TICK SURVEILLANCE

Collection, identification and packing

Ministry of Environment, Forest and Climate Change

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Contents

1. Introduction

Kyasanur Forest Disease (KFD) is a tick-borne viral haemorrhagic fever transmitted through the bite of ticks infected with the KFD virus (KFDV). The disease was first identified in 1956 in the Kyasanur Forest of Shivamogga District, Karnataka, India, from which it derives its name. KFD cases have since been reported in States along the Western Ghats, including Karnataka, Kerala, Tamil Nadu, Goa, and Maharashtra.

Tick development follows a seasonal cycle in which certain life stages are predominantly found in specific months. These different life stages feed on multiple host species: larvae feed mainly on small animals like rodents, nymphs feed mainly on medium-sized animals like monkeys and also on humans, and adult ticks feed mainly on large animals like cattle.

For a comprehensive KFDV surveillance and disease management, therefore, a combination of virus surveillance in humans, in sentinel species like monkeys and in ticks is vital to monitor and understand the regional distribution of the disease.

By combining the three aspects within the scope of a One Health approach, the dimensions of human health, animal health and environmental health are taken into account. This approach helps identify high-risk areas and seasons for transmission, supports ecological surveillance, risk mapping, early warnings regarding outbreaks, and guides public health actions, control measures, and awareness programmes.

This manual focuses on tick surveillance and is meant for professionals involved in this field. It provides concise information on tick taxonomy, morphology, surveillance methods, selection criteria for surveillance regions, personal protection measures, specimen storage and transport, as well as key laboratory facilities for KFDV diagnostic. In addition to KFDV surveillance, the described practices can also be applied to tick surveillance for other purposes.

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2. Guidelines for identifying and prioritising areas, seasons and methodologies for tick surveillance

Effective tick surveillance for KFD requires a risk-based approach that integrates seasonal tick activity patterns with relevant ecological and epidemiological factors. Surveillance timing and frequency should be aligned with the periods of highest activity of tick life stages associated with disease transmission, while area selection should prioritise locations with known human or animal cases and environments that favour tick – host interactions. Integrating these temporal and spatial considerations ensures efficient use of resources, improves early detection of KFDV circulation, and strengthens the assessment of transmission risk to humans.

2.1 Selection of appropriate surveillance season and frequency

All the life stages of ticks are present in the environment throughout the year. Adult ticks infesting livestock and other large mammals are found in higher numbers following the monsoon season, approximately from July to January.

The nymphal stage of tick species that can transmit the KFDV to humans and monkeys is active in Goa mainly between November and March. However, the KFD transmission season varies across the affected States and can extend from November to June.

Surveillance activities should commence in October, slightly ahead of the peak nymphal season, in which the risk of disease transmission to humans is highest. In villages and adjacent forest areas that have recorded confirmed KFD cases within the past year, tick sampling should be conducted monthly mainly between October and March. In addition, environmental sampling and sampling on livestock can be conducted throughout the year (with exception of rainy season when environmental sampling is not recommended) to provide general information regarding tick density and prevalence of KFDV in the tick population.

Surveillance priorities and sampling frequency should be adapted according to the ecological and epidemiological risk level of each area **(Table 1)**. The risk categories and their corresponding surveillance frequencies are summarised below:

Table 1: Tick surveillance priority based on the risk categories

Risk category	Description	Surveillance priority
High risk	KFD-positive cases (humans, monkeys or ticks) in the past year, suitable tick habitat, and high forest – human interface	Monthly[1]
Moderate risk	Adjacent to endemic areas, suitable ecology, last KFD cases (humans, monkeys or ticks) detected between one and five years ago	Quarterly surveillance

[1] Environmental sampling (flagging and dragging) is not recommended during the rainy season.

Low risk	Limited forest coverage, low host density, last KFD cases (humans, monkeys or ticks) detected more than five years ago or no cases detected yet	Annual surveillance

2.2 Selection of areas

Area selection for tick surveillance should be based on epidemiological data, where available, combined with ecological and livestock criteria. In areas without reported KFD cases, site selection should rely primarily on ecological and livestock factors.

From a practical point of view, villages including their adjacent plantation and forest areas can be considered as scale of operation for area selection. Following the prioritisation of villages, specific sampling sites should be identified, with preference given to forested areas, plantations, and livestock grazing zones, where tick densities are typically higher.

2.2.1 Selection based on epidemiological data

Tick sampling sites within a given area should be selected using epidemiological evidence, including:

- Reported human KFD cases
- Laboratory-confirmed monkey deaths related to a KFD infection
- KFD-positive tick samples

2.2.2 Selection based on ecological and livestock criteria

As a second criteria, sampling locations should be identified where conditions favour tick – host interactions. Priority should be given to locations with the following characteristics:

- Proximity to forest edges (human – forest interface)
- Regular livestock grazing in forested or semi-forested areas
- Presence of mixed livestock
- Reports of tick infestation on domestic animals
- Recorded monkey deaths in nearby forest patches

3. Personal protective measures during tick collection

Individuals conducting tick surveillance face a risk of tick bites. Ticks are frequently found on the head, neck, groin and underarms but may attach anywhere on the body, including the torso, arms, legs and ankles **(Figure 1)**.

