Training manual

on

Advances in risk analysis and GIS based prediction modeling of livestock parasitic diseases

23rd October to 1st November, 2017

Training Manual

Compiled and Edited by

Dr. P. P. Sengupta
Dr. K. P. Suresh
Dr. Siju Susan Jacob
Mr. A. G. S. Chandu

Organized by

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Post Box No. 6450,
Yelahanka, Bengaluru-560064
Acknowledgement

We wish to express our sincere gratitude to Indian Council of Agricultural Research for selecting our training proposal and sponsoring the programme.

We are grateful to the DDG (AS), DDG (HRD), ADG (HRD), ADG (AH), for their support, encouragement and best wishes.

We wish to express our gratitude to our beloved Director Dr. Parimal Roy for his guidance and constant encouragement.

Our sincere thanks are due to the authorities of the institutes/universities who granted deputation of the participants.

The accommodation and canteen facility provided by SRS, IVRI, Bengaluru and NBAIR, Bengaluru are gratefully acknowledged.

It is our pleasant duty to express our gratitude to all the external and internal faculty members of this training programme, for taking pain to preparation of write-up, delivering lecture presentation and practical demonstration. Our sincere thanks are also due to all the committee members of this training programme for their generous help.

We offer our sincere thanks to all the scientific, technical, administrative, financial, supporting staffs, JRFs, contractual staffs who assist us to run the training programme smoothly.

Dr. P.P. Sengupta (Course Director)
Dr. K.P. Suresh (Course Co-ordinator)
Dr. S.S. Jacob (Course Co-ordinator)
MESSAGE

I am immensely pleased to know that ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), Bengaluru is organizing an ICAR-sponsored Short Course on ‘Advances in Risk Analysis and GIS Based Prediction Modelling of Livestock Parasitic Diseases’ from 23rd October to 1st November, 2017 at Bengaluru.

Parasitic diseases although, cause heavy economic losses of the resource poor livestock farmers quite often, they are largely ignored as mostly they cause subacute and chronic disease conditions. The topic of the Short Course is a priority thrust area and expected to provide important platform for exchange of scientific information and intervention procedures/methods to be used for prediction modelling of parasitic diseases for their control and also help to identify the various risk factors for development of precision models.

I congratulate the organizers for taking this initiative in conducting this course which will definitely benefit the scientists/stakeholders for better animal health management practices for control of the parasitic diseases.

I wish this endeavour a grand success.

(Joykrushna Jena)

Place: New Delhi
Date: 13.10.2017
MESSAGE

It gives me immense pleasure that National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), Bengaluru is organizing ICAR-sponsored short course on ‘Advances in risk analysis and GIS based prediction modelling of livestock parasitic diseases’ from 23rd October to 1st November, 2017.

Epidemiology essentially meant to understand the distribution and determinants of health related states or events in specified populations and the application of this study to control health problems. Understanding the association between risk factors (exposures) and diseases occurrence is of extreme importance in order to strategise the control measures against diseases. I hope that this short course will enlighten the scientists to strengthen their knowledge in the area of epidemiology and disease informatics.

Best wishes for the short course

(M.B.Chetti)
MESSAGE

It is heartening to know that National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), Bengaluru is organising ICAR-sponsored short course on ‘Advances in risk analysis and GIS based prediction modelling of livestock parasitic diseases’ from 23 October to 1 November, 2017.

Being a tropical country, India is embraced with different parasitic diseases causing severe production losses. It is essential to adopt a concrete surveillance system based on accurate analysis of risk factors and prediction systems for prevention and control of these diseases. I am sure that the short course pertaining to epidemiological studies of parasitic diseases will help the scientists from different institutes to analyse the epidemiological data and to draw proper conclusions and future suggestions for the effective control of parasitic diseases.

I wish the short course all the success and compliment the organizers for selecting the appropriate theme for deliberations.

(Ashok Kumar)
Dr. Parimal Roy  
Director  

MESSAGE

I am pleased that ICAR- National Institute of Veterinary Epidemiology and Disease informatics (ICAR-NIVEDI) Bengaluru is organising an ICAR- sponsored short course on ‘Advances in risk analysis and GIS based prediction modelling of livestock parasitic diseases’ from 23rd October to 1st November, 2017.

NIVEDI under ICAR has a mandate to conduct R & D in the field of Veterinary Epidemiology and animal disease surveillance for the entire country. Its role is extremely pivotal in developing models for risk analysis, animal disease forecasting, forewarning, need based diagnostics and economic impact analysis of the diseases. As technology development and HRD process, institute has been conducting plethora of training programmes related to epidemiology, economic impact module for assessment, sampling frame, disease diagnosis, application of ELISA, offline and online forecasting softwares for various stakeholders associated with animal health especially scientists and veterinary professionals drawn from various parts of the country as part of capacity building in the area. I hope this short course will also help all the participants from different institutes to strengthen their capabilities in the area of epidemiology.
I wish the participants a pleasant stay and I wish the short course a great success.
The occurrence of parasitic diseases is based on multiple factors and often these factors are interactive. Understanding these risk factors that precipitate the occurrence of the disease is of extreme importance in order to strategize the control measures. Epidemiological data collection and analysis is the basis for formulating prevention and control policy as well as detecting the new emerging or re-emerging disease/pathogens. Geographic Information System (GIS) is employed to better understanding of epidemiology of livestock disease at macro level. These tools are very important for developing risk maps and forecasting models of livestock disease so as to take timely preventive measures in order to minimize the morbidity and mortality. Further, the livestock parasitic diseases have numerous impacts like productivity loss, loss of income, treatment and control costs etc., In animals, more specifically, loss of production (milk/meat/egg), loss of drought power, opportunity cost of labor, and treatment cost are important.

This short course is aimed at refreshing and strengthening skills of researchers in epidemiological data collection & analysis, risk factor analysis and prediction modeling associated with livestock parasitic diseases. I hope that, this training course, will strengthen the participant’s skills in collection and analysis of epidemiological data. The training will also refresh participants skills in sampling techniques and sample size estimation/requirement for epidemiological studies, epidemiological data collection (host, agent and environment) and tools required for data collection (using GPS), risk factors identifications (using questionnaire) for livestock diseases, generation of various epidemiological Maps (using GIS), etc., and participants will be able to assess the economic loss and its projection on their respective working diseases.

I express my sincere gratitude to the Human Resource Division of ICAR for giving us the opportunity to conduct this short course ‘Advances in risk analysis and GIS based prediction modeling of livestock parasitic diseases’ from 23rd October to 1st November, 2017 by sponsoring.

I extend warm welcome to all the participants to our institute and wish them a comfortable stay in this garden city and enriching their knowledge along with their learning experiences at ICAR-NIVEDI.

With warm regards,

P. P. Sengupta
(Course Director)
ICAR-NIVEDI
Convergence of Animal Health and Research Par Excellence

**Historical Background**

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), (Formerly, Project Directorate on Animal Disease Monitoring and Surveillance, PD_ADMAS) under the Indian Council of Agricultural Research (ICAR), a pioneer research institute in veterinary epidemiology is carrying out disease surveillance, monitoring and analysis of livestock diseases in India through 32 collaborative centers of AICRP_ADMAS located in different states of the country.

The AICRP on animal disease monitoring and surveillance (AICRP-ADMAS) initiated by the ICAR, made a humble beginning during the VIIth five-year plan and became fully functional in 1987 with establishment of four Regional Research Units (RRUs) at Bengaluru, Hyderabad, Pune and Ludhiana. The Central Coordinating Unit (CCU) was established at the Institute of Animal Health and Veterinary Biologicals, Bengaluru to co-ordinate research activities of the regional units. In the VIIIth plan, the institute was strengthened with support of ICAR and European Union by taking up the major responsibility under National Project on Rinderpest Eradication (NPRE) involving 32 state level diagnostic/disease investigation laboratories in the country. On 1st April, 2000 (during the IXth plan), the CCU was given the status of Project Directorate and named as ‘Project Directorate on Animal Disease Monitoring and Surveillance (PD_ADMAS)’ with ten collaborating units under AICRP_ADMAS component. In the Xth and XIth Five year plan period, five more collaborating units were added for providing impetus to a nationwide animal disease monitoring and surveillance.

Appreciating the contributions made by the Directorate to country’s livestock healthsector and the need to strengthen the effort, the council Rechristened PD_ADMAS as ‘National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI)’ on 25th October, 2013 (XIIth plan period) with its exclusive campus at Bengaluru. Further, during the same plan period, 17 additional collaborating units covering others states were added under AICRP_ADMAS component totaling to 32 collaborating units for providing the needed impetus to a strong nationwide animal disease monitoring and surveillance network. On 9th January, 2015, NIVEDI’s newly constructed administrative building and Biosafety Laboratory (BSL-2) was dedicated to the nation by Shri Radha Mohan Singh, Hon’ble Union Minister for Agriculture, New Delhi in the presence of Shri D. V. Sadananda Gowda, Hon’ble Minister of Law and Justice, GOI and Shri T. B. Jayachandra, Hon’ble Minister for Law, Justice & Human Rights, Parliamentary Affairs and Legislation and Animal Husbandry, Govt. of Karnataka and Dr. S. Ayyappan, Secretary DARE and Director General, ICAR. The centralized administrative and laboratory complex of the institute is located in a sprawling campus at Yelahanka, Bengaluru.

ICAR - National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), a pioneer research institute under Indian Council of Agricultural Research (ICAR) has been entrusted to conduct R&D in the field of veterinary epidemiology and surveillance of economically important livestock diseases in the entire country, its role is extremely pivotal for developing models for animal disease forewarning, forecasting, economic impact, risk assessment, and need based animal disease diagnostics. The institute has developed various technologies covering both products and processes and some of them are marketed.
and/or patented/copyright protected, which are being utilized by various institutes/organizations and different stakeholders in the country. The role of this institute in the eradication of Rinderpest disease in India and development of National Animal Disease Referral Expert System (NADRES) - interactive software for forecasting are noteworthy. The institute conducts various training programmes related to basic epidemiology, sampling frame and sampling techniques, outbreak investigation, research methodologies, disease diagnosis protocols for various stakeholders associated with animal healthcare. Overall, NIVEDI has been proving its worthiness to the Indian animal health sub-sector covering critical gaps in diagnostic techniques, animal disease modelling, economic impact assessment and analysis of animal diseases, human resource development in the form of skill development and empowerment, capacity building programme etc. Further, NIVEDI envisions to provide newer direction to undertake in-depth R&D activities on epidemiology of emerging and re-emerging, transboundary animal diseases to others involved in the sub-sector in the country, leading finally to prevention, control and eradication of the diseases for achieving animal welfare and safer animal – human interface under one health approach.

(i) Vision
Achieving freedom from animal diseases, animal welfare, food and nutritional security through healthy foods of animal origin, poverty alleviation and economic growth of rural India.

(ii) Mission
Capacity building in frontier areas of Veterinary Epidemiology: dynamics of animal diseases including zoonosis and animal healthcare intelligence.

(iii) Focus
- Improving disease monitoring and surveillance through development of penside diagnostics
- Risk assessment for occurrence of economically important animal diseases
- Adapting strategies to improve animal disease data quality
- Understanding the threat from animal diseases in the background of climate change and globalization
- Developing early warning system and disease modeling/forecasting
- Understanding economic impacts of animal diseases and the management strategies
- Promoting innovations and improving human resource capacity
- Fostering linkages and collaborations with public and private, national and international organizations
- Improving knowledge management system

(iv) Mandate of Institute
- Epidemiology, informatics and economics of animal diseases including zoonosis
- Surveillance, forecasting and forewarning for management of animal diseases including Zoonosis
- Repository and Capacity Development

(v) AICRP on ADMAS
All AICRP collaborating units are extensively working on animal disease diagnosis, outbreak investigation, disease reporting, pathogen characterization and mapping etc., with major focus on bacterial (Brucellosis, Leptospirosis, Mastitis, Haemorragic Septicaemia, Anthrax, Black Quarter, Enterotoxaemia), viral (Infectious Bovine Rhinotracheitis, Bluetongue, classical Swine Fever, Peste des Petits Ruminants and Sheep and Goat Pox) and parasitic Trypanosomosis, Theileriosis, Babesiosis, Fascioliosis and Amphisomiosis) diseases of economic importance with the following mandates.
Mandates of AICRP on ADMAS

❖ Sero-monitoring of animal diseases based on sample frame,
❖ Investigation of endemic, emerging and re-emerging animal disease outbreaks using innovative technologies,
❖ Strengthening of National Livestock Serum Repository,
❖ Effective updating of NADRES with active disease data and climatic and non-climatic risk-factors,
❖ Utilization of forecasting models through NADRES for forecasting and forewarning of animal diseases,
❖ Analysis on economic losses due to animal diseases and the control measures adopted for their management, and
❖ Surveillance of diseases/pathogens of companion, laboratory and wild animals.
LECTURE NOTES
<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Topics</th>
<th>Page No:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Impact of Parasitic Diseases in Farm Animals</td>
<td>1-2</td>
</tr>
<tr>
<td></td>
<td><em>P.P. Sengupta and Siju Susan Jacob</em></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Disease Pattern of Major Parasitic Diseases</td>
<td>3-15</td>
</tr>
<tr>
<td></td>
<td><em>P.P. Sengupta and Siju Susan Jacob</em></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Disease pattern of major helminth parasitic diseases in animals</td>
<td>16-21</td>
</tr>
<tr>
<td></td>
<td><em>Siju Susan Jacob and P.P. Sengupta</em></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>National Animal Disease Referral Expert System (NADRES)</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td><em>Divakar Hemadri, K. P. Suresh and Sharana Gouda S. Patil</em></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Basic epidemiological concepts- Siju Susan Jacob and P. P. Sengupta</td>
<td>23-27</td>
</tr>
<tr>
<td>6</td>
<td>Geographical Epidemiology, Spatial Analysis and Livestock Disease</td>
<td>28-32</td>
</tr>
<tr>
<td></td>
<td>Information System- K.P.Suresh, S.S. Patil and D. Hemadri</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>The role of vectors in the transmission of Haemoprotozoan diseases and</td>
<td>33-47</td>
</tr>
<tr>
<td></td>
<td>their control strategies- Placid E. D’Souza</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Integrated management of Parasitic diseases- P.M. Thimmareddy</td>
<td>48-51</td>
</tr>
<tr>
<td>9</td>
<td>Collection and dispatch of samples for Parasitic disease Investigatons</td>
<td>52-59</td>
</tr>
<tr>
<td></td>
<td>R.Sridevi</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Application of GIS in understanding the Spatial Epidemiology of Babesiosi</td>
<td>60-61</td>
</tr>
<tr>
<td></td>
<td>s in Karnataka</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Md. Mudassar Chanda, Siju Susan Jacob and P.P. Sengupta</em></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Disease surveillance by Enzyme-linked Immunosorbent Assays: A powerful</td>
<td>62-70</td>
</tr>
<tr>
<td></td>
<td>laboratory test- Rajeswari Shome</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Art and science of parasitic disease investigation- P. Krishnamoorthy</td>
<td>71-73</td>
</tr>
<tr>
<td>13</td>
<td>Application of Geographical Information Systems (GIS) in Natural</td>
<td>74-79</td>
</tr>
<tr>
<td></td>
<td>Resource Management- S. Srinivas</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Detection of microbe genome by real time quantitative polymerase chain</td>
<td>80-82</td>
</tr>
<tr>
<td></td>
<td>reaction (qRT-PCR)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>G.B. Manjunatha Reddy, Yogisharadhya R and Apsana R</em></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Establishment and Maintenance of National Livestock Serum Bank</td>
<td>83-87</td>
</tr>
<tr>
<td></td>
<td>Divakar Hemadri</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Livestock disease impact assessment – An introduction</td>
<td>88-90</td>
</tr>
<tr>
<td></td>
<td>G. Govindaraj</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Concept of antimicrobial resistance- B. R. Shome</td>
<td>91-95</td>
</tr>
<tr>
<td>18</td>
<td>Emergence of Anthelmintic Resistance- constraint to livestock sector</td>
<td>96-105</td>
</tr>
<tr>
<td></td>
<td><em>Dr. Siju Susan Jacob and Dr. P. P. Sengupta</em></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Gene Sequence Editing</td>
<td>106-108</td>
</tr>
<tr>
<td></td>
<td><em>M. Nagalingam, V. Balamurugan, Rajeswari Shome</em></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>DNA Sequencing methods and its application- V. Balamurugan</td>
<td>109-114</td>
</tr>
<tr>
<td>21</td>
<td>Molecular Epidemiology of Parasitic Diseases</td>
<td>115-119</td>
</tr>
<tr>
<td></td>
<td><em>Dr. Siju Susan Jacob and Dr. P. P. Sengupta</em></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Animal Quarantine and Certification Services (AQCS)</td>
<td>120-122</td>
</tr>
<tr>
<td></td>
<td><em>S.S. Patil, K.P. Suresh, D. Hemadri, GBM Reddy and Parimal Roy</em></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Identification, Isolation and Maintenance of Parasite in vivo and in</td>
<td>123-126</td>
</tr>
<tr>
<td></td>
<td>vitro- P.P. Sengupta and Siju Susan Jacob</td>
<td></td>
</tr>
</tbody>
</table>
Impact of Parasitic Diseases in Farm Animals

Dr. P.P. Sengupta and Dr. Siju Susan Jacob

ICAR- National Institute of Veterinary Epidemiology & Disease Informatics, Bengaluru 560064

Introduction

Being a tropical country India is embraced with so many parasitic diseases in farm animals. In any veterinary dispensary in the country almost 70% cases are of parasitic in nature. The impact of parasitic diseases on the livelihood of the farm owners is tremendous. Sometimes parasitic diseases of animals are transmissible to human also.

1. Mortality vs. Morbidity: Unlike bacterial and viral diseases, most of the parasitic diseases are highly endemic and chronic in nature. In most of the cases mortality is less but morbidity is high in shape of losses in body weight gain, meat/ milk/ egg production, reproductive failure, draughtability etc. Unfortunately, as there is no visible loss (death), many times the losses have been ignored. But a minor daily losses can be impacted a huge cumulative losses for a prolong period and may be sometime more than the mortality loss. Say for example the losses due to surra have been estimated in Philippines where identical husbandry practices (like India) are followed. In a moderately endemic area it was US $88, $84, $151, $7, $114 per animal/year respectively for buffaloes, cattle, horses, goats/sheep and pigs (Dobson et al., 2009).

