III. MATERIALS AND METHODS

This chapter deals with materials and methods that are followed in the study under the following headings:

1. Study area
2. Pre-documentation Survey
3. Documentation of Ethno veterinary Practices
4. Identification and preparation of suitable herbs and herbal formulation for treatment of selected diseases
5. On-farm clinical evaluation
6. Experimental studies.

3.1 Study Area

In order to undertake documentation and clinical evaluation of selected ethno veterinary practices, two districts namely Cuddalore and Nagapattinam were selected. The district of Cuddalore in Tamil Nadu is located in North Latitude between 15° 5/11’ and 12° 35’, East Longitude between 78° 38’ and 80°. The district of Nagapattinam in Tamil Nadu lies on the shores of the Bay of Bengal between latitude 10.7906°N and Longitude 79.8428°E an area of 2,715 square kilometres (1,048 sq mi). The District capital, Nagapattinam lies on the eastern coast, 350 kilometers down south of the State capital Chennai and of Tiruchirappalli. The district has a coastline of 187 kilometres (116 mi). (Karan et al., 2011).

3.1.1 Pre-documentation Survey

Pre-documentation survey is an important activity before starting a study as it would bring out the needs and present situation of our study in the particular
locality. The data were collected through a well defined pre-tested questionnaires in line with the principles suggested by Monitoring and Evaluation format of National Agricultural Innovation Project as recommended by NAARM, Hyderabad. Two different questionnaires were used to collect the village profile and farm household profile. Stratified random sampling method were used to distribute the sample size based on number of marginal, small and landless agricultural labours.

Cuddalore district has seven revenue taluks and two taluks viz. Chidambaram and Kattumannarkoil taluks were randomly selected for the study. From Chidambaram taluk, three villages viz. Manalur, Pichavaram, and Chidambaram were randomly selected. From Kattumannarkoil taluk, three villages viz. Sozhatharam, pudayur and vattathur were randomly selected. From each village, 25 respondents were selected for the documentation of ethno veterinary practices. Hence data were collected from 150 respondents from Cuddalore district.

Nagapattinam district has eight revenue taluks and two taluks viz. Sirkali and Tharangambadi taluks were randomly selected for the study. From Sirkali taluk, three villages viz. Sirkali, Erukkur, and Puthur were randomly selected. From Tharangambadi taluk, three villages viz. Thirukadayur, Keezhaiyur and Manalmedu were randomly selected. From each village, 25 respondents were selected for the documentation of ethno veterinary practices. Hence data were collected from 150 respondents from Nagapattinam district.

A total of 300 respondents from both Cuddalore and Nagapattinam district were interviewed for the documentation of ethno veterinary practices.
3.1.1.1 Training needs for sustainable poultry production

Cuddalore District in Tamil Nadu State was selected considering its less progress in poultry enterprise. One hundred respondents were selected by simple random sampling procedure for data collection. The training needs of poultry entrepreneurs were assessed in the study by the use of a three point rating scale with points 'much needed', 'somewhat needed' and 'not at all needed' with scores of 3,2 and 1 respectively. The frequencies of each response categories were found out and multiplied by the score in respective to it. The training need index for each operation was calculated by dividing the total score assigned to each operation by all the respondents by the maximum possible score that could be assigned to the operation by all the respondents. Based on the magnitude of the training need index, the operations were ranked.

Similarly the preferences of type, duration, season, venue and frequency of training were also found out. Here also, the preference index was calculated by dividing the actual score assigned by all the respondents to each operation by the maximum possible score that could be assigned and accordingly the ranking was done.

3.1.1.2 Knowledge level of ethno veterinary practices among the goat keepers

The study was conducted in Nagapattinam district of Tamil Nadu state. The Nagapattinam district was identified by the planning commission of India as one of the disadvantaged districts of Tamil Nadu and the farmers are engaged in goat rearing activities. Hence a low cost treatment facility to goat farmers will be of much use and therefore an initiative to assess their knowledge level on ethno veterinary practices which is dependent on medicinal plants and herbs before proceeding for new developmental ventures. Two coastal blocks were randomly selected viz. Keelayur and Sembanarkoil where goat rearing is practiced. Two
panchayats from each block viz. Vilundamavadi and Vettaikaraniruppu of Keelayur block and Pillaiperumanallur and Marutham pallam of Sembanarkoil block were also randomly selected for the study. A total of 100 respondents with 25 respondents from each panchayat involved in goat rearing were selected for the study. The extent of knowledge possessed by the respondent at the time of the interview was measured using a set of questionnaire in the regional language designed for this purpose. A knowledge test was devised. Reliability and validity were worked out and after the pilot study, a final knowledge test was arrived. The summation of scores for correct replies was considered as the knowledge level of that particular farmer. Delinious – Hodges cumulative $\sqrt{f}$ method as explained by Delinious and Gurney (1951) was used to categorise the respondents into different herd size and level of knowledge.