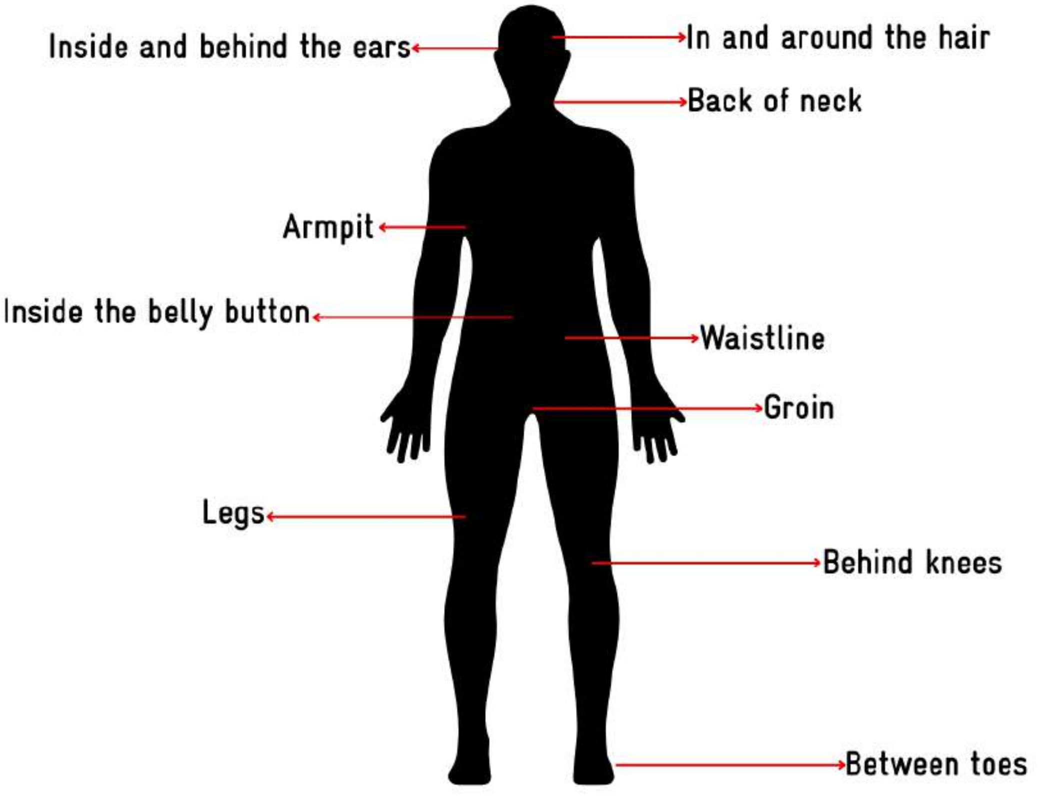


Figure 1: Key body regions to examine for ticks after surveillance | Source for Human anatomy: vectorstock.com, licensed for use

To reduce the risk of tick bites, it is essential to follow personal protective measures. It is important to note that an attached tick typically requires 24-48 hours before transmission of the KFDV occurs. Regular tick checks are therefore an effective means of reducing the risk of infection by enabling removal before virus transmission takes place.

- Use tick repellent.
- Use closed footwear during tick collection.
- White (ideal) or light-coloured clothing (trousers, shirts, and socks) should be worn so that ticks are easier to detect against a light background. Especially for environmental sampling, full body recommended (Figure 2).
- Long pants should always be tucked into long socks and/or boots, and long-sleeved shirts should be tucked into gloves.
- Upon returning home, carefully inspect clothing and footwear, and thoroughly examine the skin for any attached ticks. A hot water shower should be taken immediately to help remove any ticks that may be attached to the skin or body hair. Clothing used in the field must be washed without delay.

- If a tick attaches despite preventive measures, it should be removed using the technique described in **Chapter 4.1.1**.

- In case of a bite, the bite site should be monitored. If local symptoms like redness and swelling or general symptoms like fever develop, the individual should seek medical attention.

In countries where vaccinations against tick-borne diseases are available, these are recommended for professionals involved in tick surveillance.

Figure 2: Tick sampling team wearing personal protective equipment (PPE) | © GIZ / Stefanie Preuss

4. Standard operating procedures (SOP) for tick surveillance

4.1 Tick collection

Tick surveillance, including laboratory testing of collected ticks for the presence of KFDV, enables the development of risk maps that highlight high-risk transmission areas. This data supports decision making for disease prevention and control and can serve as an early warning signal. If even a single tick pool tests positive for KFDV, the sampling location should be categorised as at-risk for future human transmission.

All team members involved in tick surveillance should be properly trained in the methodology they are expected to use. Personal protective measures should always be taken as described in **Chapter 3**. An overview of useful material for tick sampling can be found in **Annexure I**.

Prior to tick collection, a data sheet (paper format or digital form) should be filled out with details of the collectors, sampling site, geo coordinates, and, in the case of animal sampling, also information related to the animal like identification marks, colour, gender, or any other ID of the particular animal (**Annexure II** for animal sampling and **Annexure III** for environmental sampling). This information supports assessment of host–tick relationships, identification of high-risk areas, evaluation of acaricide effectiveness, and understanding of the role of cattle in maintaining and dispersing KFD vectors within endemic regions.

Tick surveillance for KFD should include both direct sampling from livestock and environmental sampling from vegetation or surrounding habitats. Environmental sampling provides a more comprehensive understanding of tick presence, abundance, species diversity, and spatial distribution.

4.1.1 Animal sampling

Tick collection from livestock (often adult ticks are found) is a key component of tick surveillance in KFD-endemic and at-risk areas, as domestic animals serve as important hosts for several tick species and contribute to their maintenance and spread. Systematic examination of the animal body enables assessment of tick species composition, life stages, and infestation intensity, generating essential data for understanding vector distribution and disease risk.

In addition to sampling from livestock, sampling from further animal groups, e.g. rodents which carry mostly larvae and nymphs, can provide a broader overview of different life stages among the tick populations.

To ensure standardised, safe, and reliable sampling, tick collection from livestock should follow specific steps.

Figure 3: Direct collection of ticks from livestock | © GIZ / Stefanie Preuss

- Ticks should be carefully collected from animals with fine-tipped forceps **(Figure 3)**
- The ticks are grasped as close as possible to the skin surface, followed by gently pulling away from the skin without twisting or jerking **(Figure 4a and 4b)**. Pulling strongly, may cause the mouthparts to detach and remain embedded in the skin. **Table 2** indicates the locations on the animal body where ticks can be found most frequently.
- Care must be taken to avoid puncturing, crushing, or squeezing the ticks, as their body fluids may contain infectious agents.
- For additional protection against tick body fluids, use a tissue or gloves when handling ticks.
- The same technique can be used to remove ticks from humans.

***Figure 4a**: Grasping of a tick on livestock | Source: Colourbox.com (left) and GIZ / Stefanie Preuss (right)*

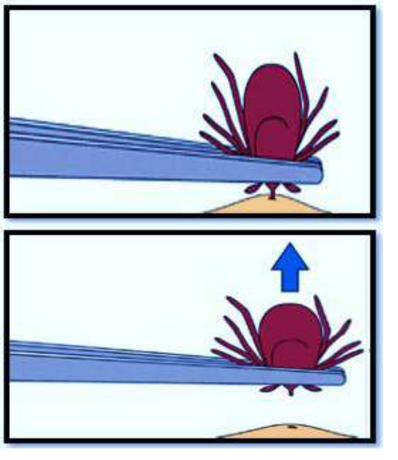


***Figure 4b**: Safe removal of ticks from human and animal skin | Source: CDC.gov*

Table 2: Common locations where ticks are found on livestock

Animal type	Locations
Cattle & Buffalo	Eye lids, back of ears, neck folds, udder, tail base
Goats & Sheep	Ears, axilla, groin, flank
Poultry	Comb, wattles

4.1.2 Environmental sampling

Environmental sampling should be undertaken in addition to animal-based tick surveillance for KFDV to comprehensively assess vector abundance, species composition, and infection risk across different ecological niches. While animal surveillance provides information on host-associated tick infestation, environmental sampling (e.g., flagging/dragging) captures questing ticks and free-living stages that are not detected on animals. This integrated approach improves early detection of virus circulation, enables identification of high-risk habitats, and supports a more accurate evaluation of spatial and seasonal dynamics of tick species associated with KFD transmission.

Nymphal and larval ticks are commonly found in leaf litter, shrubs and low vegetation, as well as on surfaces such as logs and tree trunks, where shaded conditions and high humidity are maintained. Consequently, vegetation-based sampling primarily targets these immature stages and is conducted using two standard techniques: **flagging** and **dragging**. Environmental sampling should be carried out in and around areas where tick presence is likely, including cattle sheds, footpaths connecting forests and plantations to villages, animal resting sites, grazing paths and watering points.