2. Reproductive Losses: Many time there may not be death but losses may occur due to reproductive failure. Though in India it has not been estimated but the same has been done in abroad. For example in bovine neosporosis in only California district in US it costs US$ 35 million per year (Dubey and Lindsay, 1996), whereas the figure was NZ $ 70.8 million in New Zealand and AUS $ 85 million in Australia (Reichel, 2000).

3. Intensity of infection
The intensity of parasitic infections depends upon both the quantity and the virulent sp. Involved.e.g. 4000 Trichostrongylus ~ 3000Ostertagia ~ 500 Haemonchus worms.

4. Loss of Nutrition: Many parasites like hook worms are vigorous blood suckers and thus cause blood loss. Trypanosomes uptake huge glucose from the blood of hosts.

5. Poor absorption: Some parasites remain attached with the mucosal layer of the intestine/ stomach resulting in poor enzyme secretion absorption and malnutrition. Eg. Gasterophilus larva in stomach of horse.

6. Carrier animals: Many times the animals may remain as carriers without showing any clinical sign. But in such state there will be a loss of production and the affected animal may act as the nucleus of the source of infection.

7. Zoonotic Infection: Many animal parasitic diseases are transmissible to human and are able to cause disease in humans especially those who handles the animals e.g. toxoplasmosis, cysticercosis etc. Thus in many cases the farmers or animal owners become victims.
8. **Vectors**: Many parasites can act the vector of other diseases like bacterial or viral; sometime they may be transmissible to human also. e.g. *Culicoides* transmit bluetongue virus, fleas (*Xenopsylla cheopis*) transmit *Yersinia pestis* (human plague), *Aedes* mosquito for dengue/ zika virus etc.

9. **Unaesthetic look**: In some parasitic infections, the affected animal get unaesthetic look and its market value get reduced. In mange infections the alopecia, thick keratinized skin decreased the market value of goats/sheep.

10. **Secondary infections**: Sometime parasitic infection invites other bacterial infection which altogether aggravates the condition. On skin mange infection can add secondary bacterial/mycotic infections.

11. **Suppression of Immunity**: Already existing parasitic infection may inhibit the desired host immune response after vaccination for bacterial/viral diseases (vaccine failure).

12. **Who are the ultimate losers?**
   For most of the parasitic diseases vaccines are not available. Mainly their control measure involves day to day managerial care and surveillance. Many small and marginal farmers specially who belong to below poverty line and rear small ruminants for their subsistence very often cannot afford such expense for daily management, become vulnerable to economic crisis when there is any loss due to parasitic infestations.

**References:**

Disease Pattern of Major Parasitic Diseases
Dr. P. P. Sengupta and Dr. Siju Susan Jacob

ICAR- National Institute of Veterinary Epidemiology & Disease Informatics,
Bengaluru 560064

Protozoa are ubiquitous throughout aqueous environments and the soil, and play an important role in their ecology. Farm animals are usually infected with several species of parasites and they are also confined to pasture or pens. Many times they are transmitted through vector; however some time direct transmission is also observed. Some are also transmissible to humans. The important protozoan diseases in farm ruminants are:

Babesiosis

Babesiosis is an infectious tick-borne disease of livestock that characterised by fever, anemia, haemoglobinuria and weakness. The disease also is known by such names as bovine babesiosis, piroplasmosis, Texas fever, red water fever, tick fever etc. The disease also is a hemoparasitic disease caused by protozoa of the genus *Babesia* which infects mainly ruminants. Infection of a vertebrate host is initiated by inoculation of sporozoite form of parasites into the blood stream during the taking of a blood meal by tick vectors.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Host affected</th>
<th>Geographical distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. bigemina</em></td>
<td>Cattle</td>
<td>Americas, Europe, Africa, Australia, Middle East</td>
</tr>
<tr>
<td><em>B. bovis</em></td>
<td>Cattle</td>
<td>Americas, Europe, Africa, Asia Australia</td>
</tr>
<tr>
<td><em>B. major</em></td>
<td>Cattle</td>
<td>Europe, North Africa, Middle East</td>
</tr>
<tr>
<td><em>B. jakimovi</em></td>
<td>Cattle</td>
<td>Asia</td>
</tr>
<tr>
<td><em>B. ovata</em></td>
<td>Cattle</td>
<td>Asia</td>
</tr>
<tr>
<td><em>B. divergens</em></td>
<td>Cattle</td>
<td>Europe</td>
</tr>
<tr>
<td><em>B. motasi</em></td>
<td>Sheep, goats</td>
<td>Europe, southest Asia, Middle East</td>
</tr>
<tr>
<td><em>B. ovis</em></td>
<td>Sheep, goats</td>
<td>Europe, southeast Asia, Middle East</td>
</tr>
</tbody>
</table>

Bovine babesiosis

Bovine babesiosis associated with *B. bigemina* and *B. bovis* is the most important disease of tropical and subtropical regions. Both species are transmitted transovarially by *Boophilus* ticks, but only tick larvae transmit *B. bovis*, whereas nymphs and adults transmit *B. bigemina*. In Europe, babesiosis is caused by *Babesia divergens*, an intraerythrocytic parasite that can persist for >13 months in the organs of infected cattle. The distribution of *B. divergens* reflects its triphasic telotrophic tick vector, *Ixodes ricinus*, which is widespread across Western Europe and North Africa. *B. major* occurs in Europe, North Africa and South America. *B. major* is transmitted by the three host tick *Haemaphysalis* sp. (Taylor et al., 2007).

Babesiosis in sheep and goats

In sheep and goats, babesiosis is associated with *B. ovis* and *B. motasi*, *Babesia ovis* occur in Southern Europe, former Soviet State, Middle East and Asia. *Rhipicephalus* bursa has been shown to be a vector for *B. ovis*. *Babesia motasi* occur in Southern Europe, the Middle East, the former Soviet State, southeast Asia and Africa. The parasite transmitted by ticks of the genus *Haemaphysalis* (*H. Punctata, H. Otophila*), *Dermacentor* (*D. silvarum*) and *Rhipicephalus* (*R. bursa*) (Taylor et al., 2007).

Pathogenesis
*Babesia* spp. are a various group of tickborne, obligate, intra-erythrocytic Apicomplexan parasites infecting a wide variety of animals. Ticks are most often infected transovarially. The female tick becomes infected by the ingestion of parasites during engorgement. After it drops off the host, the babesial agents reproduce within the tick’s tissues. Some of the reproducing organisms are incorporated within developing tick embryos, and the disease agents are transmitted to new hosts by the feeding of ensuing tick larvae, nymphs, or adults (Zaugg, 2009). *B. bovis* is the most pathogenic of the bovine *Babesia*. *B. bigemina* infections are not as virulent as those of *B. bovis*, however the parasites may infect 40% of the red cells (Taylor *et al.*, 2007).

### Clinical findings

#### Cattle

Incubation period is 2-3 weeks. *B. bigemina* and *B. bovis* produce acute syndromes which are clinically indistinguishable, and are characterized by high fever (41°C), anorexia, depression, weakness, cessation of rumination, and a fall in milk yield. Hemoglobinuria can be seen, the color of urine is dark-red to brown. Respiratory and heart rates are increased, and the red conjunctivates and mucous membranes change to the extreme pallor of severe anemia. Abortion occur in pregnant animals. Subacute syndrome also occurs in young animals, but fever is mild and hemoglobinuria is absent (Radostits *et al.*, 2008). In cerebral babesiosis, hyperexcitability, convulsions, opisthotonos, coma, and death, may be observed in cattle infected with either *B. bigemina* or *B. bovis*, but especially with the *B. bovis*. Central nervous system signs are caused by brain anoxia resulting from severe anemia (Zaugg, 2009).

#### Sheep

In sheep develop fever and parasitemia within 2 to 4 days; the clinical signs of the disease include anorexia, listlessness, anemia, moderate jaundice and hemoglobinuria. In general, hyperthermia returned to normal on the fourth day after the peak pyrexia, and parasitemia is eliminated within the course of the disease.

### Diagnosis

Blood smears and clinical findings are useful in acute cases of piroplasmosis, but are not sufficient in subclinical cases. The complement fixation test is used serological test for bovine babesiosis. The most commonly used tests are ELISA, PCR and a DNA probe, which can detect specific parasitemias at very low levels of infection (Radostits, 2008). Recently, the ‘reverse line blot (RLB) is found to be a versatile technique for simultaneous detection and identification of small ruminant piroplasm species, based on the recognition of specific gene regions by oligonucleotide probes.

### Treatment

After the hemoglobinuria or cerebral signs, prognosis is not well. In acute cases that PVC values are above 12%, treatment will be successful. Supportive therapy such as blood transfusions (4 L of whole blood per 250 kg of body weight), fluids, hematinsics, and prophylactic antibiotics are important (Zaugg, 2009). Babesiosis can be treated using diminazene aceturate (3-5 mg/ kg), phenemidine disethionate (8-13 mg/ kg), imidocarb dipropionate (1-3 mg/kg), and amicarbalide diisethionate (5-10 mg /kg) (Zaugg, 2009).

### Control

The control of the disease depends on effective quarantine to prevent the introduction of the vector tick. The control of ticks by dipping or spraying animals at risk with recommended acaricides. In routine surgery, Care should be taken to prevent accidental transfer of blood from one animal to another (e.g., castration, dehorning). In addition, in cattle, the selection and breeding of cattle which acquire a high degree of resistance to ticks is practiced. Widespread use of tick vaccines may also have a significant influence on the incidence of infection in cattle (Taylor *et al.*, 2007; Radostits *et al.*, 2008; Zaugg, 2009).
Theileriosis

Theileriosis is caused by *Theileria* spp. in cattle, goats, sheep and wild and captive ungulates. Theileriosis is a hemoparasitic disease caused by protozoa of the genus *Theileria* (Apicomplexa). *Theileria* species affect domestic and wild ruminants, especially in Africa, Europe, Australia, and Asia. The parasites are transmitted by tick. These parasites undergo repeated merogony in the lymphocytes ultimately releasing small merozoites, which invade the red cells to become piroplasms. Theileriosis, have a variety of tick vectors which cause infections ranged from clinically inapparent to rapidly fatal.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Host affected</th>
<th>Geographical distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. annulata</em></td>
<td>Cattle, domestic buffalo</td>
<td>Mediterranean countries, Middle East, Indian subcontinent and China</td>
</tr>
<tr>
<td><em>T. orientalis</em></td>
<td>Cattle</td>
<td>Southern Europe, Middle East, Asia, Australia</td>
</tr>
<tr>
<td><em>T. mutans</em></td>
<td>Cattle</td>
<td>Africa, Caribbean Islands</td>
</tr>
<tr>
<td><em>T. taurotragi</em></td>
<td>Cattle</td>
<td>Africa</td>
</tr>
<tr>
<td><em>T. velifera</em></td>
<td>Cattle</td>
<td>Africa</td>
</tr>
<tr>
<td><em>T. hirci</em></td>
<td>Sheep and goats</td>
<td>North and East Africa, Middle East, India</td>
</tr>
<tr>
<td><em>T. ovis</em></td>
<td>Sheep and goats</td>
<td>Europe, Africa, India</td>
</tr>
</tbody>
</table>

Epidemiology

In bovine, Tropical Theileriosis (Mediterranean coast fever), occurs in Mediterranean countries, Middle East, Indian and China, and is caused by *T. annulata* (Radostits et al., 2008). The tick vectors are *Hyalomma dentritum* in north Africa and in the Mediterranean countries, *H. dentritum* and *H. dromedari* in central Asia, and *H. marginatum* in India (Taylor et al., 2007; Radostits et al., 2008). In endemic areas indigenous cattle are relatively resistant while cross bred cattle, especially European breeds, are highly susceptible (Taylor et al., 2007). *Theileria orientalis* complex is a milder disease than East coast fever and Tropical Theileriosis, and called benign theileriosis in cattle (Radostits et al., 2008). The tick vectors are *Amblyomma variegatum*, *A. cohaerens*, *A. haemaphysal rais* and *Haemaphysalis bispinosa* are the probable vectors in Australia (Taylor et al., 2007). *Theileria hirci* causes malignant theileriosis of small ruminants, and is enzootic in North Africa, Middle East, China and India. The tick vectors are *Rhipicephalus bursa* and *Hyalomma anatolicum* (Taylor et al., 2007). *Theileria ovis* is benign theileriosis in Africa (Radostits et al., 2008). But in China *Theileria ovis* is a serious disease. The incidence and mortality rates of *Theileria ovis* in adult sheep and goats were found 17.12% and 65.78%; 8.06% and 73.33% respectively in China (Shuzhen et al., 2002).

Pathogenesis

Pathogenesis of different form of theileriosis is based on the production of schizonts in lymphocytes and piroplasms in erythrocyte. *T. parva*, *T. annulata* and *T. hirci* produce highly schizonts and piroplasma and are very pathogenic; *T. mutans*, *T. buffeli*, and *T. ovis* unusually produce schizonts but may cause varying degree of anemia when piroplasms are highly in red blood cells (Radostits et al., 2008).

Clinical findings

*Theileia* spp. are classified in to 2 groups. In first group (*T. parva* and *T. annulata*), proliferate is seen in lymphocytes but in the second group (*T. orientalis*) it is seen in erythrocytes that causes hemolytic anemia (Magona et al., 2010). In *Theileria parva* incubation period is approximately 1-3 weeks. One or two days later, the first clinical sign is generalized swelling of the superficial lymph nodes, eyes, ears and submandibular regions. After few days there is anorexia, decreased milk production, loses condition, ceases
rumination, rapid hearth beat, petechial haemorrhages under the tongue and on the vulva. In cerebral theileriosis there are localized nervous signs and convulsions, tremor, salivation and head pressing (Radostits et al., 2008). In Theileria annulata, pyrexia, anorexia, enlargement of superficial lymph nodes, nasal and ocular discharges and salivation is the most common signs. Constipation is recorded in some cases. Respiratory distress, coughing and pulmonary oedema are seen.

In Theileria orientalis clinical signs are associated with anemia, jaundice and lymphadenopathy. Clinical signs in Theileria velifera are not reported but in Theileria taurotragi mild fever and anemia are reported. In Theileria hicri which is very pathogenic in sheep and goats, in the acute form there is fever, inappetence, ceases rumination, weakness, enlargement of superficial lymph nodes, diarrhoea, jaundice, haemorrhage in submucous may occur. In chronic form there is fever, inappetence, emaciation, anemia and jaundice (Taylor et al., 2007). Theileria ovis that is benign theileriosis in sheep and goats clinically is mild (Taylor et al., 2007).

**Diagnosis**

Investigation of giemsa-stained blood smears and lymph node biopsy will reveal piroplasms in erythrocytes and schizonts in lymphocytes. For diagnosis, indirect fluorescent antibody test (IFAT) and indirect enzyme-linked immunosorbent assay (ELISA) are the most commonly used techniques. The ELISA test is more sensitive than IFAT.

**Treatment and control**

Buparvaquone is the most effective drug and the recommended dose in cattle, sheep and goat is 2.5mg /kg BW. In control of the disease use of genetically resistant breed, a judicious and selective application of acaricides at 3-week intervals and the use of vaccines are recommended.

**Trypanosomosis**

Trypanosoma evansi, a haemoflagellate extra cellular protozoan parasite causes a disease known as trypanosomosis (‘surra’). It affects a number of species of domesticated animals in Asia, Africa and central and south America. The host species affected are bubalines, bovines, dromedarines, equines, felines, canines. Recently it has also been reported several times from human host. The tryps are transmitted mechanically by haematophagous flies – Tabanus sp., Stomoxys sp. and Lyperosia sp.

Tsetse-transmitted trypanosomosis is a disease complex caused by several of these species, mainly transmitted cyclically by the genus Glossina (tsetse flies), but also mechanically by biting flies. Tsetse infest 10 million square kilometres and affect 37 countries, mostly in Africa, where it is known as ‘nagana’. The disease infects various species of mammals but, from an economic point of view, tsetse-transmitted trypanosomosis is particularly important in cattle (also referred as tsetse-fly disease in southern Africa). It is mainly caused by Trypanosoma congolense, T. vivax and, to a lesser extent, T. brucei brucei. Trypanosoma vivax is also transmitted mechanically by biting flies, among which tabanids and stomoxes are presumed to be the most important, as exemplified by its presence in South and Central America, but also as observed in some areas of Africa free or cleared of tsetse (Ethiopia, Chad, etc.). Tsetse-transmitted trypanosomosis can affect camels and is a natural barrier preventing the introduction of this mammalian species into the southern Sahel region of West Africa. Horses are also highly sensitive. Very rare human cases have been observed caused by animal Trypanosoma species. However, tsetse transmitted trypanosomosis also affects humans, causing sleeping sickness, through infection with either T. brucei gambiense or T. brucei rhodesiense. A large range of wild and domestic animals can act as reservoirs of these humans parasites; particular care must be taken for people handling biological material that can contain infective human parasites, for example in livestock.
Chagas disease is caused due to *Trypanosoma cruzi* infection. The disease is a public health threat in most Latin American countries, although cases due to blood derivatives or blood transfusion has been reported in non-endemic regions. According to WHO the overall prevalence of human *T.cruzi* infection is estimated in 18 million cases and 100 million people are living at risk. The vectors are reduvidae bugs which are haematophagus and the most important are *Triatoma infestans* (Argentina, Chile, Brazil, Bolivia, Paraguay, Uruguay, Peru), *T. sordida* (Argentina, Bolivia, Brazil, Paraguay), *Rhodnius prolis* (Colombia, Venezuela, Mexico, Central America), *T. dimidiata* (Ecuador, Mexico, Central America), and *Panstrogylus megistus* (northeast Brazil). The transmission by the vector is faecal contamination.

**Pathogenesis**

After entering through the skin, parasite reach to the bloodstream via the lymphatic system. Infection characterized with parasitemia. Some *Trypanasoma* spp. invade extravascular spaces such as the ocular aqeous humor and cerebral spinal fluid. Some trypanasoma spp. may produce hemolysin that causes anemia in the host. Then, phagocytic activity increased because of the massive erythrocyte failure.

**Clinical findings**

Clinical findings are based on the speed of onset of anemia and the grade of organ impairment. Trypanasomosis can be acute, subacute, or chronic. In acute form abortion, drop in milk, depression, anorexia can be seen. Hyperemic mucous membranes and lacrimation also can occur. In subacute form clinical signs include weight loss, enlargement of lymph nodes and dry hair coat. In chronic form dull, dry hair coat, inelastic skin, lethargy, pale mucous membranes and exercise intolerance may be seen.