3.2 Documentation of Ethno veterinary Practices

Documentation of selected ethno veterinary practices was done at localities where pre-documentation survey was undertaken at Cuddalore and Nagapattinam district of Tamil Nadu. The number of respondents was restricted to 150 members per district totaling 300 respondents both in Cuddalore and Nagapattinam districts.

Rapid Assessment of ethno veterinary health traditions is a participatory method developed in order to document and assess ethno veterinary knowledge in a rapid and cost efficient way. In this process, the traditional health practices are assessed through a method of dialogue and consensus, wherein folk healers, veterinary doctors, researchers, community members and other ethno veterinary experts, Ayurvedic doctors, Botanists, participate. This participatory documentation and rapid assessment of ethno veterinary traditions which is termed as Documentation and Rapid Assessment of Local Health Traditions (DALHT) is a process by which one could select effective ethno veterinary
remedies. The methodology for DALHT was developed and implemented by Foundation for Revitalization of Local Health Traditions (FRLHT) (Anonymous 2002).

The basis of this methodology is the conservation assessment tool based on the CBSG / IUCN guidelines that has been demonstrated intensively in India, Costa Rica, Panama, Indonesia, Thailand and other countries (Sally Walker and Sanjay Molur, 1998) The process involves comprehensive documentation of health practices, desk research for finding out and compiling scientific data on these practices and assessment workshops for prioritizing and selecting practices for promotion. The assessment workshops form a pluralistic-medicine platform for a cross-cultural dialogue between traditional and the contemporary medical sciences. Following the assessment, clinical evidence is collected on a particular practice based on pilot clinical studies. Selected best remedies are made into suitable product through local enterprises.

3.2.1 Prioritization of Clinical conditions for the study

In order to ascertain the basic realities about the indigenous treatment of livestock diseases, perusal of available literatures on the subject and a personal interactive survey at farmers’ level were undertaken. Analytical Hierarchy process (AHP) is the tool employed to select the formulations and diseases. (Soan, 2004).

3.2.2 Identification of suitable herbs and herb formulation for treatment of selected diseases

3.2.3 Perusal of literature

Relevant literatures on herbs and herbal formulations for indigenous treatment aspects in livestock available in the form of research reports, popular
writing, translation of ancient manuscripts, ayurveda slogans and other concepts in this area of study were perused in detail to identify the most common herbs and herbal formulations available to conduct the study.

3.2.4 Sampling methods and distribution

Sampling method adopted is proportional stratified sampling which is an improvised sampling over simple random sampling and systematic sampling. This sampling will have more statistical efficiency.

3.3 Preparation of herbal extracts for various therapeutic purposes

3.3.1 Preparation of aqueous extract of Aloe vera

*Aloe vera* plant was collected from the herbal garden maintained by a farmer. The leaves were washed with fresh, clean water, cut into pieces and weighed. 250gm of the cut leaves was pulverized in an electric blender, soaked in 2 litres of water for 5 hours and later filtered through a 1mm mesh sieve. The filtrate was made up to 10 litres with water, making a working dilution of 25,000ppm of the water extract of *A. vera*.

3.3.2 Preparation of aqueous extract of Ocimum sanctum

The leaves of *Ocimum sanctum* were collected and identified based on morphological characters. The leaves were washed with fresh, clean water, cut into pieces and weighed. 250gm of the cut leaves was pulverized in an electric blender, soaked in 2 litres of water for 5 hours and later filtered through a 1mm mesh sieve. The filtrate was made up to 10 litres with water, making a working dilution of 25,000 ppm of the water extract of *A. vera*. 
3.3.3 Preparation of aqueous extract of *Phylanthus niruri*

Locally available young *Phylanthus niruri* plants with roots (250 gm) were collected from a single area. A decoction was prepared by boiling in water (1:4, w/v). Boiling was continued till the water level reduced to half. Pure extract was obtained by filtering through fine muslin. The extract was made up to 10 litres with water, making a working dilution of 25,000 ppm of the aqueous extract of *P. niruri*.