In contrast, adult ticks are most frequently found on livestock and other large mammals, making direct collection from animals and within cattle sheds the most effective approach. Adult ticks are only occasionally encountered on the forest floor.

Dragging and flagging techniques

Both techniques use a cloth (usually white flannel or lint) measuring approximately 1 m², which is stitched and prepared as illustrated in **Figures 5 and 6** to facilitate efficient collection of questing ticks.

Figure 5: Dragging method | © GIZ / Stefanie Preuss

Dragging method

This method is used to collect questing ticks (primarily larvae and nymphs, less adults) from low vegetation to assess tick density and species composition in a given area. The cloth is slowly dragged on a nylon thread or with a PVC pipe over grass and leaf litter, ensuring continuous contact with plant surfaces where ticks may be questing (**Figure 4**). At regular intervals (every 10–20 metres), the cloth should be stopped and carefully inspected. Ticks found on the cloth are removed using forceps and placed into vials containing 70 % ethanol for preservation (for details see Chapter 4.1.3).

Figure 6: Flagging method | © Virus Diagnostic Laboratory, Shivamogga, Karnataka

Flagging method

In this method, the cloth is attached to a stick and kept sufficiently loose to allow it to sweep over vegetation (**Figure 5**). The cloth is moved in a flagging or sweeping motion across tall grass, shrubs, and low branches where ticks wait for passing hosts. After every few sweeps, the cloth is inspected for ticks, which are removed using forceps and preserved in 70 % ethanol (for details see **Chapter 4.1.3**). Flagging is particularly effective in dense vegetation where dragging is difficult and is well suited for sampling along animal and forest trails, animal grazing areas as well as close to water bodies, forest edges and shrub-dominated areas, efficiently targeting questing ticks (primarily larvae and nymphs, less adults).

4.1.3. Sorting of samples after collection

- Screw-capped sterile vials (5ml) should be used as primary collection tubes. The vials are to be pre-filled with **70 % ethanol** (**Figure 7**) and labelled with the sample number, location (e.g., village name) and date of collection (**Figure 8**).
- Ticks should be transferred into the labelled collection vials immediately after collection **(Figure 9).** Only ticks collected from the same sampling location and on the same date should be stored together **(Figure 10).**
- Ticks derived from animals should be stored separately from ticks derived from flagging or dragging; this will help to identify the circulation of the pathogen within the questing tick population, which still pose a risk to humans and animals, in comparison to those ticks that already fed in the current life stage.
- The vials should be tightly sealed using parafilm or cellophane tape.
- Packed vials should then be placed in a leak-proof, labelled zip-lock bag indicating the date of collection, location, and collector's name. The bags with the samples need to be placed in a cold box keeping the temperature at 4-8 °C.
- In the laboratory, ticks collected in the primary vials (5 ml) must be sorted by species, life stage and location **(Figure 11)** using 2 ml microcentrifuge tubes (secondary vials). A maximum of five adult ticks may be stored per vial to avoid dilution of viral RNA. For larvae and nymphs, up to twenty may be added per vial.
- Species that are known to transmit the KFDV are sent to an accredited laboratory for analysis (see **Chapter 5.2.2**). Other species may be stored and processed for other purposes, based on specific needs and interests. In addition, they can be used to estimate the Tick Infestation Rate (see **Chapter 4.3**) and other entomological indices.
- If the primary field vial is labelled as sample number 1, secondary vials filled after species sorting should be labelled as 1a, 1b, 1c, and so forth.

- Any unwanted ticks must be disposed of safely through autoclaving or by chemical disinfection, as described in **Chapter 4.2**.
- After handling the ticks, hands should be washed with soap and water.
- Details for long-term storage can be found in **Chapter 5.3**.

Figure 7: Filling the Eppendorf tubes with Ethanol | © GIZ / Stefanie Preuss

Figure 8: Labelled Eppendorf tubes for tick collection | © GIZ / Stefanie Preuss

Figure 9: Placing the collected tick into an Eppendorf tube prefilled with Ethanol | © GIZ / Stefanie Preuss

Figure 10: Tick pool from one animal | © GIZ / Stefanie Preuss

Figure 11: Identification of ticks via light microscope (left) or stereo microscope (right), connected to a monitor for easier visualisation of details | © GIZ / Stefanie Preuss

4.2 Discarding procedure to inactivate the KFDV

Ticks must not be discarded in regular waste as this may pose a risk of accidental exposure or spread of the virus if the tick was infected. Instead, the procedure described below needs to be followed.

Inactivation:

- Autoclaving: This is the preferred method for complete sterilisation. Autoclave ticks at 121°C for 15–20 minutes.
- Chemical disinfection: If autoclaving is not available, immerse ticks in 10 % bleach or 70 % ethanol for a minimum of 30 minutes.

Disposal:

- After inactivation, dispose of the ticks and their container as biohazardous waste, in accordance with institutional or local waste-management regulations.

4.3 Entomological indices

4.3.1 Tick Infestation Rate

Tick Infestation Rate is used to measure the prevalence of tick infestation within a host population. It provides insight into how widely ticks are distributed among the hosts, regardless of the number of ticks each host carries. It is the **percentage of hosts infested with ticks** out of the total number of hosts examined during surveillance **(Table 3)**.

$$\textbf{Tick Infestation Rate: } \frac{\textbf{Number of hosts infested with ticks}}{\textbf{Total number of hosts examined}} \times 100$$

Table 3: Tick infestation level and its interpretation

Infestation Level	Tick Infestation Rate (%)	Interpretation
Low	< 25%	Few animals are infested – may point to effective control measures, high host resistance, or low tick presence.
Medium	25-50%	Moderate infestation – may point to reduced host resistance or increased tick presence.
High	> 50%	Majority of animals are infested – may point to significantly reduced host resistance or frequent contact with ticks. Improved tick control measures are required.

4.3.2 Tick Density Calculation

Tick Density Calculation[2] is used to measure the number of ticks present per unit area. It is one of the critical parameters in planning and interpreting tick collection for KFD surveillance.

When using the **flagging method**:

Tick Density per m^2 = Ticks collected after flagging for 50 times within an area of 50 m^2

When using the **dragging method**:

Tick Density = Ticks collected within 5 min of dragging in 50-linear-meter length

[2] Directorate of Health and Family Welfare Services, Government of Karnataka (2020) *Operational manual on Kyasanur Forest Disease*. Bengaluru: Government of Karnataka.

5. Tick taxonomy, identification, storage, and transportation

5.1 Tick taxonomy

Kingdom : Animalia
Phylum : Arthropoda
Class : Arachnida
Subclass : Acaria (Acari, Acarina, Acarida)
Super Order : Anactinotrichidea (Parasitoformes)
Order : Acari
Sub Order : Ixodida

Ticks are blood-feeding arachnids in the group Ixodida, which includes **hard ticks (Ixodidae), soft ticks (Argasidae),** and a rare third family (Nuttalliellidae), the evolutionary link between hard ticks and soft ticks. Together, they overall comprise 896 species worldwide.