**Diagnosis**

Diagnosis can be based on the clinical findings, presence of vectors, appearance of trypanosomes on a fresh blood smear, or a Giemsa-stained blood smear. Indirect fluorescent antibody test (IFA) and the enzyme-linked immunospecific assay (ELISA) test are used for diagnosis. Different ELISAs employing recombinant antigens and monoclonal antibody have already been developed (Sengupta *et al.*, 2014, 2016; Ligi *et al.*, 2016; Rudramurthy *et al.*, 2015;2017). PCR based assay targeting VSG and ISG gene have also been developed (Sengupta *et al.*, 2010; Rudramurthy *et al.*, 2013)

**Treatment and control**

The most common drugs that is used for treatment of trypanosomosis are:
- Diminazene aceturate 3.5-7 mg/ kg BW.
- Homidium bromide and chloride 1mg /kg BW -Pyridium bromide 2mg / kg BW.
- Isometamidium 0.25-1mg / kg BW (Radostits *et al.*, 2008).

Vector control can help to control or prevent trypanosomosis. Insecticides can be used for preventing bites by tsetse flies and other flies.

**Sarcocystosis**

Sarcocystosis caused by Sarcocystis species in cattle, sheep and goats. The names of Sarcocystis species are according to their intermediate and final.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Intermediate Host</th>
<th>Definitive Host</th>
<th>Geographical Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. bovicanis</em> (<em>S. cruzi</em>)</td>
<td>Cattle</td>
<td>Dog</td>
<td>World wide</td>
</tr>
<tr>
<td><em>S. bovifelis</em> (<em>S. hirsuta</em>)</td>
<td>Cattle</td>
<td>Cat</td>
<td>World wide</td>
</tr>
<tr>
<td><em>S. bovihominis</em> (<em>S. citoce</em></td>
<td>Cattle</td>
<td>Human</td>
<td>World wide</td>
</tr>
</tbody>
</table>
**Pathogenesis**

*Sarcocystis* spp, are protozoan parasites with a requisite two-host life cycle. Sexual reproduction phase occur in the intestine of a carnivore (dogs, cats) later, cysts in the muscles of cattle, sheep or goats. Sporocysts are shed by carnivore’s feces and then ingested by cattle, sheep, or goats. Then sporocysts hatch in the ruminant small bowel and invade the vascular endothelium during three phases of asexual reproduction. After third phase, merozoites enter the ruminant’s muscle tissue and encyst as sarcocysts.

**Clinical findings**

In cattle is usually asymptomatic, but in heavy infections in nonimmune cattle, clinical signs include fever, anorexia, anemia, weight loss, lameness, abortion and diarrhea may occur (Taylor et al., 2007; Radostits et al., 2008). Neurologic signs are occasionally seen in cattle that include ataxia, tremors, muscular weakness, hypersalivation, blindness, opisthotonos and nystagmus (Radostits et al., 2008). In infections that caused by *Sarcocystis bovicanis* in cattle there is usually loss of hair at the end of the tail (Taylor et al., 2007). In sheep and goats clinical signs may be asymptomatic. In heavy infections there is fever, loss of weight, anemia and weakness. Abortion may occur. In chronic infections clinical signs include poor weight gain, edema of the limbs, anemia and abortion. Neurological symptoms, such as depression, in coordination, hind leg paralysis and coma can be seen in Encephalitic Sarcocystosis.

**Diagnosis**

Generally *Sarcocystis* infections are diagnosed at meat inspection with grossly visible sarcocysts in the animal’s muscle. When infection is very heavy in intermediate hosts, clinical signs and histological evidence of schizonts in the blood vessels of organs and the presence of cysts in the muscles at necropsy will be used for diagnosis (Urquhart et al., 1987). Indirect hemagglutination test (IHA) and ELISA test are used for serological diagnosis. In acute form of disease titers of antibodies are not high but 1 week to 3 months later will be at diagnostic levels. For certain diagnosis immunohistochemistry, electron microscopy and PCR techniques are available (Radostits et al., 2008).

**Treatment and control**

There is no effective treatment for sarcocystosis. (Urquhart et al., 1987; Radostits et al., 2008). Treatment of infected calves and sheep with salinomycin (4 mg /kg and 1-2 mg /kg BW; respectively) has been recommended. Amprolium 100 mg /kg, for 30 days reduces the severity of infection.

After beginning nervous system symptoms in sheep, Encephalitic Sarcocystosis recovery has not been observed. Control of disease is based on protection the food supply of ruminants. Feed bunk should be kept clean, also farm dogs and cats that have access to the feed or pastures should not be fed uncooked meat (Urquhart et al., 1987).

**Neosporosis**

<table>
<thead>
<tr>
<th>S. ovicanis (S. tenella)</th>
<th>Sheep</th>
<th>Dog, fox</th>
<th>World wide</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. ovifelis (S. gigantic)</td>
<td>Sheep</td>
<td>Cat</td>
<td>World wide</td>
</tr>
<tr>
<td>S. capracanis</td>
<td>Goats</td>
<td>Dog, wolf</td>
<td>World wide</td>
</tr>
<tr>
<td>S. hircicanis</td>
<td>Goats</td>
<td>Dog</td>
<td>World wide</td>
</tr>
<tr>
<td>S. hircifelis</td>
<td>Goats</td>
<td>Cat</td>
<td>World wide</td>
</tr>
</tbody>
</table>
Neosporosis caused by *Neospora caninum* in cattle, sheep and goats. *Neospora caninum* is a protozoan parasite of the phylum Apicomplexa in the family Sarcocystidae (Radostits *et al*., 2008).

**Epidemiology**

The protozoa *Neospora caninum* is an important parasite that cause abortion in cattle, sheep and goats. The majority of *N. caninum*-positive cattle prenatally infected via their dams. Transplacental transmission is considered the major route of transmission of *N. caninum* in cattle. In nonfatal infection in the fetus, the fetus is born with neurologic disorder (Smith and George, 2009). *N. caninum* has a worldwide distribution, the prevalence of infection in cattle and sheep approaches 100% with a lower ((Taylor *et al*., 2007; Radostits *et al*., 2008). In India, it has been observed that the seroprevalence is more among cattle from unorganized herds than organized. The sero-positive cattle are 8.4 times more likely to have abortion history than their sero-negative counterparts (Sengupta *et al*., 2013).

**Pathogenesis**

Definitive host: Dogs are the final host and sexual phase occur in them but, they are also intermediate host in prenatal infections.

Intermediate host: Cattle are the major intermediate hosts and asexual reproduction phase occur in them. Infection can be transmitted from dam to calf in utero and lactogenically. Infection of cattle can also occur from the ingestion of food or water contaminated with dog feces containing *Neospora caninum* oocysts (Taylor *et al*., 2007; Radostits *et al*., 2008). *Neospora caninum* is a major cause of abortion in cattle, however, sporadic abortions can occur in beef cows that have been infected congenitally (Dubey *et al*., 2006; Taylor *et al*., 2007; Radostits *et al*., 2008).

**Asexual phase has 2 stages:**
1. Tachyzoites: Tachyzoites penetrate host cell like central nervous system, muscles, macrophages and other cells, where they divide rapidly. Tachyzoites can also be transmitted either with contaminated food and water or transplacentally to the fetus in pregnant animals. Tissue cyst containing bradyzoites that are found only in thenervous system.After the asexual phase, sexual phase occur in definite host. It results in production of oocysts, which is shed in the dog feces.
2. Tissue cysts: Infection in sheep and goats is infrequently (Radostits *et al*., 2008).

**Clinical findings**

In cattle, neosporosis causes stillbirth, fetal resorption, mummification, abortion and decreases in their milk production. Abortions in cows are seen between 5-7 month gestations. Fetus may born alive but congenitally diseased. Neurological symptoms are different because of the widespread distribution of the parasite in the central nervous system. Calves are born with neurological symptoms, which these symptoms initially are mild but after birth become progress. In calves with neurologic dysfunction clinical signs are included of unable to stand, unable to suckle, domed skull and torticollis.

**Diagnosis**

The diagnosis of neosporosis is based on the examination of maternal and fetal sera ideally combined with the examination of fetal tissues.
- Immunofluorescent antibody test (IFAT) and indirect enzyme-linked immunosorbent assay (ELISA) are used for diagnosis.
- Histopathology of fetus: In histopathological examination of brain characteristic nonsuppurative encephalitis is suggestive of *Neospora* infection and also the lesions in the heart are characteristic for diagnosis.

**Treatment and control**

At present, there is no effective treatment for bovine neosporosis.
Control of abortion in infected cattle depends on saving food and water sources and the grazing environment from feces of any animal. Aborted fetuses and placentas should be removed or incinerated. The feces of dogs should be prevented from contaminating animal foodstuffs. Congenitally infected cows are at high risk for abortion thus seropositive animals should be culling from a herd (Radostits et al., 2008).

**Coccidiosis**

Coccidiosis is a protozoan parasitic (genus *Eimeria*) disease that cause diarrhea in young animals like calves, lambs and kids (Radostits et al., 2008). There are 12 species of *Eimeria* in cattle but all of them may not be pathogenic. The important species of bovine:

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Seat of predilection</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. zuernii</em></td>
<td>Small and large intestine</td>
<td>Cattle, water buffalo</td>
</tr>
<tr>
<td><em>E. bovis</em></td>
<td>Small and large intestine</td>
<td>Cattle, water buffalo</td>
</tr>
<tr>
<td><em>E. auburnensis</em></td>
<td>Small intestine</td>
<td>Cattle, water buffalo</td>
</tr>
<tr>
<td><em>E. alabamensis</em></td>
<td>Small and large intestine</td>
<td>Cattle</td>
</tr>
<tr>
<td><em>E. ellipsoidalis</em></td>
<td>Small intestine</td>
<td>Cattle</td>
</tr>
<tr>
<td><em>E. brasiliensis</em></td>
<td>-</td>
<td>Cattle, water buffalo</td>
</tr>
<tr>
<td><em>E. wyomingensis</em></td>
<td>-</td>
<td>Cattle, water buffalo</td>
</tr>
<tr>
<td><em>E. pellita</em></td>
<td>-</td>
<td>Cattle, water buffalo</td>
</tr>
<tr>
<td><em>E. cylindrica</em></td>
<td>-</td>
<td>Cattle, water buffalo</td>
</tr>
</tbody>
</table>

The important species of ovine:

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Seat of predilection</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. crandallis</em></td>
<td>Small and large intestine</td>
</tr>
<tr>
<td><em>E. ahsata</em></td>
<td>Small intestine</td>
</tr>
<tr>
<td><em>E. faurei</em></td>
<td>Small and large intestine</td>
</tr>
<tr>
<td><em>E. intricata</em></td>
<td>Small and large intestine</td>
</tr>
<tr>
<td><em>E. ovinoidalis</em></td>
<td>Small and large intestine</td>
</tr>
<tr>
<td><em>E. parva</em></td>
<td>Small and large intestine</td>
</tr>
</tbody>
</table>

The important species of caprine:

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Seat of predilection</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. ninakohlyakimovae</em></td>
<td>Small and large intestine</td>
</tr>
<tr>
<td><em>E. caprina</em></td>
<td>Small and large intestine</td>
</tr>
<tr>
<td><em>E. alijevi</em></td>
<td>Small and large intestine</td>
</tr>
<tr>
<td><em>E. arloingi</em></td>
<td>Small intestine</td>
</tr>
<tr>
<td><em>E. christenseni</em></td>
<td>Small intestine</td>
</tr>
</tbody>
</table>

**Bovine coccidiosis:**

Coccidiosis occurs universally in young animals normally in calves between 3 weeks and 6 months of age and caused important economic losses but also has been reported in cattle aged 1 year or more. The disease occur in animals that kept in crowded and contaminated pens. The prevalence in calves is 46%, in yearling is 43% and in adult cows is
16% *Eimeria zuernii* is the most pathogenic species that causing haemorrhagic diarrhoea (Taylor *et al*., 2007).

**Ovine/ caprine coccidiosis:**

Coccidiosis in sheep and goats is caused by protozoa of the genus *Eimeria*. Coccidiosis is one of the most common, important and higher infection rates disease in lambs and kids that kept in small areas contaminated with oocysts.

**Pathogenesis**

The pathogenesis of the disease is dependent on the destroy of the crypt cells of the intestinal mucosa because in the ruminant small intestine is very long and providing a high number of host cells and is a potential for parasite replication with minimal damage. Some species that invade the large intestine, cause pathological changes, especially when large numbers of oocysts are ingested in a short period of time. In healthy nonimmune animals when number oocysts ingested is low, the animals show no clinical signs of disease but in many oocysts condition, rupture and exfoliation of intestinal cells causes loss of blood, fluid, albumin and electrolytes into the intestine. Increased permeability of mucosal capillaries of intestine can cause to hypoproteinemia and anemia. Secondary bacterial infection may cause severe enteritis.

**Clinical findings**

In most clinical cases the temperature is normal or subnormal. Sloughing of epithelial cells of intestine cause sometimes bloody diarrhea. Dehydration may occur but anemia occasionally may be seen. In severe infections, diarrhea which may be mucoid or bloody, abdominal pain, anorexia, dehydration and weight loss are the common clinical signs in coccidiosis. In the calves that infected with high number of oocysts may developed dysentery. Clinical coccidiosis occurs rarely in adult cattle. Coccidiosis in lambs is widely similar to that in calves, but dysentery do not usually occur. In infected kids clinical signs are associated with watery diarrhea, dehydration, anorexia, and weight loss.

**Diagnosis**

Diagnosis is based on history, clinical and necropsy findings, and microscopic examination of feces. Acute coccidiosis can be diagnosed by direct examination of feces but in chronic coccidiosis that very low oocysts number are seen in feces, direct examination of feces may not be adequate.

**Treatment and control**

Coccidiostats drugs are used for treatment. All animals in the flock should be treated. Trimetoprim+Sulfadoxine (16-24 mg/kg) can be used for three days by intramuscularly in lambs and kids. Amprolium (10 mg/kg BW) for 5 days or 65 mg/kg BW one dose can be effective in calves (Radostits *et al*., 2008). Diclazuril and toltrazuril are also coccidiocidal drugs. Toltrazuril is more effective than sulphadimine and amprolium in *E. bovis* and *E. zuernii* oocysts shedding in buffalo calves.

Because of the low serum vitamin C levels in lambs with coccidiosis, administration of vitamin C is suggested to be useful combined with the classical treatment.

Hygiene in the house, minimisation of predisposing factors, avoiding the overcrowding of animals in the pens, control of the pastures for parasite are important factors for the control of coccidiosis.

**Cryptosporidiosis**

*Cryptosporidium* are protozoan parasite with at least 13 species occurring in mammals, birds, and fish, but only 2 of these species are important in livestock animals (Taylor *et al*., 2007). Cryptosporidium spp. are intracellular protozoan parasites causing gastrointestinal disease and diarrhea, and they are important infection in young ruminants.
### Parasite | Seat of predilection | Host
---|---|---
*C. parvum* | Small intestine | Cattle, sheep, goats, deer, man
*C. andersoni* | Abomasum | Cattle

**Epidemiology**
Cryptosporidiosis occurs primarily in neonatal calves, but also in lambs and kids. One major species, *C. parvum*, infects both farm animals and humans. Cryptosporidiosis is a fairly prevalent disease in many countries, and the disease is one of the most economically important diseases especially in calves and kids. Calves at 1-15 days are at the highest risk. The higher percentage of oocysts excreted is observed in 7-day-old. Animals of all ages can be infected, but diarrhea occur only in young animals. In young calves there is a significant relationship between season and infection. Infection peaks occur in spring and autumn (Taylor et al., 2007).

**Pathogenesis**
*C. parvum* infection, cause a malabsorptive diarrhea. *Cryptosporium* life cycle consist of six developmental stages. After ingestion of the oocyt there is encystation, merogony, gametogony, fertilization, oocyst wall formation, and sporogony. In contrast to other protozoal agents, cryptosporidia do not require fecal excretion for sporulation to infective stages, and they sporulate in the intestine (Radostits et al., 2008).

**Clinical findings**
Infections that caused by *C. andersoni*, are usually asymptomatic, although depressed weight gain in calves and milk yields in milking cows have been reported. In *C. parvum* infections, clinical signs are characterised by anorexia, depression, weight loss, diarrhea, dehydration, high morbidity and possibly death because of dehydration. *C. parvum* can cause diarrhea in calves 5 to 15, and in lambs and kids 5 to 10 days of age. Relapses of diarrhea are quite common, and *C. parvum* usually occurs as a component of mixed infections (Xiao and Herd, 1994).

**Diagnosis**
Oocysts can be detected using Ziehl-Nielsen stained fecal smears. *Cryptosporidia* can be diagnosed by fecal flotation. A number of molecular and immunological assays are available for diagnosis for example immunofluorescence (IF) or enzyme-linked immunosorbent assays (ELISA). Cryptosporidiosis is a zoonotic disease, and human can become infected from handling infected animals or feces.

**Treatment and control**
There is no effective drug for treatment of cryptosporidiosis in ruminants. Halofuginone has efficacy in calves with diarrhea due to *C. parvum*. Halofuginone is reported to reduce oocyst shedding and the intensity of diarrhea. Oral dose of 0.1 mg / kg BW daily for 7 days can reduce oocyte excretion.
Decoquinate, 2.5 mg/ kg, can be effective in prevention of cryptosporidiosis in kids and goats. Cryptosporidiosis in young animals should be treated with fluid therapy and correction of acid-base disturbance. The control of disease is based on the minimize transmission between the source of the organism and young animals. During the course of the diarrhea, diarrheic young animals should be isolated from healthy animals (Radostits et al., 2008). The best control of cryptosporidiosis comes from calves, lambs, and kids getting adequate immunity through colostrum after birth.

**Toxoplasmosis**
*Toxoplasma gondii* is a systemic coccidian, a universal parasite that causes rarely abortion in cattle but is a major abortifacient in sheep and goats. Felids are the definitive
hosts, and there are wide range of intermediate hosts in this disease. The organism is also responsible for abortion in human. Cats can act as definitive host as symptomless. The intermediate hosts are warm blooded animals who exhibit the clinical signs including abortion, nervous disorder, hepatitis, etc. The disease is a major cause of ovine abortion in many countries. Toxoplasmosis is one of the most common parasitic infections of man and other warm blooded animals in most parts of the world (Dubey et al., 1998). The disease is a major cause of ovine abortion in many countries. Seroprevalence studies show high rates of infection in farm animals. Major importance of disease in farm animals is its zoonotic potential. Source of infection for sheep, and cattle is the oocyst passed in the feces of the cats. Cats shed oocysts by their feces. Cats infected by ingesting tissues of intermediate hosts.