3.3.4 Preparation of aqueous poly herbal extract

Locally available young *Aloe vera*, *Ocimum sanctum* and *Phylanthus niruri* plants (250 gm each) were collected from a single area. A decoction was prepared by boiling in water (1:4, w/v). Boiling was continued till the water level reduced to half. Pure extract was obtained by filtering through fine muslin. The extract was made up to 10 litres with water, making a working dilution of 25,000 ppm of the aqueous extract of aqueous poly herbal extract.

3.3.5 Preparation of herbal medicine for mastitis

*Aloe vera* : 500 gm  
*Curcuma longa* (Turmeric) : 100 gm  
Chalk piece : 50 gm  
Juice of *Citrus aurantifolia* : 10 ml.

The above materials were pulverised and ground and made into a paste. The udder was cleaned and washed with warm water initially. After drying the prepared herbal paste was applied externally.
3.3.6 Preparation of herbal medicine for Foot and Mouth disease

3.3.6.1 Herbal medicine for internal use

Trigonella foenum (fenugreek) : 50 gm
Leaves of Coriandrum sativum (coriander) : 100 gm
Cuminum cymimum : 50 gm
Powdered Curcuma longa (turmeric) : 10 gm
Leaves of Azadirachta indica (neem) : 100 gm
Shreds of Cocos nucifera (coconut shreds) : 100 gm
Jaggery : 50 gm

The above herbal plants were ground well and formed as a bolus and given to the cow affected with foot and mouth disease for 3 times a day for 5 days.

3.3.6.2 Herbal medicine for external use

Leaves of Acalypha indica (Kuppaimeni) : 100 gm
Powdered Curcuma longa (turmeric) : 10 gm
Allium sativum (garlic) : 20 gm
Common salt : q.s

The above mixture was ground well and the paste was mixed in castor oil and applied on the affected areas.

3.4. Clinical evaluation of selected ethno veterinary practices

3.4.1 Mastitis in Dairy cows

3.4.1.1 Clinical examination of the animal affected by mastitis

Clinical examination and physical examination of the mammary gland was examined as per the procedure suggested by Sharma et al. (2009)
3.4.1.2 Estimation of blood parameters and serum Chemistry

Blood and milk samples were taken from cows diagnosed with subclinical mastitis, and from healthy cows. Blood samples were collected by jugular venepuncture with the use of heparin as anticoagulant. After collection, all the sample was immediately transferred to the laboratory for performing somatic cell counting and determination of biochemical parameters.

3.4.1.3 California Mastitis Test

California mastitis test was performed by using the kit supplied by B.V.Biologicals, India. Fresh, unrefrigerated milk was subjected to this test to ascertain mastitis as per the procedure detailed by Sharma et al., (2009) and the results were noted.

3.4.1.4 Surf field mastitis test

This test is cheap and has enough sensitivity to detect all cases of subclinical mastitis (Muhammad et al., 1995). The advantage of this test is that the readily available household detergent may be used as the reagent. The reaction of somatic cells DNA with detergent and leads to the formation of gel of varying degree depending upon the number of somatic cells in the milk. 3% surf solution was prepared. Equal quantity of 3% surf solution and milk was taken in a paddle. The mixture was swirled for about one minute and then examined visually for the presence of small floccules or gel. The formation of floccules or gel indicates positive for intra mammary infection. Absence of floccules or gel indicates negative infection.

3.4.1.5 Somatic Cell Count

The test milk samples were thoroughly mixed by gentle shaking of the vials and 0.01 ml of milk was taken on the pre-drawn 1 cm² marked area over a grease
free clean glass slide which was uniformly smeared with a standard sterilized platinum loop. The smear is dried and stained with the modified Newman’s Lampert stain by keeping the slide in the staining solution for 1 to 2 minutes. The smear was gently washed in tap water and dried. The dried smears were then examined under the oil immersion lens of the microscope.

3.4.1.6 Estimation of milk pH

Milk pH was estimated immediately after milking using an electronic pH meter and mass screening with pH paper supplied by Indian Herbs Ltd.

3.4.2 Foot and Mouth Disease (FMD) in Dairy cattle

3.4.2.1 Clinical examination of the animal affected by Foot and Mouth Disease

Clinical examination and physical examination of the animal which was affected by FMD was performed as per standard clinical procedures.

3.4.2.2 Estimation of blood parameters and serum Chemistry

Blood and milk samples were taken from cows diagnosed with FMD before and after herbal treatment. Blood samples were collected by jugular venepuncture with the use of heparin as anticoagulant. After collection, all the sample was immediately transferred to the laboratory for studying the haematological and biochemical parameters.