Ticks have four pairs of legs in nymphs and adults, but only three pairs of legs in larvae. In comparison to mites, which also belong to arachnids, ticks are larger and can easily be recognised with the naked eye.

Table 4: Tick families and genera

Ixodidae	Argasidae	Nuttalliellidae
It consists of **702 species** worldwide. Of these, 88 species belonging to 7 genera are recorded in India.	It consists of **193 species** worldwide. Of these, 19 species belonging to 3 genera are recorded in India.	This family contains only a **single species** and it is not recorded in India.
Ixodes – 11 *Amblyomma* – 12 *Rhipicephalus* – 8 *Dermacentor* – 3 *Haemaphysalis* – 44 *Nosomma* – 1 *Hyalomma* – 9	*Argas* – 10 *Ornithodoros* – 7 *Otobius* – 2	*Nuttalliella namaqua*

5.2 Tick identification

5.2.1 General tick morphology

Hard ticks (family *Ixodidae*) are characterised by the presence of a hard, chitinous dorsal shield known as the ***scutum***, from which the family derives its name. When unfed, their bodies are oval and dorsoventrally flattened, becoming swollen following blood engorgement. The tick body is divided into two main regions: the ***capitulum*** (mouthparts) and the ***idiosoma*** (main body). In hard ticks, the capitulum is clearly visible from the dorsal surface, a key feature distinguishing them from soft ticks. It bears the palps, chelicerae, and the *hypostome*, which is armed with backward-pointing teeth that firmly anchor the tick to the host's skin **(Figure 12).**

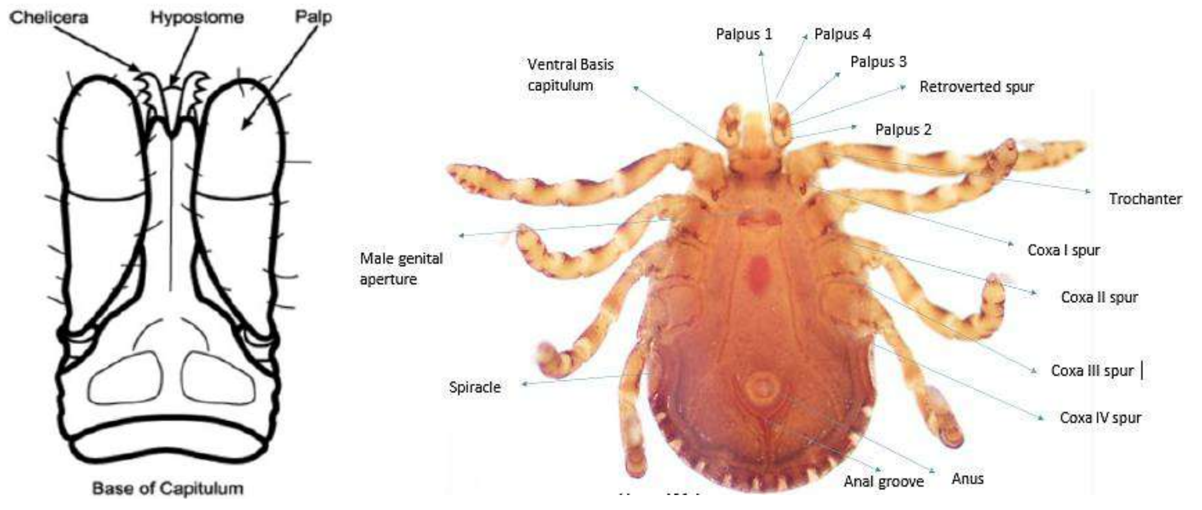


Figure 12: Taxonomical characteristics of hard ticks and their mouthparts | © ICMR Vector Control Research Centre

Adult hard ticks and nymphs possess four pairs of legs, whereas larvae have three pairs. Respiration occurs through a pair of spiracular plates located near the fourth pair of legs. In some species, additional features such as eyes and festoons (grooves along the posterior body margin) may be present. Sexual differentiation in ticks becomes apparent only after moulting to the adult stage, as males and females cannot be distinguished during the immature stages. Sexual dimorphism is pronounced in hard ticks (**Figure 13**): males possess a complete **scutum (conscutum)** covering the entire dorsum, whereas females have a partial ***scutum*** and a large, expandable posterior region known as the ***alloscutum***, which allows flexibility during blood feeding.