**Pathogenesis**

The whole developmental process of *Toxoplasma gondii* requires two hosts, the definitive host, cat and the intermediate host (all warm blooded animals). There are three stages of *Toxoplasma gondii* that are infectious for all hosts: tachyzoites, bradyzoites, and oocysts.

- **Tachyzoites**: This form of the parasite present during the acute stage of infection in the intermediate host.
- **Bradyzoites**: Present in the tissue cysts. Tissue cysts can develop in lungs, liver, and kidneys, and they are more prevalent in muscular and neural tissues, including the brain, eye, skeletal, and cardiac muscle.
- **Oocysts**: Present only in cat feces and containing sporozoites. Cats shed oocysts after ingesting tachyzoites, bradyzoites, or oocysts. Oocysts are the infective form of importance in livestock animals.

**Clinical findings**

There are a few reports of clinical toxoplasmosis in cattle associated with fever, dyspnea, nervous signs and abortion. Toxoplasma is not significant in bovine abortion. In small ruminants, particularly in sheep clinical signs include fever, abortion, prenatal mortality in lambs, generalized tremor, and dyspnea.

**Diagnosis**

Diagnosis is based on the clinical signs, presence of the characteristic small white necrotic foci in cotyledons. ELISA test is also used to detect antibodies in fetal and maternal serum. Indirect fluorescent antibody test can be used for fluid collected from the fetal thorax or abdomen. Serological examination of dam can be used for diagnosis.

**Treatment and control**

Combination of sulfamethazine (SMZ) and pyrimethamine (PMA) can be effective in pregnant ewes. The drugs are effective against the proliferating parasites in the acute stage of the disease (Radostits et al., 2008). The combined dose of (SMZ) and (PMA) is administered over 3 days for 3 periods, commencing on days 110, 115 and 130 of gestation. SMZ is injected s.c. at an initial dose of 5 ml/10kg body wt on the first day of a given treatment period with subsequent doses of 2.5 ml/10kg on each of the next 2 days. PMA is administered at 2 mg/kg on the first day and at 1mg/kg on the subsequent 2 days of each treatment period (Buxton et al., 1993).

Because the cats are the only definitive host of toxoplasmosis, it should be kept away from animals farm, and also feeding and drinking water should be prevented from contamination by cat feces. Aborted fetus and excreta, must be strictly disinfected. It is important to reduce the risk for human disease associated with consumption of infected meat.

**References:**


Introduction:

Parasitic nematodes (helminths) pose a significant and widespread problem for grazing livestock with implications for animal welfare and food production. The effect of this infection is determined by a combination of factors of which the varying susceptibility of the host species, the pathogenicity of the parasite species, the host/parasite interaction, and the infective dose are the most important. The economic losses are closely associated with the extent to which the pathogenic effect of helminth infections influences the production of the individual host. This may vary considerably from clinical disease including mortality to chronic production losses which may appear as eg. reduced growth rate, weight loss and/or reduced fecundity or it may go unnoticed. In addition to the impact on animal health and production, control measures are costly and often time-consuming. A major concern is the development of resistance by worms to many of the chemicals used to control them.

Transmission of helminth parasitic diseases

Helminths are worldwide in distribution; infection is most common and most serious in poor countries. The distribution of these diseases is determined by climate, hygiene, diet, and exposure to vectors. The mode of transmission varies with the type of worm; it may involve ingestion of eggs or larvae, penetration by larvae, bite of vectors, or ingestion of stages in the meat of intermediate hosts. The simplest way of transmission is by accidental ingestion of infective eggs (Ascaris, Echinococcus, Trichuris) or larvae (some hookworms). Other worms have larvae that actively penetrate the skin (hookworms, schistosomes, Strongyloides). Direct host-to-host transfer in helminth infections is restricted to a few parasites where prenatal infections from the mother to the growing embryo may occur and to transmission via skin penetration. Some of the filaroid worms are transmitted by vectors. In several cases, infection requires an intermediate host vector. In some cases the intermediate vector transmits infective stages when it bites the host to take a blood meal (the arthropod vectors of filarial worms); in other cases, the larvae are contained in the tissues of the
intermediate host and are taken in when the final host eats that intermediate host (Clonorchis in fish, tapeworms in meat and fish etc.).

In addition to the helminth infections which cause direct economic losses due to reduced animal production yet another dimension is added by the fact that several helminth infections can be transmitted to man (zoonoses). With regard to their host range some helminth species, do not discriminate between humans and animals at all, while others have complex lifecycles which require man as either final or intermediate host in order to complete their life-cycle. Some of these diseases may be very harmful to man and a considerable amount of effort has over the years been put into the control of these through e.g. meat inspection legislation. The level of control vary from region to region and depends to a large extend upon religious and socio-economical considerations, inclusive the organizational infrastructure of the society.

Factors responsible for the occurrence of disease

Although the reasons for the occurrence of economically significant helminthiases may be multiple and interactive, the vast majority occur for one of four basic reasons. These are:

in an already infected environment

(1) an increase in the infecting mass;

(2) an alteration in susceptibility of existing stock;

(3) the introduction of susceptible stock;

in a clean environment

(4) the introduction of infection.

Helminthiasis due to increase in the infecting mass in an infected environment

This category involves helminthiases which occur seasonally in grazing animals, usually after at least one parasitic generation. The level of contamination of the environment is influenced by several factors including biotic potential of the helminth, stock management, host immune status and hypobiosis.

Biotic potential: Biotic potential may be defined as the capacity of an organism for biological success as measured by its fecundity. A wide spectrum of biotic potential exists with nematodes such as Haemonchus contortus and Ascaris suum producing many thousands of eggs daily, and others such as Trichostrongylus spp. only a few hundred. Obviously, heavy contamination of the environment occurs with parasites of high biotic potential and disease problems due to these parasites are particularly common.
Stock management: The density of stocking can influence the level of pasture contamination and is particularly important in nematode and cestode infections where no multiplication of the parasite takes place outside the final host; it is less important in trematode infections such as liver fluke where multiplication occurs in the intermediate snail host. In nematode infections it has the greatest influence during the period when climatic conditions are optimal for development of the contaminating eggs or larvae to the infective stage.

Immune status of the host: The influence of stocking density will be greatest if the grazing stock are all fully susceptible, e.g. segregated dairy calves or weaned lambs, or the ratio of susceptible to immune stock per unit area is wide. In certain instances the resumption of development by arrested larval stages as a result of the Peri-Parturient Relaxation of Immunity results in transmission from the dam to its young, either prenatally or via colostrum in the immediate post-natal period. Host immunity can also limit the level of contamination by modifying the development of new infections or expelling existing burdens.

Hypobiosis: Larvae may become arrested in development within the host as a manifestation of acquired immunity. The hypobiosis is a most useful life cycle adaptation to ensure persistence and it facilitates the synchronization of the nematode life cycle to changing host and environmental conditions. It also enables the parasite to have available large numbers of infective forms at points in the host life cycle that coincide with the presence of susceptible neonates, thus ensuring transmission.

Helminthiasis due to altered susceptibility of existing stock in the infected environment

Altered effects of an existing infection: This is observed principally in adolescent or adult stock which are harbouring burdens of helminths below the threshold usually associated with disease; the onset is insidious and is reflected by sub-optimal productivity, the so-called sub-clinical helminthiases. Current biological concepts on the dynamics of host--parasite relationships support the theory that adult worm populations are maintained by a balance between the loss of existing worms and the acquisition of new infection, the rate at which worms are lost being proportional to the level of new infection acquired; consequently, if infected animals are subjected to a low or nil challenge then the existing burdens would decrease slowly or persist for a considerable period. The effects of these persistent low burdens can be influenced by various dietary and host factors like Change of diet, Mineral or trace element deficiencies, Pregnancy and lactation etc.

Altered susceptibility to the acquisition of new infections: Apart from the effect of late pregnancy and lactation in relaxing the immune vigilance of animals to helminths, other
pathogens and drugs can also have a suppressing effect on the immune system and so enhance the establishment and pathogenic effect of fresh helminth infections; conversely, certain drugs can enhance the immune response and reduce the establishment and pathogenic effects. Sometimes the development of a particularly strong immunity may be economically detrimental probably due to hypersensitivity reactions following challenge.

**Helminthiasis resulting from the movement of susceptible stock into an infected environment**

Clinical outbreaks with a high morbidity fall into this category, usually occurring within a few weeks of exposure, i.e. without a further parasitic generation, and involving animals from a similar age group. Since immunity to most helminths is slowly acquired, juveniles are ultra-susceptible, particularly following segregation in the post-weaning phase; however, helminth- naive adults moved from non-endemic to endemic areas are also at risk as significant age immunity occurs to only a few helminth species. Another factor which contributes to this situation is the great longevity of many helminth infective stages which results in the maintenance of a highly infective environment between the introductions of successive batches of livestock.

**Helminthiasis due to the introduction of infection into a clean environment**

**The role of infected stock**

One of the current trends in the international livestock arena is the movement of quality breeding stock from country to country, or continent to continent. Quarantine restrictions and vaccination requirements on such global animal travel are severe in respect of the epidemic diseases, but limited for the non-contagious diseases such as the helminthiases. Where the transmission of larval stages occurs via the dam's milk the importation of infected female livestock can result in the establishment of a hitherto exotic species without the completion of a further parasitic generation.

**The role of effluent and vectors**

The transfer of infection from one farm to another via manure is happening in most of the countries. The introduction of vectors into a suitable new environment has also been shown to be associated with the subsequent occurrence of helminth disease.

**Effect of climate on disease pattern of helminth infections**

The influence of climate on the development and mortality of the free-living stages of helminthes of livestock has been extensively studied and climate change might therefore be expected to affect parasite transmission. In line with the changes in climatic patterns, parallel
changes in prevalence, seasonality and geographic distribution of most major helminthes of livestock is evident particularly *Haemonchus* spp., *Teladorsagia* spp., *Nematodirus* spp., *Fasciola* spp., and *Paraphistomum* spp., since survival and development of free-living stages is chiefly affected by temperature and moisture. Therefore, the transmission rates, prevalence, intensity and pathogenicity of helminthes are expected to increase with increasing temperature, but only up to the extent of development and acquiring of immunity in the hosts to helminthes.

The current climate change scenario is expected to cause widespread shift in the pattern of a number of helminthes and alter the life cycle dynamics of vectors and parasites as well as dramatically influence the transmission potential of the vectors resulting in introduction of diseases into new areas (emergence) and or cause dramatic increase of the disease incidence in already endemic areas (re-emergence).

Global climate warming produces ecological perturbations, which cause geographical and phonological shifts, and alteration in the dynamics of parasite transmission, increasing the potential for host switching. The increased infection rates of *Haemonchus contortus*, *Teladorsagia circumcincta*, *Nematodirus battus* and *Fasciola hepatica* in temperate climates has been attributed to climate change, since the survival of the free-living stages is chiefly affected by temperature and moisture, and larval development rate is highly temperature dependent.

**Conclusions:**

Disease pattern of helminth infections of livestock is so complex and is varying with each parasite. Understanding the disease pattern is extremely important to strategist the control measures. Global climate change is a dominant factor for current and future trends in helminth diseases in livestock with both direct and indirect impacts on livestock production, animal health and welfare. However, the study of the effects of climate change on the helminth diseases of livestock is still in infancy. There are still gaps in our knowledge in relation to the biology of parasites and pathogens and how they will respond to changing climatic conditions.

**Further reading**

National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI) has the mandate to carry out research activities in the area of veterinary epidemiology and disease informatics. With the eradication of rinderpest successfully, India has not only proved its ability to face the challenges, but also to succeed, despite various limitations. Similar efforts are needed to control and eradicate the diseases like FMD, PPR, Brucellosis, CSF, BT, HS etc., which cause huge economic loss annually to the livestock industry. In this context, it is worth to note that the livestock and fisheries sector contribute about 5% of the national GDP. Though there has been a steady increase in contribution of this sector to the national GDP, the real potential of this sector is yet to be realized. In order to achieve higher growth, the livestock production system should rely on quality diagnostics, vaccines and effective surveillance programs for health related issues. In a way, disease surveillance is central to animal health for better productivity as it helps in

- early detection of disease and dissemination of early warning
- for Planning and monitoring of disease control programs
- for providing sound animal health advice to farmers
- for certification of exports;
- for international reporting and verification of freedom from diseases.

Finally, it is particularly vital for animal disease emergency preparedness. ICAR-NIVEDI, in its quest for achieving better livestock health, has developed an interactive web portal named “National Animal Disease Referral Expert System (NADRES)”. The portal, which was developed initially from the financial support of two National Agricultural Technology Projects (NATP) titled “weather based animal disease forecast (WB_ADF)” and “animal health information system through disease monitoring and surveillance (AHIS_DMS)” projects. The portal, which was launched in the year 2005, has undergone many structural and functional changes over the years without compromising the quality of the output. The portal is interactive and allows the public to access livestock disease forewarning (n=13) at the district level for entire country two months in advance. The portal is built on mySQL platform, which stores the administrator provided disease information and other relevant meteorological and risk factor information. The stored information is then subjected sophisticated statistical analysis for providing disease forewarning and country wide district level disease map on a GIS platform. The detailed information about NADRES will be provided during the presentation.
Basic epidemiological concepts
Dr. Siju Susan Jacob and Dr. P. P. Sengupta
ICAR- National Institute of Veterinary Epidemiology and Disease Informatics,
Ramagondanahalli, Yelahanka, Bangalore- 64

Epidemiology: The word epidemiology comes from Greek words ‘epi’, meaning on or upon, ‘demos’, meaning people and ‘logos’, meaning the study of. The basis for any epidemiological investigations is the assumption that disease does not occur in a random fashion because one of the main objectives of epidemiologist is to identify causal relationships between potential risk factors and outcomes such as disease or productivity losses. Both types of losses are assumed to be influenced by multiple, potentially interacting factors. Epidemiological investigations focus on general population and disease aspects as well as on causation of the disease.

Definition of epidemiology:

‘Epidemiology is the study of distribution and determinants of health-related states or events in specified populations and the application of this study to the control of health problems.’

Key terms in this definition reflect some of the important principles of epidemiology.

Study: Epidemiology is the basic science of public health. It is a highly quantitative discipline based on principles of statistics and research methodologies. Epidemiology is data-driven and relies on a systematic and unbiased approach to the collection, analysis and interpretation of data. Basic epidemiologic methods tend to rely on careful observation and use of valid comparison groups to assess whether what was observed, such as the number of cases of disease in a particular area during a particular time period or the frequency of an exposure among persons with disease, differs from what might be expected.

Distribution: Epidemiology is concerned with the frequency and pattern of health events in a population. Frequency refers not only to the number of health events, but also to the relationship of that number to the size of the population. The resulting rate allows epidemiologists to compare disease occurrence across different populations.

Pattern refers to the occurrence of health-related events by time, place and person. Time patterns may be annual, seasonal, weekly, daily, hourly or any other breakdown of time that may influence disease occurrence. Place patterns include geographic variation, urban/rural differences, and location. Personal characteristics include demographic factors which may be related to risk of illness, injury, or disability such as age, sex and socio-economic status, as well as behaviors and environmental exposures.

Determinants: Epidemiologists search for causes or factors that are associated with increased risk or probability of disease. A determinant is any factor or variable that can affect the frequency with which a disease occurs in a population. Determinants can be broadly classified as being either intrinsic or extrinsic in nature. Intrinsic determinants are physical or physiological characteristics of the host or disease agent which are generally determined genetically. Extrinsic determinants are normally associated with some form of environmental influence on the host or disease agent. They may also include interventions made by man into the disease process by the use of drugs, vaccines, dips, movement controls and quarantines.

Health-Related States: Early epidemiological study focused solely on infectious diseases. Today epidemiology studies a variety of health-related events, which includes chronic
disease, environmental problems, behavioral problems, and injuries, in addition to infectious diseases.

**Populations:** A population can be defined as the complete collection of individuals that have some particular characteristic(s) in common. Depending on the characteristic(s) being considered, a population can be very large or very small. For example, one may wish to study a particular disease in a particular cattle population in a particular country. That cattle population could consist of: All the cattle in the country or all the dairy cattle in the country or all the dairy cattle of a certain breed in the country etc.

**Types of epidemiological investigations:**

- **Observational study**
  - In observational study, the distribution of an exposure and/or an outcome is examined without any attempt by the investigator to influence them.

- **Experimental study**
  - A study designed to test a hypothesis by modifying an exposure within the study population.

- **Aggregated data**
Data concerning exposures and outcomes in which the unit of analysis is the population rather than individual animals.

Example for aggregated data is examining the data on the overall incidence of bovine spongiform encephalopathy cases in different countries rather than in individual animals.

**Individual-based data**

Data concerning exposures and outcomes derived from individual animals within the population.

**Randomised controlled trial**

It is the study in which individual animals are randomly assigned to receive the exposure under investigation or to be a control who does not receive the exposure. For example, in a randomised controlled trial to investigate the effect of kennel cough vaccine in dogs coming to a boarding kennel, dogs were randomly assigned to receive the vaccine or to be a control (no vaccine).

**Quasi experimental study**

In this study the investigator does not have full control over the allocation of the exposure. For example, a comparison of the incidence of bovine tuberculosis before and after the introduction of a control programme would be a quasi-experimental study.

**Cross-sectional study**

In cross-sectional study, the prevalence of an exposure and/or an outcome is measured in a given population at a specified point in time. The data may be analysed to find an association between the exposure and the outcome. For example, prevalence of *Mycobacterium paratuberculosis* infection in a particular herd could be determined by a descriptive cross-sectional survey. If data on possible risk factors for paratuberculosis infection are collected from the same animals at the same time then they could be used in an analytic study to look for associations between the risk factors and the disease.

**Descriptive epidemiology:** It can be used to measure the burden of disease within a population. The 5W’s of descriptive epidemiology include, *What* = health issue of concern, *Who* = animals, *Where* = place, *When* = time and *Why/how* = causes, risk factors, modes of transmission.

