxide (NaOH) or potassium hydroxide (KOH) (1 KOH pellet per 5 mL of water) at concentrations of 5–10%. Bleaching agents like sodium hypochlorite (bleach) can also be used, but high concentrations should be avoided as they can damage the specimen. The specimen can be left in the solution for several hours at room temperature, with heating to speed up the clearing process. However, care must be taken not to over-bleach the specimen, as it may become too pale. Clearing time can vary widely between specimens. Occasionally, air bubbles may become trapped in the specimen, requiring its removal and treatment under a microscope using the same method as for "pumping out" body contents. Once cleared, the specimen should be transferred to distilled water acidified with glacial acetic acid or 15% hydrochloric acid to neutralise the caustic solution and prevent further tissue deterioration.

Mounting Procedures

After completing the decolouring procedure, the specimen is ready for mounting. First, the centre of the slide must be determined. This can be done by placing a blank glass slide on a sheet of paper and outlining the slide's shape. Then, the slide should be removed and diagonal lines must be drawn from corner to corner. The point where the lines intersect is the exact centre of the slide. Once these steps are finished, including the decolouring and centring of the slide, a temporary mounting medium must be selected. There are three common types: Hoyer's medium, Berlese's medium, and methylcellulose. Of these, Hoyer's medium is preferred, as it can preserve specimens for one to three years. To prepare Hoyer's Medium, the following ingredients are used:

Distilled water: 50 mL

Gum Arabic (crystalline): 30 g

Chloral hydrate: 200 g

Glycerine: 20 mL

The arthropod specimen is mounted in the following manner:

1. Place a few drops of the mounting medium in the centre of the slide.
2. Carefully position the arthropod on the slide, ensuring that it is centred correctly.
3. Add enough medium to fully cover the specimen and the area that will be covered by the coverslip.
4. Coat one edge of the cover glass with the medium to improve adhesion between the cover glass and the slide.
5. Gently place the coverslip on the slide. If air bubbles form in the medium, remove them by lightly tapping the coverslip

or by gently heating the slide over a Bunsen burner. Take care not to crush the specimen while tapping the glass.

6. Allow the slide to dry in an incubator at 40 ºC for four days, then let it air dry for an additional two weeks.
7. If the medium shrinks, add more around the edge of the coverslip, and it will be pulled underneath the glass. To prevent the medium from drying out or cracking, seal the edges of the coverslip with shellac or nail polish.

Permanent Slide Mounts

For permanent slide mounts, the killing and decolouring procedures used for temporary mounts can be applied. However, unlike temporary media, permanent mounting media are insoluble in water, so specimens must first be dehydrated. This is done by placing the specimens in a series of alcohol solutions with increasing concentrations: 70%, 90%, and 95% ethyl alcohol. The choice of alcohol concentrations and the order of dehydration depend on the specimen's delicacy. Soft-bodied specimens should be gradually processed through the alcohol series, while specimens with tougher exteriors can be directly placed in higher alcohol concentrations without harm. After dehydration, the specimen should be rinsed in xylene, which is compatible with permanent mounting media.

a. Preliminary Procedures: The initial steps for permanent mounts are identical to those for temporary mounts. Various permanent mounting media are available, with Canada balsam being one of the most commonly used. Once the desired medium is chosen, the mounting process follows the same procedure as for temporary mounts. However, permanent mounts should be dried at 50 ºC for two weeks to ensure proper setting.

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

Fleas should be positioned with the ventral side facing up and the head pointing to the right. Lice, bedbugs, and mites should be placed with the ventral side facing up and the head directed downward, towards the mounter. If preferred, two specimens of the same species can be mounted on a single slide—one with the dorsal side up and the other with the ventral side up. The correct mounting positions for a flea, louse, and mite are illustrated in Figure 15.

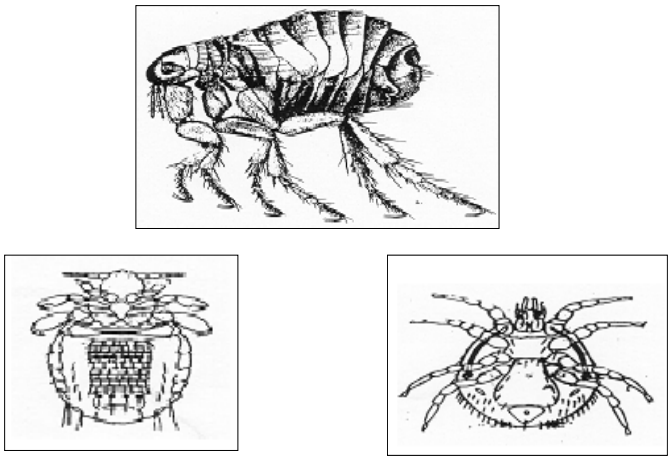


Figure 15.Correct Mounting Positions for a Flea, a Louse, and a Mite

Mounting Mites in Methylcellulose

1. Kill the specimen by immersing it in 70% alcohol. Then, place the specimen in a 10% lactic acid solution for 10 to 30 minutes, depending on the size of the specimen. You can handle up to ten specimens at once.
2. Transfer the specimen to methylcellulose medium and gently heat it, being careful not to boil. Repeat the heating process as needed until the specimen is properly cleared and relaxed, and the legs are straightened.
3. Place the specimen on a slide containing methylcellulose, positioning it ventral side up with the head facing down. Apply the coverslip, ensuring that only one specimen is mounted per slide.