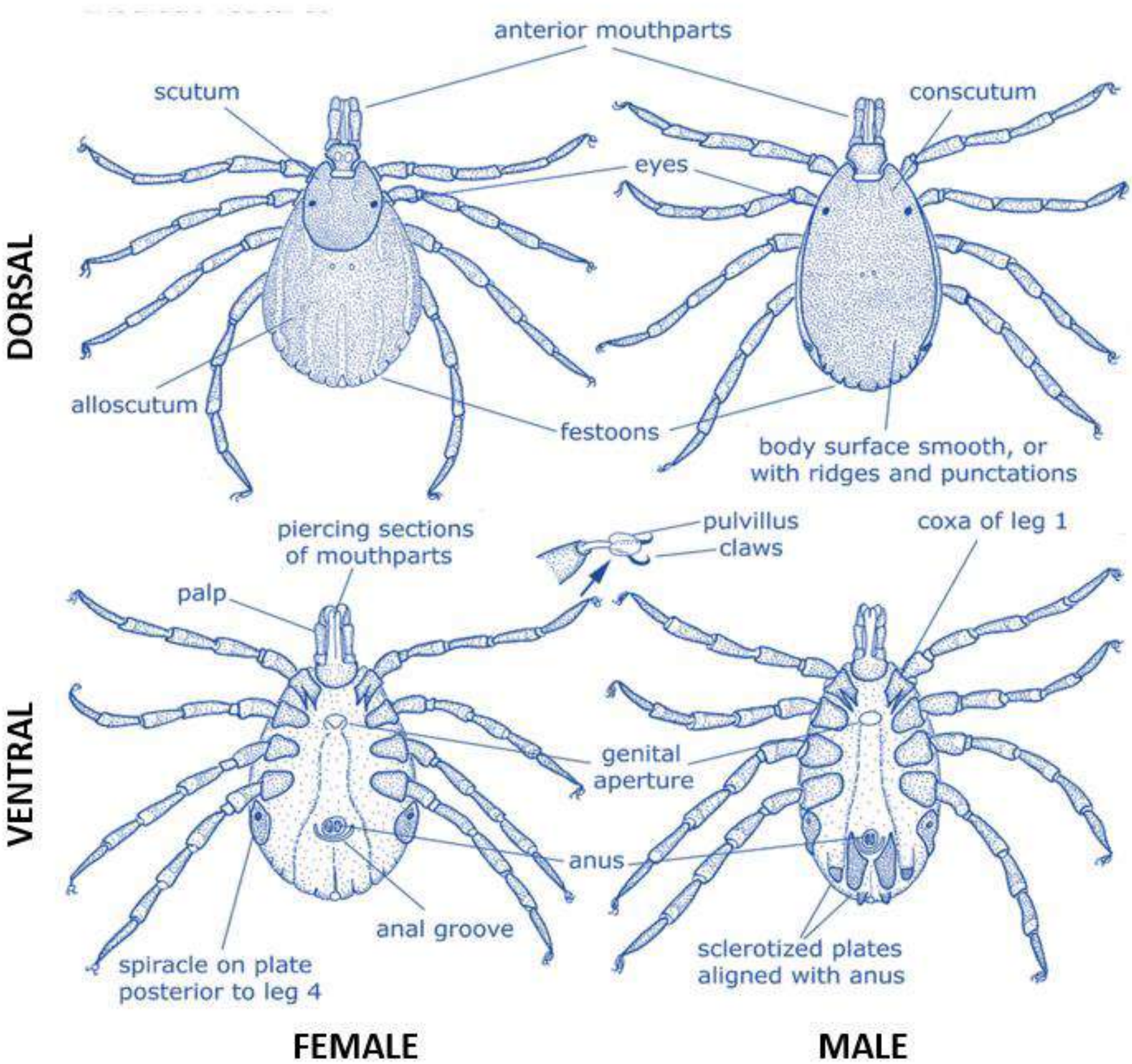


Figure 13: Hard ticks (Male and Female) | Source: Barker and Walker, 2014. Ticks of Australia. Zootaxa.

In contrast, **soft ticks** (family *Argasidae,* **Figure 14**) lack a hard dorsal *scutum* and have a leathery, wrinkled body surface. Their bodies are more rounded and dorsoventrally flattened, showing only minor visible changes after feeding compared to hard ticks. The capitulum is located on the ventral surface and is not visible dorsally, which is a key feature for distinguishing soft ticks from hard ticks. Although soft ticks also possess palps, chelicerae, and a *hypostome*, the *hypostome* is generally less heavily toothed. Spiracular plates are situated behind the third or fourth pair of legs. Eyes are usually present, while festoons are absent. As in hard ticks, larvae have three pairs of legs, and both nymphs and adults have four pairs. Sexual dimorphism is less pronounced in soft ticks, and males and females are often difficult to distinguish externally. Soft ticks typically feed rapidly and repeatedly, taking short blood meals and spending most of their life cycle off the host in sheltered environments.

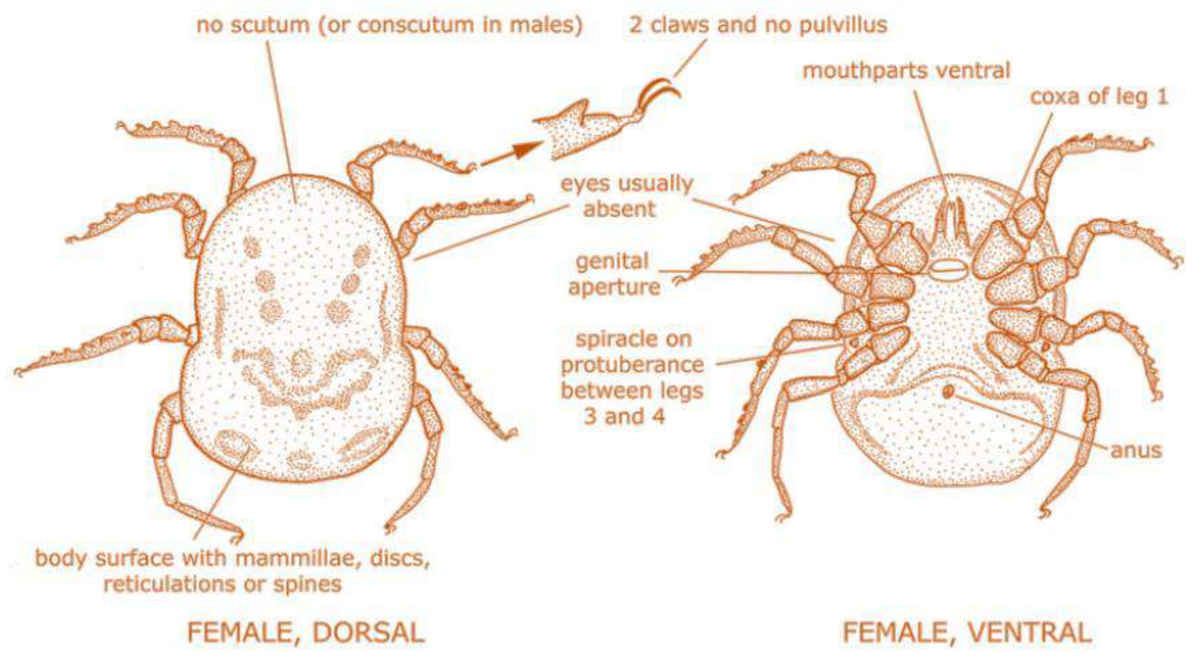


Figure 14: Soft ticks (Female dorsal & ventral) | Source: Barker and Walker, 201 4. Ticks of Australia. Zootaxa.