For example, descriptive epidemiology can be used to examine changing pattern of pre-weaning mortality in a particular country over the last 10 years, or to describe differences in the prevalence of hip dysplasia between various dog breeds, or to compare the incidence of bovine tuberculosis in different countries.

**Analytical epidemiology:**

With analytical epidemiology, risk factors for a disease or an outcome of a disease can be investigated. In analytical epidemiology, statistical analysis of epidemiological data will be attempted to establish relationships between causative factors and incidence of disease.

**Cohort study**
In cohort study one or more groups of individual animals are followed up over a period of time to determine the frequency of a particular outcome.

For example, in a cohort study of the effect of the bovine spongiform encephalopathy (BSE) status of the mother cow on the risk of BSE in her offspring, calves from diseased and non-diseased cows were followed-up until disease occurrence. The frequency of BSE could then be compared between calves having a dam with or without BSE.

**Case-control study**

In case control study individuals with and without the outcome of interest are identified. Their status with respect to exposures of interest is then determined in order to look for associations between these exposures and the outcome of interest.

For example, a case-control study can be used to determine risk factors for bovine tuberculosis in cattle at the herd level. For this identifying the herds with and without bovine tuberculosis and obtain information concerning exposures of interest (purchase, neighbours, wildlife) is required. After that analysis of the data to find out whether the presence of TB in neighbouring herds or frequent purchase of cattle was associated with bovine tuberculosis presence in the herd.

**Measures of health**

A fundamental task in epidemiology is to quantify the occurrence of disease. This can be done by counting the number of affected animals. Quantifying the levels of disease in a population is important since it allows to determine the economic importance of diseases and to prioritize the resources for disease control activities. The proportion and ratio are the key terms required for the quantification of diseases in a population:

A **proportion** is a fraction in which the numerator is included in the denominator. In a herd of 100 cattle over a 12-month period if 58 animals are identified as diseased, then the proportion diseased animals is 58 ÷ 100 = 0.58 = 58%.

A **ratio** defines the relative size of two quantities expressed by dividing one (numerator) by the other (denominator). The odds of disease (a ratio) in the above herd of 100 cattle can be calculated as 58:42 or 1.4 to 1.

Measures of health include the following terms:

The term **morbidity** is used to refer to the extent of disease or disease frequency within a defined population. Morbidity can be expressed as either **prevalence** or **incidence**. **Prevalence** refers to the number of cases of a given disease or attribute that exists in a population at a specified point in time. Two types of prevalence are (1) **point prevalence**: equals the number of disease cases in a population at a single point in time (2) **period prevalence**: equals the proportion of the population with a given disease or condition over a specific period of time.

**Incidence** measures how frequently initially susceptible individuals become disease cases as they are observed over time. An incident case occurs when an individual changes from being susceptible to being diseased.
**Attack rates** are usually used in outbreak situations where the period of risk is limited and all cases arising from exposure are likely to occur within the risk period. Attack rate is defined as the number of cases divided by the number of animals exposed.

**Secondary attack rates** are used to describe infectiousness. The assumption is that there is spread of an agent within an aggregation of individuals and that not all cases are a result of a common-source exposure. Secondary attack rates are the number of cases at the end of the study period less the number of initial (primary) cases divided by the size of the population that were initially at risk.

**Mortality** is an example of incidence where death is the outcome of interest. Cause-specific mortality risk is the incidence risk of fatal cases of a particular disease in a population at risk of death from that disease.

**Case fatality rate** refers to the incidence of death among animals which develop the disease.

Case fatality risk reflects the prognosis of disease among cases, while mortality reflects the burden of deaths from the disease in the population.
Geographical Epidemiology, Spatial Analysis and Livestock Disease Information System.

K.P. Suresh, S.S. Patil and D. Hemadri

ICAR-National Institute of Veterinary Epidemiology & Disease Informatics

Place is an important element in understanding the geographic distribution of livestock diseases and means of formulating and testing etiological hypothesis. The term ‘place’ is usually considered as a surrogate for interaction of genetic factors, species type or host and environment. In the prospective of veterinary health practices, knowledge that the veterinary health issues is concentrated in identifiable places is essential for the efficient distribution of resources for prevention, minimizing the disease burden or prevalence, treatment or amelioration. In recent years, availability of geographical information system and databases provide the excellent environment to explore the disease pattern in the given study area.

Age, breed, species type, environment material and other variables vary from one place to another and hence influences the varying risk of disease incidence. The observed differences of risk of diseases is likely to be confounded by these variables and hence the comparison of risk variability is an important issue in the process of disease epidemiology. The adjustment of potential confounding variables is important for evaluation of spread of risk of disease incidence.

Data for Spatial analysis

Point and layer (area) data are two important types of spatial data. Each item in the veterinary health data system viz., population, breed, environmental exposure, morbidity and mortality relate to a point data while data like village, herd, state, districts, blocks etc. indicated as layer data. The data for spatial analysis comes from different sources, it is advisable collect these data taking help of veterinary epidemiologists or Bio-statisticians. It is necessary to ensure that precise and complete point or area data are used in spatial epidemiology. The data related to diagnosis, collection, coding and reporting of a given health events may vary between geographical regions and time.

The quality of data must be given important in spatial analysis of data, presence of missing cases, underreporting, under ascertainment of cases, inaccurate baseline population data, may lead to inaccurate or misleading interpretation of estimated high or low risk. The confidentiality is also an important issue in spatial epidemiology, breaching the confidentiality may cause concern, particularly when it discloses the area with high rates of morbidity/mortality, outbreaks or risk of disease incidence.

Disease Clustering

Scanning for disease clustering is one of the important topic in geographical epidemiology, involves the assessment local or global concentration of disease events. There are two types of clustering, general clustering and Specific clustering. Analysis of disease events for overall assessment of clustering tendency and its spatial autocorrelation refers to general clustering, whereas the specific clustering methods are designed to investigate the exact location of clustering. Methods for detection of clusters in Point format data are more compared to areal format of data, and are usually divided into three groups, global, localized.
and focused. Large number of statistical tests available to assess the different kinds of clusters in point format data.

A Cluster is being a bounded group of occurrences, i) of a disease already known to occur characteristically in clusters, or ii) of sufficient size and concentrations to be unlikely to have occurred by chance, or iii) related to each other through some social or biological mechanism or having common relationship with some other event or conditions. The statistics of disease clustering are useful detect and monitor potential public hazards.

**Disease mapping**

Mapping of disease events is one of the best method for better visualization for exploring the complex structure of data. Data visualization is not only creating interest and also attract the attention of viewer and provide the way for discovering the pattern. Disease mapping is one of the tools of geographical epidemiology, fulfilling the need to generate accurate and precise maps of disease events. For Example, dot or dot density maps are used display point data, while areal data were presented by Choropleth maps and for continuous surface data, contour maps or isopleth maps are used. In the veterinary epidemiology, the presentation of maps is established as a basic tool for analysis and interpretation. The selection of appropriate of administrative unit for mapping viz., village, block, district or state, selection of suitable data classification in the map viz., high risk, moderate risk, low risk no risk etc., and selection of colour schemes are the important issues associated with creation of disease maps.

**Ecological analysis**

Ecological analysis refers to the analysis of association of disease incidence or outbreak with covariates of interest viz. anthropogenic variables, socio-economic variables, host related information, environmental variables and remote sensing variables etc. In the ecological analysis, the variables are defined on aggregated groups of individuals rather than individuals themselves. In the ecological analysis, the important issue to be attended is scale of covariate. The optimum choice of scale is a trade-off between making the groups (regions) large enough to have stable estimates and small enough to make them homogenous in terms of their socio-economic and other important covariates. For large regions, there is greater possibility that associations measured at the aggregate level will differ from the same association measured at individual level. This can lead to a problem known as ecological fallacy, or ecological bias, in this scenario, inference is made incorrectly at individual -level association from one that is observed at regional level. On other hand, if the regions or units chosen are too small, the results may show spurious spatial correlation or pattern due to random variation in small number of disease events.

The uncertainty induced by the aggregation procedure may result from scale dependency data and cause modifiable unit problem. Hence it is important to consider the scale of analysis of ecological variables and analyze the data at two or more levels of aggregation. It is possible to overcome the issues like scale dependency and ecological bias, by the se of multi-level approach using individual level and group level together.

**Geographical epidemiology**

Geographical epidemiology is defined as the description of spatial pattern of livestock disease events such as outbreak, incidence, prevalence, mortality etc. It is an analytical epidemiology wherein specific hypothesis will be formulated for testing of etiology of disease events.
Disease mapping, disease clustering and ecological analysis are important predominant methods of geographical epidemiology. Most of the geographical epidemiological studies depends on scale of ecological variables, leads to serious spatial problem when the results of geographic aggregate level data are combined with those at individual level. The combination of aggregate and individual level data analysis is possible with multilevel modelling, hierarchical regression, contextual analysis. Multilevel modelling is more powerful and a new technique, that can be used to determine the ecological effects explained by individual risk factors.

Geographical Information systems

Geographical Information System (GIS) can be defined as software system for the automated system to capture, storage, retrieval, analysis and display of spatio-temporal data. GIS has dramatically changed the ability of epidemiologists and public health specialists to work with spatial data. The advantages of GIS include an ability to operate repetitive tasks, handle large volume of data and quickly compare the spatial & temporal data. GIS has traditionally been used for maps when analyzing associations between locations, environment and disease events, recently GIS is used in the surveillance and monitoring of vector-borne diseases, water-borne disease, environmental health, modelling the exposures, prediction of disease events and analysis of disease policy and planning.

Veterinary geographic information system provides a strong framework for increasing our ability to monitor the disease pattern and identify their causes. The evolution of GIS from early disease maps to digital maps is a journey long in the making and continues to evolve. These maps have enabled us to gain insight about temporal and spatial pattern of livestock diseases and increasing knowledge of worldwide health issues.

Global Positions systems

Global Positions systems (GPS) is a system consists of group of powered satellites orbiting earth every 12 hour and transmitting radio pulses at every time intervals. To determine the position of specified location in three dimensions, latitude, longitude and elevation, a receiver needs signals from at least four satellites. GPS has become standard method for data capturing in geographical epidemiology and public health studies. Using GPS, it is currently possible to obtain the latitude and longitude of location at higher resolution. The impact of GPS in providing spatially referenced case data is potentially greatest in the developing countries where the absence of quality paper maps in that data are not often collectible. The other technical advance that has made obtaining spatially referenced case data increasingly easy for geocoding using postal codes.

Disease modelling and Smoothing

A disaster, precipitated by a natural hazard, can be defined as a “serious disruption of the functioning of community or society or livestock system causing widespread human, animal, material, economic or environmental losses which exceed the ability of the affected community or society to cope using its own resources ( ISDR, 2004, Terminology: basic terms of disaster risk reduction, International strategy for disease reduction, Geneva ). The expression of ‘early warning’ is used in many fields to mean the provision of information on an emerging dangerous circumstance where the information can enable action in advance to reduce the risks involved. Early warning systems exist for natural geophysical and biological hazards, complex socio-political emergencies, industrial hazards, and many other related fields.
Early identification of an infectious disease outbreak is an important first step towards implementing effective disease interventions and reducing resulting mortality and morbidity. The geographic and seasonal distribution of many infectious diseases are associated with climate and therefore the possibility of using seasonal climate forecasts as predictive indicators in disease early warning system (EWS) is an interest of focus. Geographic Information system (GIS), remote sensing (RS) and Global Positioning system (GPS) are the three commonly used veterinary geo-informatics technologies employed in this digital era for rapid communication of data for better management of animal diseases.

The ability to detect disease incidence or outbreaks early is important to minimize morbidity as well as mortality through timely implementation of disease prevention and control measures. The attacks on world trade center & terrorist attack using anthrax spores in 2001, as well as recent SARS outbreaks, have motivated many public health authorities to develop system for early detection of outbreak using non-diagnostic information, often derived from electronic data collected for other purposes. Emerging infectious disease poses a threat to livestock population, climate change like increased temperature and altered rainfall patterns are likely to increase the burden of vector-borne diseases resulting into emergence of zoonotic diseases. Forecasting is the monitoring of specific risk parameters helping to predict the situation that could lead to occurrence of disease and its subsequent spread. The forecasting of disease helps to predict the course of disease, warn health care workers and adopt control measures to prevent disease outbreaks.

Spatial autocorrelation

Spatial autocorrelation refers to lack of independence of neighboring areas. The correlation or dependency refers to the index for geographically close areas are more related than those areas that are geographically distant in respect of disease events. Detecting the spatial dependency would help researchers to justify the application of statistical models or smoothing techniques for disease mapping of rare disease or mapping is done for small boundaries. Spatial autocorrelation statistics provide a very useful summary information about the spatial arrangement of disease events in a map. Moran I and Geary’s C statistic are two commonly used spatial autocorrelation statistics for detecting the global clustering in continuous areal data. There is also many spatial autocorrelation statistics available like Getis and Ord’s G, which measures the local clustering.

Mobile Phone Technology

Global spread of mobile phones and networks at higher internet bandwidth, this technology is increasingly used to communicate early warning services and coordinate preparation activities. SMS alerts can be used to alert the local veterinary officers about the possible of occurrences of disease outbreaks. Advanced mobiles phones can also be used for video conferencing of disease information activities.

Early warning systems are combinations of tools and process embedded within institutional structures coordinated by national or international agencies. These systems are composed of four elements depending upon they focus on specific hazard or many, namely, knowledge of risk, a technical monitoring and warning services, dissemination of meaningful warnings to at-risk areas, and farmers awareness and preparedness to act. Warning services lie at the core of these systems, and how well they operate depends on having a sound scientific basis for predicting and forecasting. As early warning systems grow in geographical coverage and sophistication, false alarms to in rise. High false alarms can undermine the
public confidence, breed mistrust, dilute the impact of alerts and reduce the credibility of future warnings.

Further readings

The role of vectors in the transmission of Haemoprotozoan diseases and their control strategies

Placid E. D’Souza, Professor cum Director
CAFT in Veterinary Parasitology, Veterinary College, KVAFSU
Hebbal Bengaluru-24

The importance of vectors in the fields of natural history, veterinary and medical sciences and public health departments has long been realized. Vectors are assuming great economic significance since they have through the course of time become most versatile as far as their disease transmission ability is concerned. The association of insects with disease and pestilence has been recognized since the biblical plagues. It was only just over 100 years ago that a hematophagous arthropod was implicated in disease transmission. Since then, the life cycles and transmission of numerous arthropod borne infections have been studied in detail both in the field and in the laboratory.

The year 1879 was a landmark one as Manson discovered the development of microfilaria in mosquitoes. In the year 1893 the discovery of the role of ticks in transmission of Texas cattle fever by Smith and Kilbourne was another major landmark in the history of arthropod-borne diseases.

The term "Arthropod" refers to invertebrate animals, externally parasitic on larger animals; many of them blood feeders in at least one stage of their life cycles. The ecological relationships between them and their hosts may be exceedingly complex, involving ectoparasites as vectors of parasites, and in some cases as reservoirs of infection as well. In their role as vectors and also as bloodsucking parasites, they have a great impact on the ecology of animal and human populations.

The term 'Vector' refers to one which spreads pathogens (Latin: vectum to convey). A vector can transmit the disease agent either mechanically or cyclically. The major type of diseases that could have affected the early nomadic tribes must have been the arthropod-borne ones like typhus, sleeping sickness, malaria, tick-borne relapsing fever, tularaemia etc. A vector, according to Croll (1973) is a host which is essential for the life cycle of the pathogen, but does no more than a hypodermic needle. As an example he cited the relation between Trypanosoma evansi and the tabanid fly. James and Harwood (1969) classified vectors into mechanical carriers and obligate vectors. If an intermediate host bites or otherwise seeks out a definitive host of a pathogen it should also be considered as an obligate vector.

Feeding habits of the vector, its association with specific host species, timing of sampling, duration available for the pathogen within the vector, availability of a susceptible host for maintaining the infection chain are some of the factors controlling perpetuation of the disease. Each factor is further influenced by many subfactors needing a thorough knowledge of the parasite in question, vector species and their distribution, types of definitive hosts and their genetic makeup.

In mechanical transmission two types of vectors operate in disease transmission: 1. The first type collect the agent in their mouth parts and directly transfer it to the next host Eg. Blood sucking arthropods described as living needle. 2. The others are those vectors which pick up the causal agent in their legs, body hairs or proboscis as by flies and moths. They are
designated as living swabs. In both the cases infection is being brought about by mere surface contact with the contaminated media.

Infectivity of a mechanical vector declines sharply with time and becomes totally inefficient within a short time. Mechanical transmission is a character of the pathogen and not of the vector. Elimination of the vector need not totally eliminate the infection but will only reduce the incidence rate. Another factor which influences the efficacy of spread of a disease here is the role of an organic medium which favours the spread of mechanical transmission.

In biological transmission, intervention of a vector seems mandatory for an efficient spread of the pathogen. The role of insects, therefore, is very important here and their control is a major weapon in disease control. Biologically transmitted pathogens undergo a cycle of development in the vector. Consequently there is a period of incubation during which the infection does not spread. When this period is over, the vector is infective and remains so for the rest of its life. Vectors transmitting pathogens by biological means are in strict sense the definitive hosts. Broadly, five factors influence the process of dissemination and establishment of the disease.

1. The manner in which the agent is released from the vertebrate host: If the pathogen is restricted to blood or tissues and are to be transmitted by blood sucking or biting arthropods, the process is designated as ‘active egress’. If the agent escapes from the host in feces, urine, tear etc. and other excretions and are transmitted by coprophagous insects, the process is called ‘Passive egress’.

2. Mode of vector infection: This depends on the manner of egress of the agent from the host, habits of the vector, vector’s innate characters. Vectors are blood sucking, tissue biting or those feeding on contaminated exudates. Attraction of vectors to certain species of hosts or to certain individuals is influenced by body odour differences, body temperature, certain chemicals emanated from the host, level of CO2 in exhaled air, skin colour etc. Studies have shown that such factors greatly influenced preferential affinity of vectors to specific hosts and thereby the character of the epidemics. The mode of infection is also dependent on the barriers present in various organs of the vector, which indirectly depends on the concentration of the agent in the host and the time of feeding.

3. Course of infection in the vector: The agents have different predilection sites inside the vector and the cause of infection depended on (a) the time required to amplify inside the vector, (b) whether the agent actively multiplied or remained unaltered for long periods.