9. Outbreak Investigation

Scrub Typhus

When investigating an outbreak of scrub typhus, two critical parameters require special attention: 1) the total number of confirmed cases, and 2) the chigger index. These two factors should be analysed in relation to the time of year when cases occur. The chigger index, which is linked to the presence of the vector trombiculid mite (*Leptotrombidium deliense* group), plays a significant role in understanding the disease transmission dynamics.

Mite collection should be conducted in both domestic and wild environments using the methods described earlier.

The chigger index, which indicates the number of chiggers infesting a single host, is essential for estimating the occurrence of scrub typhus. The chigger index required for a single case of scrub typhus to occur in a month has been estimated to be 0.6 chiggers per *Suncus murinus* and 0.6 chiggers per *Rattus* species. Therefore, the chigger index value of 0.6 can be considered to be the critical threshold necessary for a single case of scrub typhus to occur.

Kyasanur Forest Disease

The important features that deserve careful attention for investigations during an outbreak are as follows:

1. The presence of monkeys in the area must be confirmed, as KFD outbreaks in humans are typically preceded by epizootics in the local monkey population.
2. Any monkey deaths in the area must be thoroughly investigated.
3. Ticks found on or near dead monkeys should be carefully collected.
4. The presence of known KFD vectors should be confirmed, especially in the affected area.
5. The natural hosts of tick vectors, both domestic and wild animals, must be identified.
6. The source of tick infestation in humans should be determined.
7. If the area has no history of the disease and lacks known vector species, the investigation should prioritise virus isolation and transmission experiments to identify the vector species responsible.

Plague

During a plague outbreak, immediate actions must be taken, including proper diagnosis, quarantine, treatment, and implementation of prevention and control measures. Additionally, the following entomological investigations are necessary:

- Identification of fleas on rodents and isolation of the plague pathogen through microscopic and culture examination
- Active surveillance in areas known to be natural plague foci, with a flea index greater than one
- Reporting of rat falls
- Investigation of suspected or confirmed cases of bubonic plague

10. Prevention & Control Measures

Community Awareness

Awareness should be raised within the community, particularly in endemic areas, about tick- and mite-borne diseases. Various agencies can be approached to provide information or speakers for training medical, pest management, or other personnel. Additional educational methods include distributing brochures, pamphlets, and fact sheets to new personnel; publishing periodic notices in the installation newspaper or daily plans, especially during warm months and the fall hunting season; and posting warning signs in tick-infested woods or other areas frequently visited by troops, hunters, or hikers.

Personal Protection

- Proper clothing should be worn to prevent ticks from reaching the skin, thus reducing the risk of bites.
- Pants should be tucked into boots or socks, and shirts should be tucked into pants.
- Sitting or lying on areas with thick brush or scrub should be avoided.
- Hanging clothes on bushes or trees should be avoided.
- Pets must be regularly treated to prevent tick infestations.
- Long sleeves and a hat are recommended, especially when crouching or crawling through bushes or undergrowth. Light-coloured clothing makes ticks easier to spot.
- Use of repellents such as DEET 33% (N,N-diethyl-m-toluamide or N,N-diethyl-3-methylbenzamide) and permethrin is advised to prevent tick bites. These provide up to 12 hours of protection, depending on environmental conditions, and should be applied as a thin film over exposed skin, following the product instructions.
- Permethrin should be applied to the lower pant legs, waistband, crotch, shirt sleeves, collar, and front placket, but never directly to the skin.
- Permethrin aerosol (0.5% permethrin) should be sprayed generously over the outside of the uniform, ensuring it is damp but not wet.

Environmental Management

- In suitable areas, clearing edge habitats through leaf litter removal, mechanical brush control, and mowing or burning vegetation can effectively control ticks and mites in residential and recreational areas.
- Removing low-growing vegetation and brush eliminates the support ticks need to reach hosts, reducing tick attachment rates.
- Clearing leaf litter and underbrush removes tick habitats and lowers the population of small mammal hosts, such as deer, mice and meadow voles.
- Mowing lawns and grassy areas to a height of less than 6 inches (16 cm) significantly reduces the chances of human-tick contact.
- Where environmentally permissible, controlled burning has been shown to decrease tick populations for up to a year.
- Thinning early successional shrubs and grasses in early to mid-fall can stress the overwintering tick population and reduce their survival. This should be done late enough to prevent regrowth.