3.4.3 Coccidiosis in Broiler Chicken

3.4.3.1 Preparation of Aloe vera gel for the treatment of Coccidiosis

Aloe vera gel was extracted from the leaf of the Aloe vera by making a cut using a pocket- knife, the juice was allowed to drip in a glass jar. After extraction, the gel was poured in a pot, heated on fire to evaporate water, and the residue was
rolled into a ball and dried. The dry ball was ground into fine powder and stored in an air-tight container to avoid oxidation. Infected birds were housed in cages constructed out of wire mesh and metal poles.

3.4.3.2 Preparation of Broiler chicken for Clinical evaluation

The cages were divided into three distinct compartments with ten birds in each cage of 1 m² floor and feeding area. Wood shavings were used as bedding material for the birds. Feeders and drinkers made out of plastic containers were used i.e. one drinker and a feeder per two birds. Feeders and drinkers were tightly secured in each cage to avoid spillage during feeding. Broiler starter and finisher mash were fed to birds *ad libitum* as per routine management practices.

3.4.3.3 Design of clinical evaluation

Birds naturally infected with coccidiosis were grouped into two and cages in a separate place in cages.

- Healthy birds : 10 birds
- Birds treated with *Aloe vera* : 10 birds
- Birds treated with amprolium : 10 birds

One group of the infected bird was treated with the aloe extract and the other group was given standard treatment for coccidiosis using allopathic drugs. One gram of aloe powder was used to treat ten birds in a cage for seven days. The optimal dosage rate of administering the aloe drug was based on previous research work (Gundidza, 2001). After the first seven days of treatment one gram of dropping was taken from each treatment for coccidia Oocyst analysis. The results were tabulated.
3.4.3.4 Counting of Oocysts

A sample of one gram of droppings was taken from each specimen, then mixed with 9.5 ml of physiological saline, sieved in a 150 micro-mesh to remove debris and poured in test tube and centrifuged at 1.5 RPM for 5 minutes. Heavy material in the droppings settled at the bottom and the supernatant was discarded. The material that remained in the test tube was mixed with 15 ml Aluminium Nitrate and 10 % (1.5 ml) of the contents were poured on a fill chamber. The number of oocysts were counted under a microscope and results were recorded.

3.5 Experimental study

Experimental studies were conducted in white wistar rats in order to evaluate the sequence of changes that has taken place due to the administration of single fresh herbal extract and fresh poly herbal extract.

3.5.1 Experimental animals

The Institutional Animal Ethics Committee of Raja Muthiah Medical College, Annamalai University approved the animal studies. The experimental study was carried out in 72 white wistar rats (*Rattus norvegicus*) of either sex weighing between 150 to 200 grams, aged between three and six months were kept in open top cages of standard dimensions with stainless steel top grills.

Housing conditions of experimental animals

All the animals were kept in an identical environmental condition to prevent the influence of external factors during the period of study. The maximum number of animals in each cage was six. Dried rice husk was opted as a bedding material. Bedding material was changed on every alternate days. All the animals were kept under standard management conditions as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals
(CPCSEA), and were maintained under a controlled environment with temperature at 23 ± 2°C, relative humidity at 55 ± 5 %, and a 12-hours/12 hours light/dark cycle throughout the experiment. The animals were fed on a standard pellet diet. The animals were housed in experimental cages for one week before the commencement of the experimental study so as to enable them to acclimatize to the environment.

3.5.3 Feeding the experimental animals

The animals were fed with balanced commercial brown coloured pelleted rat feed to avoid any nutritional deficiency and subsequent interference with the experimental study. Food and water were provided ad libitum.

3.5.3.1 Nutritional pellet for the experimental animals


3.5.3.2 Proximate analysis of the feed

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Proximate Principle</th>
<th>Value (%)</th>
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<tbody>
<tr>
<td>1</td>
<td>Moisture</td>
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</tr>
<tr>
<td>2</td>
<td>Crude Protein</td>
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</tr>
<tr>
<td>3</td>
<td>Crude fat</td>
<td>03.65</td>
</tr>
<tr>
<td>4</td>
<td>Crude fibre</td>
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</tr>
<tr>
<td>5</td>
<td>Calcium</td>
<td>01.26</td>
</tr>
<tr>
<td>6</td>
<td>Phosphorus</td>
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</tr>
<tr>
<td>7</td>
<td>Total ash</td>
<td>06.88</td>
</tr>
</tbody>
</table>
3.5.3.3 Oral feeding of aqueous extract of *Aloe vera*

3.5.3.4 Experimental design (*Aloe vera* Group)

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
<th>Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Control group</td>
<td>6</td>
</tr>
<tr>
<td>Group 2</td>
<td>Fed with 50 ppm of aqueous <em>Aloe vera</em> extract</td>
<td>6</td>
</tr>
<tr>
<td>Group 3</td>
<td>Fed with 100 ppm of aqueous <em>Aloe vera</em> extract</td>
<td>6</td>
</tr>
</tbody>
</table>

The aqueous extract of the preparation was fed to the experimental animals in appropriate concentration as mentioned above according to its body weight. Blood samples were collected on 15 days, 30 days, 45 days and 60 days of experiment.