4. Manner of release of the pathogen from the vector: This is dependent on the action of the vector itself. In mosquitoes, the pathogen is automatically injected while feeding, in ticks they are expelled along with saliva while feeding, fleas disgorge plague bacilli by vomiting: bugs discard the pathogen by defecation while feeding or shortly thereafter, lice and mites which have the pathogen in their tissues contaminate the wounds created by scratching process of the host in turn resulting in crushing of the vectors. Some vectors are ingested by the host and the pathogen leaves the vector by heat or while being digested (Snail, earthworm).

5. Combination of circumstances required for infecting the vertebrate host: Vectors may go in search of hosts (mosquitoes, flies) or merely place themselves in vantage perches (ticks, lice, mites) such as on leaf blades, branches, shrubs and wait for a potential host to approach the site. They are activated by vibration during the passage of the host. The height of plant...
and the other sites selected by different stages of the vectors often determine the species of
host encountered.

The transmission of pathogens by vectors is also, profoundly influenced by a number of other
parameters viz.,

1. Vector density
2. Population density of the host
3. Environmental conditions
4. Spectrum of pathogen susceptibility of the vector
5. Geographic distribution of specific species and sub-species of the vector

Such knowledge will immensely help us in understanding vector-borne diseases and in
designing strategies for their control. Vector science involves the study of vector-pathogen
and vector-vertebrate interactions. Vector-pathogen association ranges from facultative
relationships to highly specific obligatory relationships. In obligatory relationships, the
pathogens do multiply in the vector and show a definite infective cycle. Evolution of blood
feeding habit (haematophagy) is one of the greatest attributes of vectors which transmit
pathogens of vertebrates including man. Many evidences especially the studies on Diptera
indicate that haematophagy in insects originated at a time much before mammals were
differentiated into an ecologically important group.

It is interesting and suggestive to note that even to this date many blood sucking
groups among Diptera (flies) still use amphibians and reptiles as hosts. At least three major
routes could be suggested for the evolution of haematophagy.

1. It could have developed as an offshoot of the scavenging and predatory habits exhibited
by the progenitors of the arthropods in question which must have been inhabitants of lair or
nest of the would be hosts. The blood sucking Triatomine bugs and their close relatives, the
reduvid bugs illustrate the evolution of haematophagy from predatory habits. Fleas, the
larvae of which are detritous feeders and adults blood sucking could be one of the examples
development of haematophagy from scavenging habit.
2. A possible second route of evolution of blood sucking habit would be chance feeding
encounters perhaps initially on proteinaceous secretions carried a step further into a more
purposive relationship.
3. Secondary haematophagy, a third possible route in the evolution of blood feeding habit is
seen in many Diptera. Fanniaflavipalpis and Hydrotaeaarmipes are reported to be in
association with and imbibing blood made available by tabanids.

The role of vectors in the transmission of many important parasitic diseases is well
established. Vector borne diseases account for more than half of the disease problems
encountered in animals and seriously affects productivity and health. In spite of the
predominance of vectors and vector borne parasitic infections in tropical countries including
India this area of study has generally remained neglected. Research work has been largely
confined to the study of incidence or superficial aspects of biology and taxonomy of vectors.
It is needless to state that vector control could lead to effective eradication of serious parasitic
problems such as Theileriosis, Babesiosis, Trypanosomosis and many other commonly
encountered infections in the field. Attention with regards to the control of vectors has always focused on the use of chemicals even though they have undesirable effects on the environment. Many a time constant use of pesticides has led to its inefficacy and there has been a serious problem of pestilence in livestock farms and in human dwellings. This has more and more prompted the consideration of alternate methods of control such as biological, genetic and other methods. There have however been great strides in the application of immunological and biotechnological methods to develop vaccines against vectors and vector borne parasites.

The vaccines developed against Theileriaannulata, Babesia bovis and Boophilusmicroplus bear testimony to this statement. In order to combat vector borne disease either the parasites or the vector should be controlled. It is generally believed that vector control is more appropriate. The control of vectors faces two important challenges namely - the development of resistance to insecticides and pesticides and the environmental pollution caused due to the constant application of insecticides and pesticides. Therefore it is desirable to have alternate strategies, which do not involve resistance among the vectors. Genetic control or autocidal control is one such method.

The main objectives of vector control are to keep the vector density at the low level to minimise vector-reservoir contact and to curtail the longevity of vector species to interrupt disease transmission.

This approach which should be carried out prudently and skillfully is naturalistic and involves an attempt to extend and intensity natural factors which limit vector breeding, survival and contact with man. But these measures have many constraints and limitations viz.,

i) Selective applications,

ii) Require high degree of inter-sectoral coordination,

iii) Capital investment of some of the methods is high,

iv) Maintenance is very essential

v) Active and sustained community involvement.

**Different approaches and systems in the control of arthropod pests and vectors**

Arthropod parasites (ectoparasites) are a major cause of production losses in livestock throughout the world. In addition, many arthropod species act as vectors of disease for both animals and humans. Treatment with various drugs to reduce or eliminate ectoparasites is therefore often required to maintain health and to prevent economic loss in food animals. The choice and use of ectoparasiticides depends to a large extent on husbandry and management practices, as well as on the type of ectoparasite causing the infestation. Accurate identification of the parasite or correct diagnosis based on clinical signs is necessary for selection of the appropriate drug. The selected agent can be administered or applied directly to the animal, or introduced into the environment to reduce the arthropod population to a level that is no longer of economic or health consequence. Parasites that live permanently on the skin, such as lice, keds, and mites, are controlled by directly treating the host. Semi-permanent parasites (ticks, flies, etc) are less easily controlled because only a small proportion of the population can be treated at any one time, and other hosts may maintain them.
Some tick and mite species stay on the host only long enough to feed, which may be as short as 30 min, or as long as 21 days. Biting flies, such as the horn fly, can be found continuously on the backs and undersides of cattle, where they suck blood up to 20 times a day; other biting flies (such as stable flies and horse flies) and mosquitos feed to repletion, then leave the animal to lay eggs. Non biting flies, such as the face fly or the house fly, may visit infrequently but can be very annoying and may transmit disease agents. Larvae of certain blowflies live on the skin or in tissues of sheep and other animals and cause cutaneous myiasis. Larvae of other flies spend several months inside animals, eg, nasal bots in the nasal passages of sheep and goats, bots in the stomach of horses, and cattle grubs or warbles in the spinal canal, back, or esophageal tissues.

**Personal protection measures**

1. **Anti-Adult measures**

Several personal protection measures are available for providing protection against insect bite. They can be used as supplementary measures in remote and inaccessible areas or against exophilic and endophagic vector species depending upon their feasibility, cost effectiveness and sustainability.

i. **Physical method:** This include protective clothing's, use of bed nets, screening of windows/doors etc.

ii. Repellents these are chemicals that prevent insect damage to plants or animals by rendering them unattractive, unpalatable, or offensive. Thus, repellents include a wide range of chemicals, from volatile substances active in the vapour phase to protect humans against biting flies, and mosquitoes DMP (Dimethyl phthalate) and DEET (Diethyl toluamide) to persistent chemicals such as Bordeaux mixture which act as feeding deterrents to foliage feeders. Many species of ticks throughout the world are serious pests of man and animals. Current methods of protection from attack by ticks depend upon personal protection through the use of repellents.

**Space spraying by mist/ Thermal fogging or ULV spray**

Space spraying has been successfully used to control the out breaks of vector- borne diseases such as malaria, dengue, Japanese encephalitis, western equine encephalitis etc. The space spray is usually undertaken to control the resting population of mosquitoes either by using the natural pyrethrin extract diluted in Kerosene oil or malathion during out break situations to interrupt the disease transmission by crisis. This is done in the form of mist, chemical fogging or ULV spray.

2. **Anti larval measures**

Anti larval measures are used as an adjunct to other methods of control and are rarely used as main method of control except against container breeding species or against those mosquito species which bred in confined or specific small water bodies such as Aedes aegypti, Anopheles stephensi and An. sundaicus. Anti larval measures can also be tried in an area where vector species are resistant to commonly used insecticide or exhibit exophily and exophagy or under those situations where adulticide measures are not cost effective or tend to endanger the environment.

Antilarval measures in tropical countries are mainly used in urban or peri-urban areas. These measures can be used in certain specialised situation like mining, irrigation, wells,
tanks etc. if they are operationally feasible and cost effective. The basic idea of all anti larval measures is to prevent, reduce or eliminate the breeding places.

i) M.L.O. (Mosqitolarvicidal oil) - Oiling is done in situations where breeding is temporary and permanent measures may not be cost effective. Oiling of a breeding sites kill the larvae by choking their spiracles with oil film and cutting the oxygen supply. It also deters the adult mosquitoes from egg laying.

ii) Paris green (Copper aceto-arsenite) - It has been successfully used in malaria control programme for the control of anopheline and culicine breeding. It is applied as dust or granular formulation.

iii) Abate (Temephos and Baytex (Fenthion) - Widely used under urban Malaria Scheme for the control of breeding of anopheline and culicines mosquitoes.

**Genetic control mechanisms**

Despite the advantage of insecticides for pest control, the use of insecticide has been ecologically unsound, leading to disadvantages such as insect pest resistance, outbreak of secondary pests, adverse effects on non target organisms, objectionable pesticide residues, and direct hazards to the user and environmental pollution caused due to the constant application of insecticide and pesticide. Therefore, it is desirable to have alternate strategies which do not involve resistance among the vectors. Genetic control or autocidal control is one such method.

The use of insects to control populations of their own kind through the transfer of damaged genetic material represents a new approach that could prove to be useful for the control of a number of major insect pests throughout the world. The principal requirement for the success of all genetic control methods is the production of sufficient numbers of healthy, competitive (though genetically different) insects and their release in the right place at the right time for them to mate successfully with wild insects. Success will depend on the knowledge of how to rear the species on a large scale, how to sterilize or otherwise genetically manipulate without affecting mating ability and competitiveness and a detailed acquaintance with the general ecology and bionomics of the insect to be controlled (Davidson, 1974).

**Translocations**

A chromosomal translocation involves the breakage of two non-homologous chromosomes and the reattachment of the broken parts to the wrong partners. During meiosis, in the translocation heterozygotes, the synoptic forces between homologous loci result in the formation of a cross shaped figure (Belling and Blakesies, 1924). It is the subsequent segregation pattern of this configuration which can lead to reduced fertility of translocation heterozygotes and which may be utilized for insect control naturally occurring translocations are rare but can be induced either by applying chemicals or radiations.

**Genetic sexing**

In genetic control programme(s), semi-sterile males are released into natural population. Since the females of the insects are potential vectors and cause biting nuisance, they should be eliminated during early development stages by genetic methods. This will also help to lower the cost of mass production of males for release purposes.
Genetic sexing mechanisms utilising conditional lethal (eg. insecticide susceptible gene or temperature sensitive gene) and radiation induced Y-linked translocation and an inversion been carried out for a number of mosquito vectors. These include An. gambiae species A; the dieldrin resistant semi-dominant autosomal gene was translocate to the Y-chromosome via radiation induced translocation (Curtis et al., 1976).

Sterile Insect technique

The Sterile Insect Technique (SIT) is based on the induction of sexual sterility in males through the use of radiation or chemical sterilants and on inundating natural populations with such males (Knipling, 1955, 1959). Such techniques have been extensively used for the control of insect vectors of diseases, insect of veterinary importance and agricultural pest.

The major success has been achieved in the control of the screw worm, Cochliomyiahominivorax (Coquerel), a major pest to livestock in the Southern United States. In this case control was effected through the competition for mating between sterile mass-reared males and the natural population of males. At present the screw worm has been eradicated from the southeastern United States.

Cytoplasmic incompatibility

Sterility in certain crosses between strains of Cx. pipienscomplex was first reported by Marshall (1938). Laven (1967a) made extensive studies between certain allopatric populations of Cx. pipiens. Such an incompatibility which can be either uni or bi-directional and which is maternally transmitted has been ascribed to the presence of a rickettsial endosymbiont, Wolbachia species in the gonads. Thus, incompatibility is due to the death of the sperm nucleus in an incompatible egg cytoplasm before karyogamy occurs. As a result no progeny are produced from such incompatible crosses.

Refractoriness to disease transmission

The production of genetically defined lines of vector mosquitoes refractory to the development of parasite and thus incapable of transmitting the infection is one possible method of controlling vector borne diseases. Apart from selection of malaria refractoriness by old fashion animal breeding technique, ideas have also been presented as to how refractoriness might have been contrived by genetic engineering method (WHO, 1996). These suggestions include the introduction of gene coding for a transmission blocking single chain antibody (Winger et al., 1987) namely an antibody against Plasmodium gamete, zygotes or oocysts which are produced in or on the stomach of the mosquito.

Integrated vector control

The integrated vector control may be defined as the application of one or more than one vector control method. Simultaneously or consequently in a given area to control vector borne diseases when available options are selected on the basis of epidemiological paradigm, vector behaviour, human behaviour and environmental aspects, it become selective vector control. This approach is quite appropriate but requires effective planning, technical, competence, managerial skills and sound understanding of vector and its environment.
Community participation

WHO Aima Ata Declaration (1973) envisaged that community participation should be considered as a crucial component of Primary Health Care (PHC). The idea of community participation was developed with the fond hope that it will make disease control of communicable diseases was considered advantageous to improve the quality of preventive care, reduce morbidity and mortality due to communicable diseases and encourage the participation of people. Involvement of community for the success of any vector control programme assumed still greater significance as the problem revolves mainly around man and his environment. The community will perceive the impact of control measures which will stimulate their active involvement in PHC socially, culturally and technically.

Consequent upon the resurgence of Kala-azar in Bihar in 1977 Plague in Maharashtra and Gujarat in 1994 and Dengue/DHF in Delhi, Haryana and Punjab during 1996, it was observed that the public health education about vector-borne diseases is poor and community participation was practically nil. It is felt that for the control of vector-borne diseases, community may be motivated to co-operate and participate for the effective implementation of vector control strategy. After motivation, the community would be able to extend their full co-operation in getting their dwelling units, cattle sheds etc., sprayed with insecticide and should restrain from mud plastering the insecticide treated surface for a minimum period of two months for the retention of residual effect of insecticide. Besides, the community may be motivated to undertake bio-environmental measures like removal of garbage from in and around the houses, pigsties, cattle sheds and filling of all cracks and crevices. The shelters should be made more ventilated and lighted to prevent the breeding, resting and feeding of vector species.

For the success of vector control programme, there has to be frequent interactions between the health workers and the people so that they may accept the control programme as the "People's Programme" and only this approach will be fruitful for the effective implementation of vector control strategy vis a vis control of vector-borne diseases in various parts of the country.

Chemical control

The most common method of control of all arthropods is the use of insecticides or acaricides. The agents are classed as follows:

**Organochlorines**: The insecticidal properties of the best known representative of this class of insecticides, DDT was made by the Swiss Scientist Paul Muller. For this discovery, he was awarded the Nobel Prize for Medicine in 1948. DDT was introduced in the market in 1944. DDT works by opening the sodium channels in the nerve cells of the insect but this agent which was used for a long time effectively is now banned due to environmental and food contamination.

**Organophosphates**: The next large class developed was the organophosphates, which bind to acetyl cholinesterase and other cholinesterases. This results in disruption of nerve impulses, killing the insect or interfering with its ability to carry on normal functions. Organophosphate insecticides and chemical warfare nerve agents (such as sarin, tabun and soman) work in the same way. Organophosphates have an accumulative toxic effect to wildlife, so multiple exposures to the chemicals amplify the toxicity.
**Carbamates**: Carbamate insecticides have similar toxic mechanisms to organophosphates, but have a much shorter duration of action and are thus somewhat less toxic.

**Pyrethroids**: A pyrethroid is an organic compound similar to the natural pyrethrins produced by the flowers of pyrethrums (Chrysanthemum cinerariaefolium and C. coccineum). Pyrethroids now are the main commercially used insecticides. In the concentrations used in such products, they may also have insect repellent properties and are generally harmless to human beings in low doses but can harm sensitive individuals. They are usually broken apart by sunlight and the atmosphere in one or two days, and do not significantly affect groundwater quality. Pyrethroids are however toxic to aquatic organisms and beneficial insects. These are non persistent sodium channel modulators, and are much less acutely toxic than organophosphates and carbamates. Compounds in this group are often applied against household pests and animal pests as well.

**Macrocyclic Lactones (Avermectins and Milbemycins)**: Avermectins and the structurally related milbemycins, collectively referred to as macrocyclic lactones, are fermentation products of Streptomyces avermitilis and Streptomyces cyanogriseus, respectively. Avermectins differ from each other chemically in side chain substitutions on the lactone ring, while milbemycins differ from the avermectins through the absence of a sugar moiety from the lactone skeleton. A number of macrocyclic lactone compounds are available for use and include the avermectins such as abamectin, doramectin, eprinomectin, ivermectin, and selamectin, and the milbemycins, moxidectin and milbemycin oxime. These compounds are active against a wide range of nematodes and arthropods and, as such, are often referred to as endectocides.

**Neonicotinoids**: Neonicotinoids are synthetic analogues of the natural insecticide nicotine (with a much lower acute mammalian toxicity and greater field persistence). These chemicals are nicotinic acetylcholine receptor agonists. Broad-spectrum systemic insecticides, they have a rapid action (minutes-hours). They are applied as sprays, drenches -often as substitutes for organophosphates and carbamates. Treated insects exhibit leg tremors, rapid wing motion, stylet withdrawal, disoriented movement, paralysis and death.

Ryanoids: Ryanoids are synthetic chemicals with the same mode of action as ryanodine, a natural insecticide extracted from Ryaniaspeciosa (Flacourtiaceae). They bind to calcium channels in cardiac and skeletal muscle, blocking nervous transmission. Apparently only one such insecticide is currently registered, Rynaxypyr, in the generic name chlorantraniliprole.

**Insect Growth Regulators (IGRs)**: Many IGRs are labeled "reduced risk" by the Environmental Protection Agency, meaning that they target juvenile harmful insect populations while causing less detrimental effects to beneficial insects. Unlike classic insecticides, IGRs do not affect an insect's nervous system and are thus more worker-friendly within closed environments. IGRs are also more compatible with pest management systems that use biological controls. In addition, while insects can become resistant to insecticides, they are less likely to become resistant to IGRs. As an insect grows, it undergoes a process called moulting, where it grows a new exoskeleton under its old one and then sheds to allow the new one to swell to a new size and harden. IGRs prevent an insect from reaching maturity by interfering with the moulting process. This in turn curbs infestations because immature insects cannot reproduce. Because IGRs work by interfering with an insect's moulting process, they take longer to kill than traditional insecticides.