5.2.2 Morphological characteristics of *Hard ticks (*Ixodidae*)*

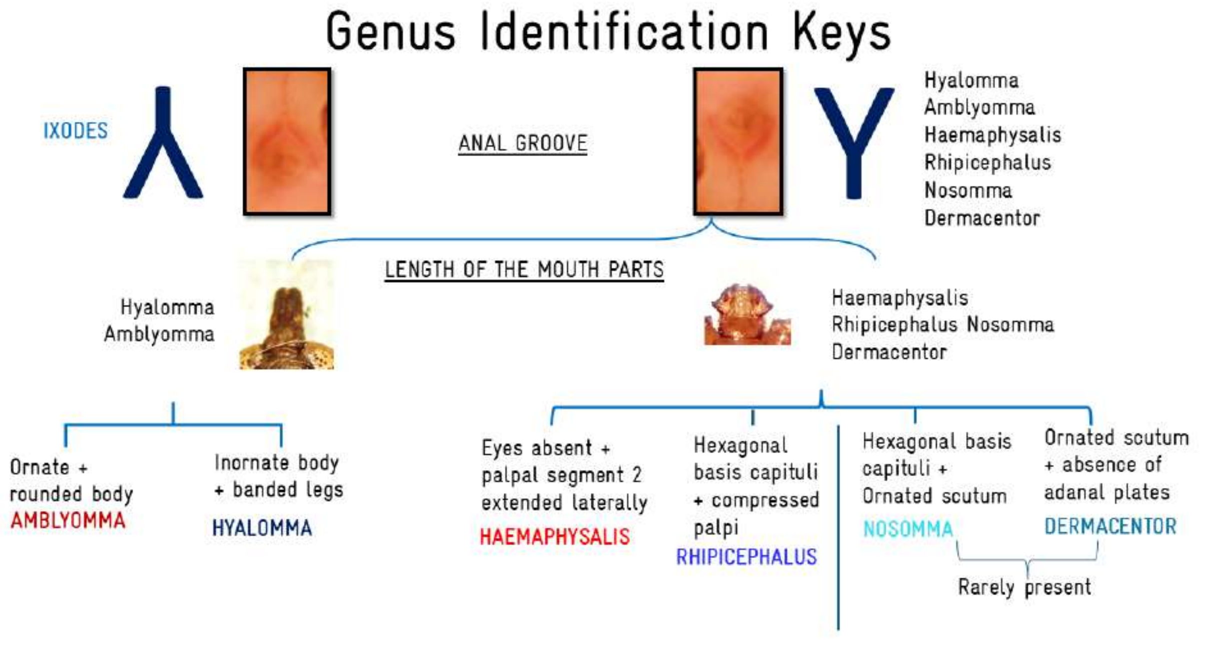
Hard tick identification is based on stable external morphological characters, including the presence or absence of eyes and festoons, the shape of the *basis capituli*, palpal morphology, scutal ornamentation, and the position of the anal groove. These diagnostic features enable rapid differentiation of the seven major ixodid genera (**Table 5**), namely, *Amblyomma, Hyalomma, Rhipicephalus, Dermacentor, Haemaphysalis, Ixodes,* and *Nosomma.* The following table presents a genus-level morphological identification matrix for hard ticks commonly encountered in India.

Tick species belonging to the genera *Haemaphysalis, Ixodes, Dermacentor, Rhipicephalus, Amblyomma,* and *Ornithodoros* have been identified as vectors of KFDV, with *Haemaphysalis spinigera* recognised as the principal vector. However, species belongs to Haemaphysalis serves as a primary vector. In KFD-endemic regions, *H. spinigera*, is the most abundant species (56.64%), followed by *Haemaphysalis turturis* (35.94%) and *Haemaphysalis. bispinosa* (3.96%) (R. Balasubramanian et al., 2019).

Table 5: Morphological characteristics of hard ticks (distinctive features marked in blue) followed by the flow chart (genus level identification keys)

Morphological characteristic	*Amblyomma*	*Hyalomma*	*Rhipicephalus*	*Dermacentor*	*Haemaphysalis*	*Ixodes*	*Nosomma*
Eyes	Present	Present	Present	Present	Absent	Absent	Present
Festoons	Present	Present	Present	Present	Present	Absent	Present

Basis capituli	Rectangular	Rectangular	Hexagonal	Rectangular	Rectangular	Rectangular	Hexagonal
Palps	Long	Long & slender	Short	Short	Short, broad, laterally flared	Long	Short
Scutum ornamentation	Ornate	Inornate	Inornate	Ornate	Inornate	Inornate	Inornate
Anal groove	Posterior	Posterior	Posterior	Posterior	Posterior	Anterior	Posterior



5.2.3 Morphological characteristics of *Haemaphysalis*

Among hard ticks, species belonging to the genus *Haemaphysalis* can be recognised by their rectangular basis ***capituli*** (base of the mouthparts), which bears backward-extending lateral posterior projections, a feature that distinguishes them from the genera *Rhipicephalus* (hexagonal basis capituli).

The palps are short and laterally flared in *Haemaphysalis*, with the second palpal segment usually shorter and broader than the third. The scutum is typically inornate and punctate, and eyes are generally absent. Another diagnostic characteristic is the anal groove, which lies posterior to the anus.

Members of this genus are three-host ticks which complete their life cycle by feeding on three different hosts. The larva feeds on the first host, the nymph feeds on a second host, and the adult feeds on a third host before reproducing. After feeding at each stage, the tick drops off the host to moult in the environment before seeking the next host. These ticks feed on a wide range of wild and domestic mammals and may occasionally infest humans.

H. spinigera **(Figure 16)** and *H. turturis* **(Figure 17)** are important tick species encountered in KFD-endemic regions of India, with *H. spinigera* recognised as the principal vector of KFDV. Although *H. bispinosa* (**Figure 18**) is only occasionally reported as a vector, it is commonly abundant on livestock. Accurate identification of these closely related species is critical for effective tick surveillance and risk assessment.

Each species exhibits distinct morphological characteristics across developmental stages, particularly in the nymphal and adult stages, including features of the basis *capituli*, palpal segments, salience (a prominent outward projection of palpal segment II) and coxal spurs. The key morphological characteristics used for species-level identification are described in **Table 6.**

Table 6: Key morphological characteristics for identification for *H. spinigera, H. turturis and H. bispinosa*

	Haemaphysalis spinigera	*Haemaphysalis turturis*	*Haemaphysalis bispinosa*
Nymph	• **Broad salience** • No recurvature in palps • **Palpal segment III with sharp ventral spur** and reaching about **1/4** distance to the basal margin of palpal segment II	• No salience • **Palps broader with external profile showing pronounced recurvature** • **Palpal segment III with strong ventral spur** extending **1/2** distance to the basal margin of palpal segment II	• No salience • No recurvature in palps • **Palpal segment III with blunt ventral spur** extending about **1/4** distance to basal margin of the palpal segment II
Female	• **Broad salience** • **Strong & sharp spur at Palpal segment II** • Dorso-basal margin of palpal segment III with median retroverted spur	• No salience • **Palpal segment II extends little beyond lateral margin of basis capituli** • Palpal segment III dorsobasal-internal margin **without spur or,** if present, **not extending 1/2 the length of segment II**	• No salience • No spur in palpal segment II • **Palpal segment III with dorsal medial retroverted spur**

Male	• **Broad salience** • Coxa IV with greatly elongated sharp spurs (a pair), much exceeding the length of other coxal spurs	• No salience • No spur in Coxa III & IV	• No salience • No spur in Coxa III & IV
# Refer the illustration **(Figure 15)** for the better clarification on the principal characters and terminology used in the table			

Note:

* The blue coloured points indicate the important identification character of the respective species.

* Examination of the ventral side of the specimen is sufficient for identification of *Haemaphysalis spinigera*, as all key diagnostic characteristics required for species determination are present on the ventral surface.