3.5.3.5 Oral feeding of aqueous extract of *Ocimum sanctum* leaf

3.5.3.6 Experimental design (*Ocimum sanctum* Group)

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
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<tr>
<td>Group 3</td>
<td>Fed with 100 ppm of aqueous <em>Ocimum sanctum</em> extract</td>
<td>6</td>
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</tbody>
</table>

The aqueous extract of the preparation was fed to the experimental animals in appropriate concentration as mentioned above according to its body weight. Blood samples were collected on 15 days, 30 days, 45 days and 60 days of experiment.
3.5.6 Oral feeding of aqueous extract of *Phylanthus niruri*

3.5.6.1 Experimental design (*Phylanthus niruri* Group)

- **Group 1**: Control group - 6 animals
- **Group 2**: Fed with 50 ppm of aqueous *Phylanthus niruri* extract - 6 animals.
- **Group 3**: Fed with 100 ppm of aqueous *Phylanthus niruri* extract - 6 animals.

The aqueous extract of the preparation was fed to the experimental animals in appropriate concentration as mentioned above according to its body weight. Blood samples were collected on 15 days, 30 days, 45 days and 60 days of experiment.

3.5.7 Oral feeding of aqueous Poly herbal extract

3.5.7.1 Experimental design (Poly herbal extract Group)

- **Group 1**: Control group - 6 animals
- **Group 2**: Fed with 50 ppm of aqueous Poly herbal extract - 6 animals
- **Group 3**: Fed with 100 ppm of aqueous Poly herbal extract - 6 animals.

3.5.8 Clinico -Pathological studies

3.5.8.1 Collection of blood for haematological and enzyme studies

The rats were put on dorsal recumbency and the skin on the ribs and sternum was held taut using the left thumb and index fingers. Using a 22G needle attached to a 5ml syringe, 3ml of blood was aspirated from the heart into labeled
tubes containing the sodium salt of ethylenediamine tetraacetic acid (Na-EDTA) anticoagulant, and gently mixed to prevent clotting.

3.5.8.2 Evaluation of Haematological parameters and indices

Packed cell volume (PCV), haemoglobin (Hb) concentration, erythrocyte (RBC), total leucocyte (WBC), Mean corpuscular volume (MCV) and Mean corpuscular haemoglobin concentration (MCHC) were determined by standard techniques (Jain, 1986). After the centrifugation of the unclotted blood at 1200 rpm for 10 minutes, the plasma was carefully removed and used for the estimation of the enzymes.

3.5.8.3 Evaluation of serum chemistry

Total protein, albumin, globulin, urea and creatinine asdescribed by Ogunsanmi et al., (1994). Others include activities of plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT), measured by the procedures of Reitman and Frankel (1957), alkaline phosphatase (ALP) by a modified method of Frajola et al. (1965) and Aspartate amino transferase (AST) and Alanine amino transferase (ALT) by the method of Szas (1969). The plasma total cholesterol and triglyceride were determined as described by Toro and Ackermann (1975).

3.5.8.4 Collection of tissues for histopathological studies

The rats were anaesthetized and dissected using standard procedure and gross changes in the organs were observed and recorded. Sections of the intestines, liver, lungs, kidney, heart and brain of each rat were harvested into labelled sample
bottles containing 10% phosphate-buffered formalin fixative for 24 hours. They were thereafter, trimmed and dehydrated in graded concentrations of xylene, embedded in wax and sectioned at 5μ and fixed on to clean, grease-free glass slides. The thin sections were stained with haematoxylin and eosin (H&E) for histologic examination under the light microscope.

3.5.9 Statistical Analysis

The means, standard deviations and differences between the various measured parameters were calculated using appropriate statistical packages to see whether there is any significance or not. ANOVA was used with P value <0.05. For analyzing the experimental studies, the result obtained are expressed as mean ± S.D of six rats in each group.