Insect growth regulator is a term coined to include insect hormone mimics and an earlier class of chemicals, the benzoylehnylureas, which inhibit chitin (exoskeleton)
biosynthesis in insects. Diflubenzuron is a member of the latter class, used primarily to control caterpillars which are pests. The most successful insecticides in this class are the juvenoids (juvenile hormone analogues). Of these, methoprene is most widely used. It has no observable acute toxicity in rats, and is approved by WHO for use in drinking water cisterns to combat malaria. Most of its uses are to combat insects where the adult is the pest, including mosquitoes, several fly species, and fleas. Two very similar products, hydroprene and kinoprene are used for controlling species such as cockroaches and white flies. Methoprene has been registered with the EPA since 1975, and there are virtually no reports of resistance. A more recent type of IGR is the ecdysone agonist tebufenozide (MIMIC), which is used in forestry and other applications for control of caterpillars, which are far more sensitive to its hormonal effects than other insect orders.

**Chitin synthesis inhibitors:** Chitin synthesis inhibitors work by preventing the formation of chitin, a carbohydrate needed to form the insect’s exoskeleton. With these inhibitors, an insect grows normally until it molts. The inhibitors prevent the new exoskeleton from forming properly, causing the insect to die. Death may be quick, or take up to several days depending on the insect. Chitin synthesis inhibitors can also kill eggs by disrupting normal embryonic development. Chitin synthesis inhibitors affect insects for longer periods of time than hormonal IGRs. These are also quicker acting but can affect predaceous insects, arthropods and even fish.

**Antifeedants:** Many plants have evolved substances, like polygodial, which prevent insects from eating, but do not kill them directly. The insect often remains nearby, where it dies of starvation. Since antifeedants are nontoxic, they would be ideal as insecticides in agriculture. Much agrochemical research is devoted to make them cheap enough for commercial use.

Approaches to Biological Control: Natural enemies have been utilized in the management of insect pests for centuries. However, the last 100 years has seen a dramatic increase in their use as well as our understanding of how they can better be manipulated as part of effective, safe, pest management systems.

Recent efforts to reduce broad spectrum toxins added to the environment have brought biological insecticides back into vogue. An example is the development and increase in use of Bacillus thuringiensis, a bacterial disease of Lepidopterans and some other insects.

Toxins produced by different strains of this bacterium are used as a larvicide against caterpillars, beetles, and mosquitoes. Toxins from Saccharopolysporaspinosa are isolated from fermentations and sold as Spinosad. Because these toxins have little effect on other organisms, they are considered more environmentally friendly than synthetic pesticides. The toxin from B. thuringiensis (Bt toxin) has been incorporated directly into plants through the use of genetic engineering. Other biological insecticides include products based on entomopathogenic fungi (e.g. Beauveriabassiana, Metarhiziumanisopliae).

**Role of filamentous fungi in Tick control,**

Some of the fungi used were as follows: Metarhiziumflavoviridae, Metarhiziumanisopliae, Hirsutellathompsoni, BeauveriabassianaPaeclomycesfumosoroseus and Verticilliumlecarii.

Use of leguminous plants in tick control: Stylosanthescabra, Stylosantheshumilus, Stylosantheshamata and Aqueous and Methanolic extracts were used for the ticks such as
Rhipicephalus sanguineus, Rhipicephalus (Boophilus) microplus, Haemaphysalis intermedia, (Fernandes et al 1999).

Methods of treatment:

Products are available for both parenteral administration and for topical application by various methods including dips, sprays, pour-ons, spot-ons, dusting powders, and ear tags. The method used depends on the target parasite and host. Ectoparasiticides that act systemically may be given parenterally or applied topically to the skin, where the active ingredient is absorbed percutaneously and taken up into the circulation. Many of the endectocides are now available as either SC or IM injections or as pour-on preparations acting systemically. Dusting powders have been widely used for the topical treatment of ectoparasite infestation but have been largely superseded by other methods of application. Many of the earlier organochlorine, organophosphate, and pyrethroid insecticides were formulated with an inert base, or bulking agent, for direct topical application. Accurate dosing may be difficult as the recommended dose rates are often loosely based on the size of the animal. Powders also have limited residual activity, necessitating frequent reapplication. Hand application of insecticides as washes, ointments (especially to skin or wounds to control cutaneous myiasis), dusts, spray foams, aerosols, etc, can also be done. To avoid gathering range animals for treatment, self-treatment devices, such as “back rubbers” or dust bags, may be placed in areas where cattle can rub against them.

Another widely used method is spray application of aqueous emulsions or suspensions. Cattle sheds, barns, stables, and dairies are typically sprayed or misted with insecticidal sprays. The animals may be sprayed with insecticide, both to kill and repel flies. A range of formulations is available as liquid concentrates that require dilution with water to produce an emulsion for application by spray. The use of microencapsulation techniques, in which a thin coat of chemical is applied around the active ingredients, can enhance the residual activity of sprays.

Dips are used for the control of mites, ticks, lice, keds, and flies in sheep, cattle, goats, and horses. These may either be by full-body immersion or more shallow baths that cover only the legs and lower body. With immersion or plunge dipping, animals are either made to swim in straight swim-through or circular dip baths or are cage dipped for a prescribed period of time in strict accordance to manufacturer’s instructions. Sheep dip formulations may deplete from the vat faster than the carrier fluid (stripping dips) to maintain therapeutic or prophylactic concentrations, the vat must be topped up with a higher than initial concentration of dip.

Impregnated devices include ear tags, tapes, bands, and collars in which a medium, usually plastic or some form of fabric is impregnated with the chemical, which is then slowly released onto the animal’s coat. A residual life of several months may be expected from such devices. Ear tags on cattle, eg, can provide almost season-long control of biting and nuisance flies. Horses may be treated with such tags attached to halters or with strips attached to halters or to tails. Unfortunately, in a number of areas, horn flies have become resistant to the pyrethroid insecticides that are commonly used in insecticide-impregnated ear tags.

Pour-ons and spot-ons contain the pesticide chemicals at relatively high concentration and are formulated to either penetrate the skin or act systemically or spread over the skin surface and act by contact. Pour-on treatments are usually applied along the backline of an animal or at a single spot on the shoulder blades using a specially designed applicator. They offer the obvious advantages of ease of use, speed, and accuracy of dose. Spot-on
formulations offer a convenient and simple method of application of a small amount of the active ingredient to one or more sites.

**Delivery systems:**

Consumer convenience is an important factor in product choice, especially for flea and tick control. A bewildering array of systems has historically been available powders, aerosols, sprays, shampoos, rinses, dips, spot-ons, mousses, oral tablets or liquids, and impregnated collars. However, the safety, efficacy, and ease of use of the newer spot-on, injectable, and oral application systems have rendered many of the older application technologies essentially obsolete.

Some of the insecticides used for the treatment of various parasitic infestations are as follows:

**Ultrasaber:** Pour on insecticide for cattle containing lambdacyhalothrin with piperonylbutoxide used for the control of horn flies in beef cattle and calves.

**Ultra Boss Pour-on Insecticide:** A pour-on insecticide for cattle, sheep and horses containing 5% permethrin and 5% piperonylbutoxide. Against lice, horn flies, face flies, horse flies, stable flies, mosquitos, black flies and ticks

**Taktic:** An ectoparasiticide containing amitraz for the control of ticks, mange mites, lice and keds on cattle sheep, goats and pigs by spray or dip treatment.

**Synergized Delice Pour-on Insecticide:** An insecticide containing 1% permethrin and 1% piperonylbutoxide. Controls lice, horn flies and face flies on lactating and non-lactating dairy cattle, beef cattle and calves and aids in control of horse flies, stable flies, house flies, mosquitoes and black flies as well as others.

**Saber Pour-on:** A pour-on containing 1% lambdacyhalothrin. For the control of lice and horn flies on beef cattle and calves, not for use in dairy cattle.

**Saber Extra Insecticide Ear Tags:** Insecticidal ear tags containing 10% lambdacyhalothrin and 13% piperonylbutoxide. For up to 5 months control of horn flies and up to 4 months control of face flies on beef and non-lactating dairy cattle and calves.

**Dominator insecticide ear tags:** Insecticide ear tags with 20% pirimiphos methyl. Control of horn flies (including synthetic pyrethroid-resistant horn flies) and as an aid in the control of face flies on non-lactating dairy cattle and calves.

**Butox 7.5% Pour on:** Prevention and treatment of flies, lice, keds and ticks on cattle and sheep. Contains deltamethrin in a ready to use suspension for external use.

**BUTOX 50% EC** Prevention and treatment of ticks, flies, lice and mange on cattle and sheep. Contains deltamethrin in an emulsifiable concentrate for preparation of solution for external use.

**Boss pour-on insecticide:** Pour-on insecticide containing 5% permethrin. For fly control for lactating and non-lactating dairy cattle and beef cattle and calves, and ked and lice control on sheep.

**Atroban 11% Ec:** An emulsifiable concentrate insecticidal spray for livestock and their premises. Contains permethrin. Controls horn flies, face flies, stable flies, house flies, horse flies, and black flies, mosquitos, eye gnats, mange mites, ticks, lice, and sheep keds.
Atroban 1% delice pour-on insecticide: Non-systemic pour-on insecticide for beef, lactating and non-lactating dairy cattle. Contains permethrin. Controls lice and flies on cattle and keds and lice on sheep.

Ivermectin: Ivermectin is also used in veterinary medicine. It is sometimes administered in combination with other medications to treat a broad spectrum of animal parasites. Some dog breeds (especially the Rough Collie, the Smooth Collie, the Shetland Sheepdog and the Australian Shepherd), though, have a high incidence of a certain mutation within the MDR1 gene; affected animals are particularly sensitive to the toxic effects of ivermectin. Kittens are also very sensitive. A 0.01% ivermectin topical preparation for treating ear mites and lice in cats (Acarexx) is available. Ivermectin is sometimes used as an acaricide in reptiles, both by injection and as a diluted spray. While this works well in some cases, care must be taken, as several species of reptile are very sensitive to ivermectin. Use in turtles is particularly contraindicated.

Cultural and Biological Control

These measures can be directed against both the free-living and parasitic stages of ticks. The free-living stages of most tick species, both ixodid and argasid, have specific requirements in terms of microclimate and are restricted to particular microhabitats within the ecosystems inhabited by their hosts. Destruction of these microhabitats reduces the abundance of ticks. Control of argasid ticks such as Argas persicus and A. walkerae in poultry can be achieved by eliminating cracks in walls and perches, which provide shelter to the free-living stages.

The abundance of tick species can also be reduced by removal of alternate hosts or hosts of a particular stage of the life cycle. This approach has occasionally been advocated for the control of 3-host ixodid ticks such as Rhipicephalus appendiculatus, Amblyomma hebraeum and Ixodes rubicundus in Africa, and Hyalomma spp. in southeastern Europe and Asia. Rotation of pastures or pasture spelling has been used in the control of the one host ixodid tick Boophilus (Rhipicephalus) microplus in Australia. The method could also be applied to other one host ticks, in which the duration of the spelling period is determined by the relatively short life span of the free-living larvae. However, it has minimal application to multihost ixodid ticks or argasid ticks because of the long survival periods of the unfed nymphs and adults.

Predators, including birds, rodents, shrews, ants and spiders, play a role in some areas in reducing the numbers of free-living ticks. In the New World, fire ants (Pheidole megacephala) are noteworthy tick predators. Engorged ticks may also become parasitized by the larvae of some wasps (Hymenoptera), but these have not significantly reduced tick populations.

Zebu (Bos indicus) and Sanga (a B. taurus, B. indicus crossbreed) cattle, the indigenous breeds of Asia and Africa, usually become very resistant to ixodid ticks after initial exposure. In contrast, European (B. taurus) breeds usually remain fairly susceptible. The tick resistance of Zebu breeds and their crosses is being increasingly exploited as a means of control of the parasitic stages. The introduction of Zebu cattle to Australia has revolutionized the control of B. microplus on that continent. Use of resistant cattle as a means of tick control is also becoming important in Africa and the Americas.

In Africa, infestations of ixodid ticks on livestock and wild ungulates may also be reduced by oxpeckers (Buphagusspp), which are birds that feed on attached ticks.
Among biological agents, entomopathogenic fungi played a uniquely important role in the history of microbial control of insects. Beauveriabassiana, commonly known as white muscaridine fungus, attacks a wide range of immature and adult insects. Metarhiziumanisopliae, a green muscaridine fungus was reported to infect 200 species of insects and arthropods. Both these entomopathogenic fungi are widely distributed and are soil inhabiting.

Evaluation of the biological control potential of these fungi for vectors of animal diseases has begun recently. Beauveriabassiana was used to control Boophilusmicroplus, Hyalommaanatolicum larvae, Rhipicephalus bursa, Amblyommaamericanum, Dermacentorvariabilis, larvae and adult of Muscadelomestica.

Metarhiziumanisopliae was used as potential biocontrol agent for B.microplus, I. scapularis, B. annulatus. Oil suspension of both fungi killed adults and larvae of ticks more rapidly than aqueous suspension. Between these fungi, M. anisopliae is considered suitable for the climatic conditions prevalent in our country

Other species of entomopathogenic fungi namely Beauveriatenella, Aspergillusparasiticus, Cephalosporiumcoccorum, and Paecilomycesfumosonoseus are obligate pathogens of I. ricinus and Dermacentor sp. and are one of the potential factors to reduce natural populations of ticks.

The use of entomopathogenic nematodes and semiochemicals for tick control is also indicating them as futuristic methods as per research reports.

Immunological control of ticks

Major alternatives to conventional acaricide treatments have been developed in recent years. Among the most important are various vaccines that have been commercialized for release to the agricultural community, including recombinant vaccines against midgut surface antigens, Tick GARD and GAVAC. Unfortunately, efficacy of tick control has not reached the levels that the inventors had hoped to achieve. According to data summarized by Willadsen, reduction in tick numbers was 56% for cattle in an Australian study with TickGARD and 67% for cattle in a Cuban study with Gavac. The best vaccine, recombinant Bm86, is reputed to reduce tick fecundity by 90%. However, this is the highest efficacy currently achieved, with most other vaccines showing lower levels. Several exposed antigens have been expressed as recombinant proteins so far and evaluated for their efficacy as anti-tick vaccine.

Tick vaccine against multiple tick species

A broad spectrum / universal vaccine is one that targets not only all stages of ticks but also multiple tick species and ideally, all species. Highly conserved antigens between tick species potentially possess the properties for cross resistance across all tick species and stages. Early experiments using B. microplus Bm86 demonstrated cross protection against B. annulatus and B. decoloratus infestations and partial protection against Hyalomma and Rhipicephalus spp.

An important aspect of controlling tick infestations is a reduction of the transmission of tick borne pathogens. Therefore anti-tick vaccines should reduce the incidence of tick-borne diseases through reducing vector numbers in principle. The phenomenon of reduced transmission capacity of ticks fed on immunized animal has been observed with several works. Early experiments with Bm86 vaccines resulted in a reduction in the incidence of Babesiosis together with a reduced number of tick infestation. The transmission of tick borne
encephalitis virus (TBEV) was prevented by the vaccination of the putative tick cement 64P in mice model, suggesting that 64P could be a potential candidate for transmission blocking vaccines. Because vaccines are expensive and involve considerable risk, a high level of efficacy is required to offset these negatives. Hence, there has been little industry enthusiasm for further commercialization of anti-tick vaccines. However, exciting new developments, such as the ability to disrupt the male engorgement factor or the administration of combined anti-tick and anti-pathogen agent vaccines, might change this picture.

It can thus be concluded that a combination of methods would be required to combat the problem of vectors and vector borne diseases.
Integrated management of Parasitic diseases

Dr.P.M. Thimmareddy
Professor
Centre of Advanced Faculty Training
Department of Parasitology
Veterinary College, Hebbal, Bengaluru-560024

Integrated parasite management (IPM) is a concept that is increasingly being adopted in animal parasite control. It is based on the integrated pest management philosophy already well-established in agricultural and horticultural crop protection. IPM builds on acceptance of the view that total dependence on chemical control is no longer a sustainable option because

a) **Resistance**: is limiting the use or usefulness of many existing pesticides or parasiticides.

b) **Few replacement products**: the rate of introduction of products active against resistant strains is restricted by the high cost of discovery, development, safety-testing, registration and marketing of novel chemical entities.

c) **Meat and milk residues**: are potential problems associated with chemotherapy if products are used inappropriately.

d) **Ecological concerns**: arise if significant quantities of biologically active materials are released into the environments.

The IPM model aims to manage pest or parasite populations, rather than eliminate them, by using chemicals intelligently as one component of a wider integrated control strategy based on epidemiological principles. This encompasses husbandry and hygiene measures, together with vaccines and other technological aids, if available. The use of parasiticides, when needed, is limited to sustainable strategic interventions.

**Helminth parasites**

Internal parasites can be a major problem with parasites developing resistance to chemicals and therefore alternate ways are important to consider.

1. **Nutrition**: Nutrition plays a major role and animals on a high plane of nutrition are resistant to parasites. Vitamin-A, D and B-complexus and minerals like zinc, iron, cobalt, sodium, potassium, phosphorus, etc are very essential for proper functioning of immune system.

2. **Pasture management**: An understanding of the life cycles of different parasites within the whole soil-plant animal system will help show the inter-relationship between these three components.

   a) By using controlled grazing methods that allow pastures to rest and soil life to function well, contamination can be reduced. This reduction occurs because soil organisms, including earthworms, dung beetles, and nematophagous fungi will destroy or keep a lot of the parasite eggs and larvae from developing.
b) Good grazing management includes the use of clean pasture to minimize re-infection. Clean pasture is pasture that has not been grazed by the host animal (in this case sheep and goats) for 12 months, and therefore is not contaminated with worm larvae.

c) Warmth, oxygen and moisture are the three most important things that increase the chances that larvae will survive on pasture. Knowing when your pastures are apt to be driest and coldest will help you manage them better for parasite control.

3. Immunity: Most animals develop immunity against internal parasites, though not to the level that is developed against virus and bacteria. This immunity keeps the parasites from reproducing but rarely kills them. Every farm is different. The parasite load of the animal depends on many variables—such as stocking density, time of year, the reproductive state of the animal etc.