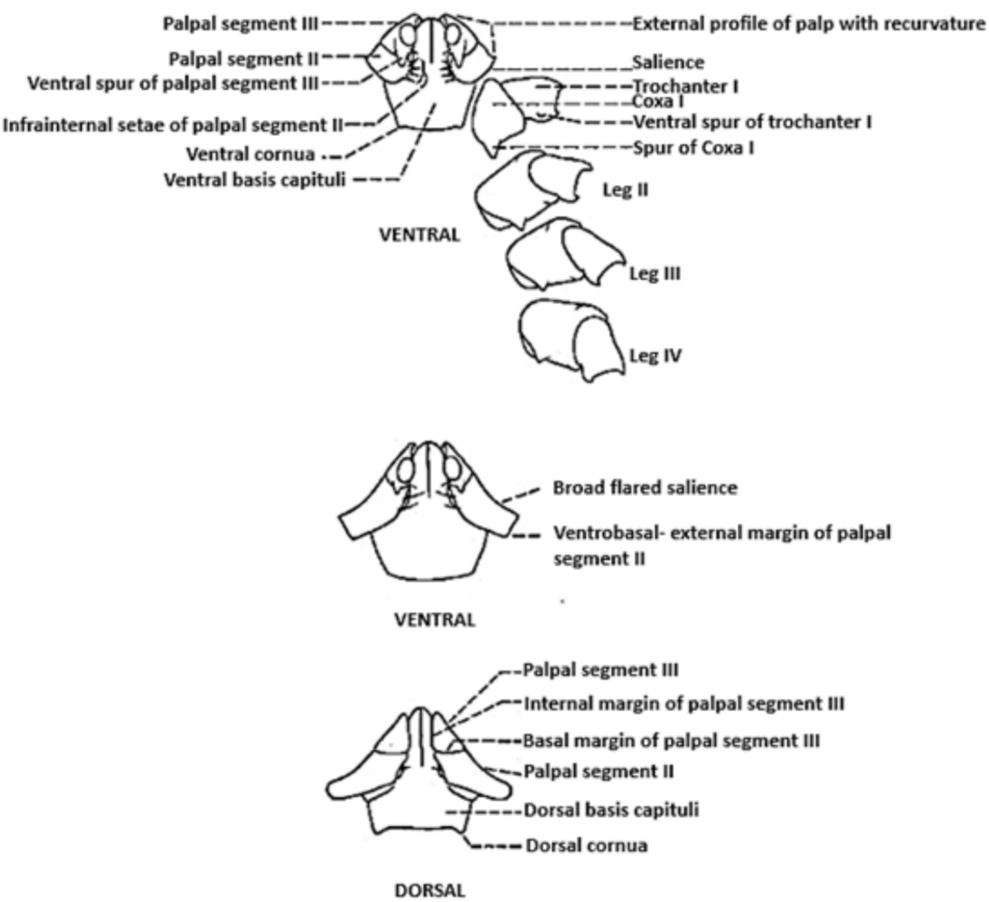


Figure 15: Illustration of principal characters and terminology used in the keys | Source: Trapido et al., 1964. Bulletin of Entomological Research

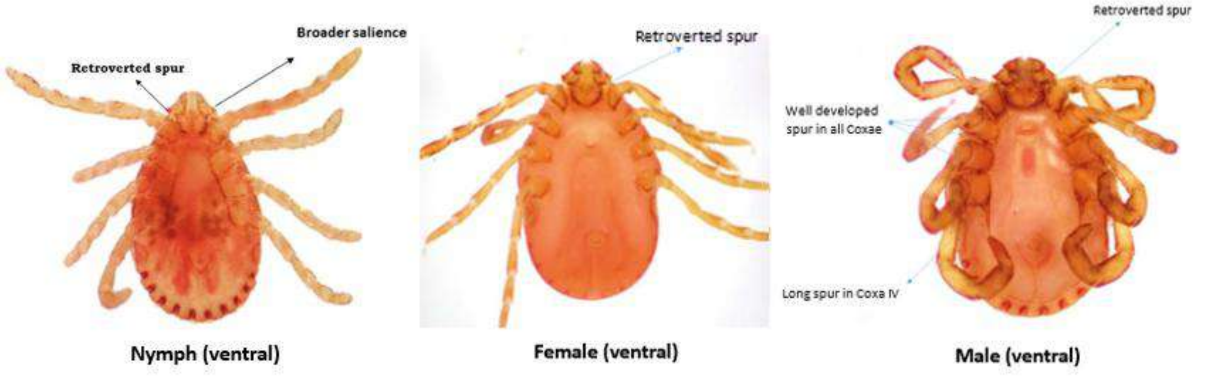


Figure 16: Haemaphysalis spinigera | © ICMR Vector Control Research Centre

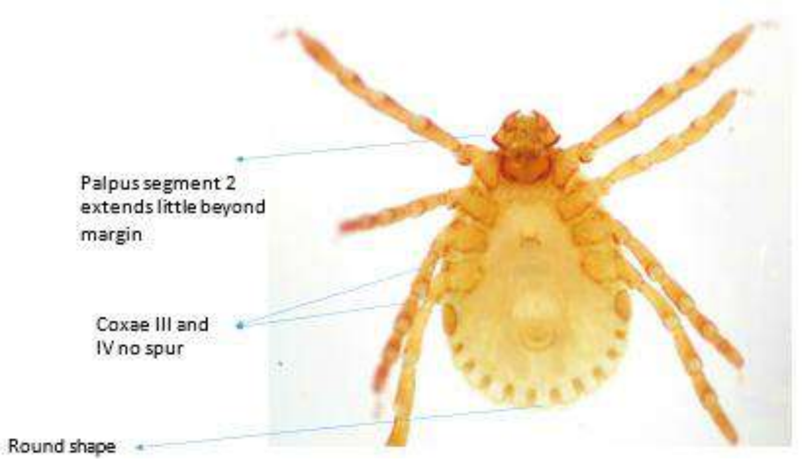


Figure 17: Haemaphysalis turturis (Female) ventral | © ICMR Vector Control Research Centre

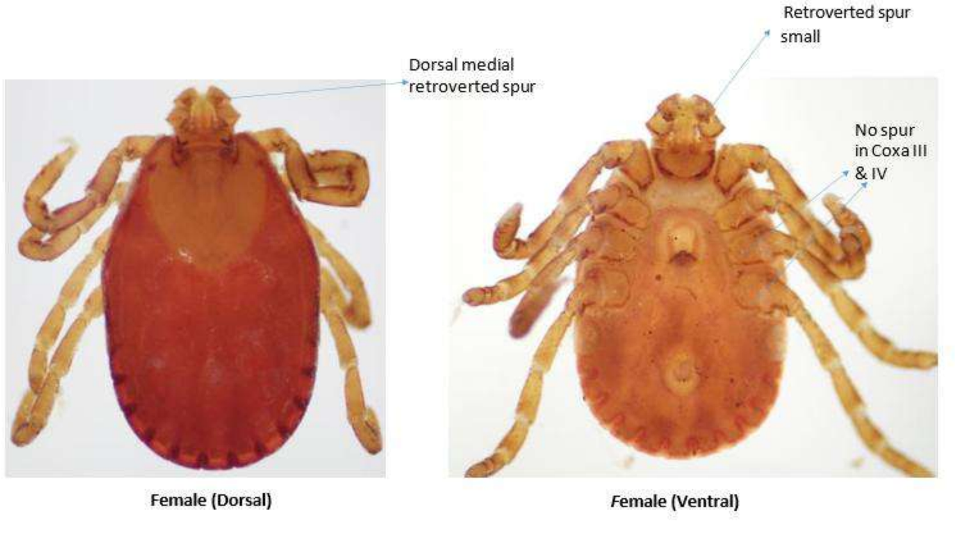


Figure 18: Haemaphysalis bispinosa (Female) ventral and dorsal view | © ICMR Vector Control Research Centre

5.3. Storage

Storing ticks in ethanol preserves the nucleic acid (genetic information) of the virus for the intended analysis while inactivating the virus – thereby reducing the risk of accidental infections – and other possible infectious agents. When placing the ticks into the vials after collection, ethanol can be used at room temperature.