Chemical Control

- The choice of acaricide formulation (e.g., dust, granule, emulsifiable concentrate) is a key factor in selecting the appropriate equipment for application. However, using acaricides directly to control ticks may not always be effective.
- A more efficient approach is to allow livestock to naturally collect ticks and then treat the animals to eliminate the parasites, rather than applying acaricides to the pastures.

Measures to Control Ticks

Area-wide acaricide application, which targets tick habitats such as leaf litter and brush, can be used to control tick populations. This can involve applying acaricides to rodents using baited tubes, boxes, or feeding stations in areas where tick-borne pathogens are endemic.

In addition, clearing vegetation to allow the area to dry for a few weeks, followed by spraying with malathion, is an effective strategy. This is especially useful when outdoor activities, such as troop camping, are planned. To further prevent chigger bites, repellents can be applied before entering forested areas for such activities.

Measures to Control Mites

One or two treatments of the forest floor with the insecticide lindane, which is highly effective in killing ticks, can be implemented. Control measures should include spraying malathion dust within a 100-meter radius of the monkey death site. Spraying should also extend along forest tracks frequently used by villagers. If human dwellings are near the infested forest, spraying should be carried out around those areas as well. It is advisable for people to use tick repellents before entering the forest.

For long-term control, efforts should focus on managing *Haemaphysalis* ticks in local cattle populations. This can be achieved by spraying the animals with benzene hexachloride (BHC) or by dipping them in an acaricidal solution to eliminate ticks effectively.

Measures to Control Fleas

Dusting the runways and burrows of rodents is one of the most effective and rapid methods for reducing flea populations. This involves applying insecticides directly to the rodent runways and at the entrances of their burrows, as well as using residual sprays in selected houses. Research has shown that fleas have developed resistance to DDT, making it less effective. As an alternative, malathion can be used for controlling fleas.

Dust formulation for flea control can be made by combining 5% malathion powder, which consists of 1 part of 25% wettable powder (WP) malathion and 4 parts of chalk powder or any inert material. This mixture is used to dust rodent runways, burrows, and areas around human dwellings.

Residual Insecticidal Spray

Malathion 25% WP

To prepare a 5% malathion suspension for application at 20 g/sq-m of the active ingredient, 2 kg of 25% wettable powder (WP) malathion should be mixed with 10 litres of water. This will result in the desired concentration for effective application.

Deltamethrin 2.5% WP

To prepare a 0.125% suspension of deltamethrin for application at 35.0 mg/sq-m of the active ingredient, 500 grams of 2.5% deltamethrin should be mixed with 10 litres of water. Two rounds of spraying should be done annually, and this method should be used only in emergency situations.

11. Susceptibility Test for Insecticide Resistance

Limited research has been conducted on techniques to assess tick susceptibility to insecticides. The current methodology involves applying a known volume of insecticide, either in an aqueous or alcoholic solution, in a series of two-fold dilutions, and observing the mortality rate after a 24-hour incubation period. This allows for the determination of LC_{50} or LC_{90} values. In KFD areas, studies have indicated that species of the *Haemaphysalis* genus show greater susceptibility to BHC (which has been banned since 1997) compared to other conventional acaricides.

When selecting a suitable laboratory test for acaricide resistance, the following criteria must be met:

- The test should be sensitive enough to detect resistance at its early stages and should cover a broad range of chemical groups, including the most recently developed active ingredients.
- The diagnostic test should be simple, cost-effective, and should provide rapid and reliable results.
- It should be suitable for standardisation across laboratories worldwide.
- The most common *in vitro* tests are bioassays conducted on larvae and engorged female ticks.
- However, none of the existing tests fully meet all the above criteria, and improving protocols for diagnosing acaricide resistance should remain an ongoing objective.

While resistant strains of ticks can be diagnosed without standardised test protocols, adopting standardised diagnostic methods would aid in global monitoring and provide a basis for comparing test results. To facilitate this, since 1975, the Food and Agriculture Organisation has promoted the use of the Larval Packet Test (LPT) for field investigations on acaricide resistance.

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Annexure I

Format for Ticks & Mites Surveys

Table 1.Details of the Collected Rodent Samples

S. No.	Cage Code/ Place	Rodent Species (Male/ Female)	Ectoparasites				Ear Pinna Collected (70% Alcohol)	Blood/ Sera Sample	Smear	Organs Collected in Media			
			Mite	Tick	Lice	Flea				Heart	Lung	Liver	Spleen

Date: **Place/ Village:**