A concentration of 70 % ethanol is optimal, as it preserves external morphological characteristics required for species identification without causing excessive shrinkage, while allowing subsequent molecular assays. Unlike formalin, which is toxic, carcinogenic, and unsuitable for nucleic acid analysis, ethanol is safer and appropriate for both morphological and molecular investigations.

However, if samples are to be stored for more than 7 days, it is recommended to use lysis buffer or a viral culture medium instead of ethanol.

Recommended temperatures for storage (for all tick stages):

- Refrigerate at 2 to 4°C if samples are to be processed (or sent to laboratory) within 24h,
- Keep frozen at -20°C if samples are to be processed after 48h and within 7 days,
- Keep frozen at -70°C if samples are to be processed after 7 days.

5.4 Packing and transportation of ticks for KFDV diagnosis

- Tick samples, particularly those suspected of containing infectious agents, must be transported using the standard triple-packaging system to prevent leakage and exposure.
  - **Primary container**: A sterile, watertight, and leak-proof sample vial or container. The lid must be sealed with parafilm to prevent leakage.
  - **Secondary container**: A robust, leak-proof container such as a zip-lock pouch, cryobox, or plastic container that encloses the primary vial.
  - **Outer container**: A rigid external container (e.g., thermocol or vaccine transport box) that holds the secondary container along with ice or gel packs to maintain the temperature at +2 to +4°C. The container should be securely sealed with adhesive tape.
- Tick samples should be transported to the designated laboratory within **24 hours**, accompanied by the Laboratory Request Form – Tick Pool (**Annexure IV**). If they can't be transported within 24 hours, they need to be stored at the temperature mentioned in **Chapter 5.3**.
- The outer package must be clearly labelled with the details of sender and receiving laboratory, including full postal address and contact telephone numbers for both parties.
- A biohazard label must be affixed to the outside of the package.
- Samples may be transported by a designated person or through an approved courier service.

6. List of accredited laboratories for KFDV diagnosis in ticks

1. National Institute of Virology (NIV)
   Microbial Containment Complex, 130/1 Sus Road, Pashan, Pune – 411021, India. Tel: 020-26006390.
   https://niv.icmr.org.in/

2. National Institute of Virology (NIV), Bengaluru Unit
   Dept. of Microbiology, RGICD Premises, Near NIMHANS, 1st Main, Someshwar Nagar, Dharmaram College Post, Bengaluru – 560029. Tel: 080-26654084 /074.
   https://niv.icmr.org.in/form/root/about-us/field-stations/icmr-niv-bangalore-unit

NIV Pune handles both monkey and tick samples for KFDV diagnosis whereas NIV Bengaluru Unit handles only tick samples.

Contact for queries related to tick surveillance activities:
Directorate of Health and Family Welfare of the State of Karnataka – Virus Diagnostic Laboratory - Shivamogga. Tel: 08182-222050. Email: ddvdlsmg@gmail.com

7. References

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15. Perumalsamy, N., Sharma, R., Elango, A., Nagarajan, S.A. and Rahi, M. (2025) '12S rRNA based phylogeny and genetic diversity of *Haemaphysalis spinigera*, the primary vector for Kyasanur forest disease in India', *Scientific Reports*, **15**, Article number: 39688.

Annexure I – Overview of useful material for tick surveillance

Sl. No	General Materials
1	70 % Ethyl Alcohol
2	Plastic dropper to fill tubes with alcohol
3	PPE kits and gloves
4	Parafilm tape
5	Marker pen
6	Fine pointed forceps
7	Fine pointed painting brushes
8	Zip lock covers (A4 and 6×4 inch sizes)
9	Thermocol box
10	Ice Packs
11	Gum Boots
12	Hand sanitizer
	Additional material for environmental tick sampling (flagging/ dragging)
1	Flag (Flannel / Satin, white colour) 1×1.5 meter
2	a. Flagging: PVC Pipe as handle (3/4-inch diameter, 2 m length) b. Dragging: PVC Pipe to keep the flag open (3/4-inch diameter, 1 m length = same as flag size)
3	Nylon thread to be inserted in the PVC Pipe for dragging (8 m)
4	15 mL Falcon tube
	Additional material for tick sampling from animals
1	Eppendorf tubes (2ml)
2	Eppendorf tube rack

Annexure II – Data sheet for tick collection from livestock

Sampling date: ....

Collector's name and designation: ....

Place (Cattle shed/ farming unit): ....

Location (Geo-coordinates): ....

Sl. No.	Village	House No./ Identification landmark	Name of the household	Livestock Sl. No.	Livestock details							Number of ticks collected
					Type	Name	Gender	Age	Colour	Identification mark	Veterinary ID No.	

Note: This data sheet is part of the 'Operational Manual Kyasanur Forest Disease', Directorate of Health and Family Welfare Services, Government of Karnataka.

Annexure III – Data sheet for tick collection from the environment (flagging / dragging)

Sampling date: ....

Collector's name and designation: ....

Place (Village): ....

Location (Geo-coordinates): ....

Sl. No.	Village	Type of locality where ticks are collected (peri-domestic, plantation, forest, others)	Number of ticks collected

Annexure IV – Laboratory request form for tick pool samples

No. KFD/TICKS/ /

To

The Deputy Director
Virus Diagnostic Laboratory
SHIVAMOGGA-577201

Sir/Madam,

I am sending pools of Ticks specimens Larva/ Nymphs / Adults with the following details for detection of KFD virus through Sri ....with a request to kindly intimate the lab results.

Sl. No.	District	Taluk	PHC	Village	Longitude-Latitude	Field No.	SVDL No.	Date of Collection	Time of collection	Hot spot collection (Yes/No)	Type of locality where tick pools are collected						Tick species	Larva (a)		Nymph (b)		Adult (c)		Total (a+b+c)		Result
											Peridomestic	Plantation	Forest	Cattle	Human body	Others		No. of pools	No. of pools Positive	No. of pools	No. of pools Positive	No. of pools	No. of pools Positive	No. of pools	No. of pools Positive	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27

SVDL No.: Shivamogga Viral Diagnostic Laboratory Number

Note: if any district is sending sample directly to NIV Pune, obtain SVDL number from VDL Shivamogga and then send to NIV Pune

Signature

Note: This data sheet is part of the 'Operational Manual Kyasanur Forest Disease', Directorate of Health and Family Welfare Services, Government of Karnataka.