4. Resistance: Healthy and well-nourished animals will be able to develop resistance and resilience to worms and other parasites much better than thin animals that do not have good availability of quality feed. Resistance is the ability of an animal to prevent the establishment and maintenance of a parasite population within the gastrointestinal tract. Some individuals and some breeds show more resistance to parasitic infection than others. Research to identify characteristics in such individuals is a hot area. Resilience is the ability of an animal to reduce production loss during parasite infestation. Both of these traits are being looked at as ways of selecting animals that will be less susceptible to parasite effects.

5. Soil organisms: There are several soil organisms that can have an impact on parasites. Managing pastures to favour populations of beneficial soil organisms will decrease parasite levels on pastures. Oxygen is the primary requirement for worm eggs and larvae to survive and develop. Earthworms have been shown to ingest worm eggs and larvae, either killing them or carrying them far enough below ground to keep them from maturing. Dung beetles ingest and disperse manure, taking it to their burrows, thus keeping eggs and larvae from developing. There are also nematophagus fungi that produce “traps” that engulf and kill parasitic larvae.

6. Strategic deworming: There will be times when chemical dewormers are the best treatment. The situation, time of the year and the location will help determine which chemical dewormer to use. Getting rid of all worms all the time is not essential for health of the animal, is rarely cost effective and can actually be detrimental since the immune system of the animal is an important defence mechanism in managing parasite effects.

7. Alternative dewormers: By using diatomaceous earth (DE), herbal and folk medicine.

Insects

Among insects, flies play a major role in causing nuisance, annoyance and transmission of diseases. Therefore integrated pest management includes physical and biological control through herbal or microbial or fungal and others.

1. Physical methods

   a) Use of net shed.

   b) Good hygiene, sanitation, waste management including heaping up of manure, wet straw, spreading of waste as thinly as possible.
c) Proper drainage, use of polystyrene beads to underground tanks and applications of biolarvicides to water accumulated bodies.

d) Confinement of animals during the peak fly activity

e) Use of insect growth regulator like methoprene which has very low level of toxicity to mammals.

f) Early warning system and prediction models: Baylis and rawlings (1998) used climate data and satellite imagery model to know the abundance of *culicoides*. In which they estimated NDVI(normalized difference vegetation index) measure of photosynthetic activity and LST (land surface temperature) a measure of temperature at earth surface. NDVI and LST as predictors help in population peaks of flies.

2. Biological control.

**Pathogens:** A micro-organism that lives and feeds (parasitically) on or in a larger host organism and thereby causes injury to it. Examples are viruses, bacteria (and their toxins), protozoa, fungi, nematodes, microsporidian *Thelokomia opacita* for control of Tabanids, mosquitoes, midges, simulids.

**Parasitoids:** A parasitic insect that lives in or on and eventually kills a larger host insect (or other arthropod). Wasps: *Spalangia cameroni* are the pathogens for house flies.

**Predators:** An animal that feeds upon other animals (prey) that is either smaller or weaker than itself. Ex. *Poecilia rectculata* (guppy fishes) as a larvivorous for control of mosquiotos and for *Culicoides* spp.

**Concept of bio-pesticide:** A compound that kills organisms by virtue of specific biological effects rather than as a broader chemical poison. Differ from bio-control agents in being passive agents, whereas biocontrol agents actively seek the pest. The rationale behind replacing conventional pesticides is that the latter are more likely to be selective and biodegradable as per FAO

**Biopesticides are classified as follows;**

1. Microbial pesticides consist of microoraganism (eg. a bacterium, fungus, virus or protozoan) as their active ingredient.

2. Plant-Incorporated-Protectants (PIPs) are pesticidal substances that plants produce from genetic material that has been added to the plant.

**Ticks**

Ticks not only cause direct pathogenic effect but transmit important diseases to animals and man. Therefore control by other methods is gaining importance.

1. **Physical methods.**

a) Caulking process involving burning of tick eggs in the cattle shed for annihilation of breeding places, to be undertaken three consecutive times at weekly interval in a season.

b) Caulking of the cracks and crevices on walls of the animal sheds by slow burning over a period of one or two days.
2. Biological methods.

a) Grooming: It is the manual removal of ticks, is widely used in the developing world, although it is rare in extensive system.

b) Pasture spelling

c) Biological control/environmental: It includes the candidates such as ants, predatory mites, chickens, parasitoid wasps, *Bacillus thuringiensis*, entomopathogenic nematodes and oxpeckers, *Buphagus africanus*.

d) Biopesticides: Research on a biopesticide derived from a strain of naturally occurring soil fungus *Metarhizium anisopliae* has confirmed the effectiveness with 100% tick mortality of the most common variety of tick within two days in laboratory condition. Control of tick populations by spraying *Metarhizium anisopliae* conidia on cattle under field conditions.

e) Vaccines so far commercially available were developed to be effective against *B. microplus* and are based on the tick midgut protein Bm86. An effective antigen against ticks is the protein Bm86, specifically directed against the cattle tick *Rhipicephalus microplus*, it stands as the basis of two commercial vaccines, TickGARD plus and gavac plus.

f) Herbal approach

g) Forecasting: early warning system using GIS information is the recent tool. Meteorological data for making the prediction model is available at district centres (612 centres) in India (Kshirsagar et al., 2013).

References


Collection and dispatch of samples for Parasitic disease Investigations

Dr.R.Sridevi, Scientist, ICAR-NIVEDI

Parasites includes nematodes, cestodes, trematodes, protozoans, arthropods etc. Generally, parasites causes chronic infections in livestock animals but causes lot of losses in terms of productivity and longevity. Although, studies on parasitic disease burden are less, the importance of damage caused by them cannot be neglected. Animals exhibit different clinical manifestations depending on the system involved but same symptoms for parasitic or bacterial or viral diseases. Now a days, climate change drastically affects parasite-host-environment interactions and hence the consequence is of immense and varied in nature. Generally, Field level investigations are common for microbiological or parasitological origin. In this scenario, presumptive diagnosis at the field level is tricky unless specific agent signature available or updated knowledge available at the field level. Here, some of the general collection and dispatch tips are provided for diseases of parasitic origin. The main aim of collection of samples is diagnosis, control and treatment/ preventive measures, hence emphasis is given in some of the points to avoid aberrant results during diagnosis.

Haemoproteozoan diseases in animals

Haemoproteozoan such as Trypanosoma, Theileria, Babesia, Anaplasma cause devastating losses to the livestock industry throughout the world. However, it is known that most of blood protozoan parasites cause anemia by inducing erythrophagocytosis. Most of the haemoproteozoan parasites are arthropod borne and is of great economic importance in Asia and has always been a formidable barrier to the survival of exotic and cross bred cattle in India.

Laboratory investigation of animal disease is critically dependent on the quality and appropriateness of the specimens collected for analysis. Sampling may be from individual animals, from animal populations, or from the environment for a variety of purposes, such as disease diagnosis, disease surveillance, health certification, and monitoring of treatment and/or vaccination responses.

To provide scientifically and statistically valid results the specimens collected must be appropriate for the intended purpose, and adequate in quality, volume, and number for the proposed testing. Additionally, the animals and tissues sampled must be appropriately representative of the condition being investigated.

Specimens must be collected using appropriate biosafety and containment measures in order to prevent contamination of the environment, animal handlers, and individuals doing the sampling as well as to prevent cross-contamination of the specimens themselves. Care should additionally be taken to avoid undue stress or injury to the animal and physical danger to those handling the animal. Biological materials should be packaged to rigorously control for leakage, and then labelled with strict adherence to the applicable regulations guiding their transport.

General considerations for Collection and despatch of samples from live/dead animals for diagnosis:

Careful consideration must be given to the collection, containment, and storage of the specimens, including biosafety measures that must be in place to prevent contamination of the environment or exposure of other animals to potentially infectious materials. Diagnosis
based on symptoms and laboratory examination of the relevant materials is essential for initiating treatment at the proper time. In general the following points should be duly considered while collecting materials for laboratory diagnosis.

All materials collected should be accompanied by full history of disease outbreak namely species affected, duration of disease, clinical signs, morbidity and mortality rates, disease suspected etc. The collected biological specimens should be transported on ice to the nearest laboratory as early as possible. If death is reported, Necropsy examination should be conducted at the earliest as putrified materials are unfit for laboratory examination. Detailed post-mortem report should be attached along with the samples collected during post-mortem. Specimen bottles with wide mouth should be used for collecting tissues. The specimen bottle should be sealed well so as to avoid leakage and clearly indicating the fixative/transport media used. The reliability of the diagnostic testing is critically dependent on the specimen(s) being appropriate, of high quality, and representative of the disease process being investigated. Prior to sampling, consideration must be given to the type of specimen(s) needed including the purpose of the testing and the test technologies to be used. The volume or quantity of specimen must be sufficient to perform initial testing, to perform any subsequent confirmatory testing and to provide sufficient residual specimen for referral or archival purposes.

Specimens must be collected according to a sound knowledge of the epidemiology and pathogenesis of the disease under investigation, or the disease syndrome to be diagnosed. This will lead to the sampling of tissues or fluids most likely to contain the infectious agent or evidence of the infection. Considerations will include the tissue predilection(s) or target organ, the duration and site of infection in each tissue type and the duration and route of shedding, or the time frame in which evidence of past infection, such as an antibody response, can be detected reliably by the tests to be deployed. These considerations will also indicate the method(s) of collection to be used.

Some laboratory tests are not compatible with specific blood anticoagulants and tissue preservatives, such as heparin, formalin, dry ice (exposure of the test sample to elevated levels of CO2), or even freezing. While it is critical to collect specimens as aseptically as possible, equal care must be taken to avoid contamination with detergents and antiseptic treatments used to clean the collection site on the animal, as these agents may interfere with the laboratory test procedures. Procedures requiring tissue culture of pathogens, as well as many molecular-based tests, can be negatively affected by chemicals or detergents commonly used in the manufacture or preparation of collection tools.

Specific considerations regarding different specimen types are as outlined below.

In selecting the anticoagulant to be used the collector must be aware of the laboratory tests, including PCR-based diagnostics, clinical chemistry which may be negatively affected by the presence of specific anticoagulants or preservatives. To be effective anticoagulants require that the collected blood be thoroughly mixed with the chosen anticoagulant during or immediately following its sampling from the animal. Whole blood should be collected aseptically, typically by venipuncture of the live animal. Depending on the animal and sampling situation jugular, caudal, brachial, cephalic, mammary veins or the vena cava may be used. Care should be taken to collect and dispense blood samples as gently as possible to prevent damage to red blood cells, which causes haemolysis.

For detection of some viral, bacterial and blood parasite DNA or RNA or antibodies against some pathogens, small amounts of whole blood can be collected from the freshly opened body cavity by dripping it on a thick Whatman-type filter paper or by touching to organs or muscles with the filter paper. The blood spots can then be dried at room
temperature and stored in plastic bags in a dry place. Adding a small silica gel pack to each plastic bag is recommended (Munson, 2000).

**For serology,** serum (clear fluid, yellow, in autolysed animals red-tinged) or plasma should be separated for the blood cells, divided into at least two sterile tubes and then refrigerated or frozen at -20° or -70° if possible until transport to a laboratory. Serum, plasma or blood from the heart of a carcass can be collected in vials during necropsy and left undisturbed for approximately 30 min to encourage clot formation, then centrifuged at approximately 2000 X G for 20 min. When a centrifuge is not available, serum can be obtained by letting the clot or blood cells settle. If blood is obtained from a live animal or a dead animal whose blood has not yet clotted, whole blood can be removed into a blood tube and stored with the tube inverted (rubber stopper down) until it clots; then the tube can be cautiously turned and the stopper can be removed with the clot attached, leaving the serum in the tube (Munson, 2000).

*Trypanosoma evansi* (Surra) infection: *Trypanosoma evansi* causes a trypanosomosis known as ‘surra’. It affects a large number of wild and domesticated animal species in Africa, Asia, and Central and South America. The principal host species varies geographically, but camels, horses, buffalos and cattle are particularly affected, although other animals, including wildlife, are also susceptible. It is an arthropod-borne disease; several species of haematophagous flies, including Tabanids and Stomoxes, are implicated in transferring infection from host to host, acting as mechanical vectors. Clinical suspicion of surra can emerge from the field in case of fever and/or anaemia. Anaemia is a reliable indicator of trypanosome infection, but it is not in itself pathognomonic. On the other hand, animals with a mild subclinical infection can have parasitaemia without evidence of anaemia (Dargantes *et al.*, 2005a). In enzootic areas, routine diagnoses can be made using parasitological techniques, while serological surveys can be carried out preferably by ELISA. CATT can be used to target individual animals for treatment with trypanocidal drugs.

Sample collection from live animals:

i) Blood sampling: *Trypanosoma evansi* is a parasite of the blood and tissues. As for other trypanosomes, it is recommended that blood for diagnosis be obtained from peripheral ear or tail vein, even if the jugular vein is most often preferred for practical reasons. However it should be realised that less than 50% of infected animals may be identified by examination of blood. Peripheral blood is obtained by puncturing a small vein in the ear or tail. Deeper samples are taken from a larger vein by syringe. Cleanse an area of the ear margin or tip of the tail with alcohol and, when dry, puncture a vein with a suitable instrument (lancet, needle). Ensure that instruments are sterilised or use disposable instruments to avoid iatrogenic transmission of the infection by residual blood.

ii) Preparation of Wet blood films: Place a small drop of blood (2–3 μl) on to a clean glass slide and place over it a cover-slip to spread the blood as a monolayer of cells.

iii) Preparation of thick blood smears (Unstained /Stained): Place a large drop of blood (10 μl) on the centre of a microscope slide and spread with a toothpick or the corner of another slide so that an area of approximately 1.0–1.25 cm in diameter is covered. Air-dry for 1 hour or longer, while protecting the slide from insects. Placing the slide in a horizontal position, stain the unfixed smear with Giemsa’s Stain (one drop of commercial Giemsa + 1 ml of phosphate buffered saline, pH 7.2), for 25 minutes. After washing and drying, examine the smears by light microscopy at a magnification of 500x with oil immersion.

iv) Preparation of Stained thin blood smears: Place a small drop of blood (3–5 μl) at one end of a clean microscope slide and draw out a thin film in the usual way. Air-dry briefly and fix
in methyl alcohol for 1 minute and allow to dry. Stain the smears in Giemsa (one drop Giemsa + 1 ml PBS, pH 7.2) for 25 minutes. Pour off stain and wash the slide in tap water and dry.

v) Lymph node biopsies or oedema fluid: Samples are usually obtained from the prescapular or precrural (subiliac) lymph nodes. Select a suitable node by palpation and cleanse the site with alcohol. Puncture the node with a suitable gauge needle, and draw lymph node material into a syringe attached to the needle. Expel lymph on to a slide, cover with a cover-slip and examine as for the fresh blood preparations. Fixed thin or thick smears can also be stored for later examination. Similar examination can be done by collection of oedema fluid. *Trypanosoma evansi* has a broad spectrum of infectivity for small rodents, and so rats and mice are often used.

**Trypanosomosis: Similar to Surra**

**Bovine babesiosis**: Babesiosis is a tick-borne disease of cattle caused by the protozoan parasites *Babesia bovis*, *B. bigemina*, *B. divergens* and others. *Babesia bovis* and *Babesia bigemina* – are widely distributed and of major importance in Africa, Asia, Australia, and Central and South America. Babesia divergens is economically important in some parts of Europe. *Rhipicephalus* (*Boophilus*) spp., the principal vectors of *B. bovis* and *B. bigemina*, are widespread in tropical and subtropical countries. The traditional method of identifying the agent in infected animals is by microscopic examination of thick and thin blood films stained with Giemsa.

Sample collection from Live animals: Samples from live animals should preferably be films made from fresh blood taken from capillaries, such as those in the tip of the ear or tip of the tail, as *B. bovis* is more common in capillary blood. *Babesia bigemina* and *B. divergens* parasites are uniformly distributed through the vasculature. If it is not possible to make fresh films from capillary blood, sterile jugular blood should be collected into an anticoagulant such as lithium heparin or ethylene diamine tetra-acetic acid (EDTA). The sample should be kept cool, preferably at 5°C, until delivery to the laboratory.

Preparation of blood smears: (i) Thin blood films are air-dried, fixed in absolute methanol for 10–60 seconds and then stained with 10% Giemsa for 15–30 minutes. It is preferable to stain blood films as soon as possible after preparation to ensure proper stain definition. (ii) Thick films are made by placing a small droplet of blood (approximately 50 μl) on to a clean glass slide and spreading this over a small area using a circular motion with the corner of another slide. This droplet is not fixed in methanol, but simply air-dried, heat-fixed at 80°C for 5 minutes, and stained in 10% Giemsa. This is a more sensitive technique for the detection of *Babesia* spp. For serological tests: Blood with anticoagulant (sodium citrate or EDTA), blood without anticoagulant for serum.

Sample collection from dead animals: Samples from dead animals should consist of thin blood films, as well as smears from cerebral cortex, kidney (freshly dead), spleen (when decomposition is evident), heart muscle, lung, and liver (Bock *et al.*, 2006; de Vos *et al.*, 2004). Organ smears are made by pressing a clean slide on to a freshly cut surface of the organ or by crushing a small sample of the tissue (particularly cerebral cortex) between two clean microscope slides drawn lengthwise to leave a film of tissue on each slide. The smear is then air-dried (assisted by gentle warming in humid climates), fixed for 10–60 seconds in absolute methanol, and stained for 15–30 minutes in 10% Giemsa. This method is especially suitable for the diagnosis of *B. bovis* infections using smears of cerebral cortex.

**Theileriosis**: Theileriae are obligate intracellular protozoan parasites that infect both wild and domestic Bovidae. They are transmitted by ixodid ticks, and have complex life cycles in both vertebrate and invertebrate hosts. The two most pathogenic and economically important
are *T. parva* and *T. Annulata*. Diagnosis of acute theileriosis is based on clinical signs, knowledge of disease, and vector distribution as well as examination of Giemsa-stained blood, lymph node and tissue impression smears. The infected animal shows enlargement of the lymph nodes, fever, a gradually increasing respiratory rate, dyspnoea and/or diarrhoea. **Samples from live animals:** Giemsa-stained biopsy smears of lymph nodes, and is a characteristic diagnostic feature of acute infections with *T. parva* and *T. annulata*. **Samples from dead animals:** Schizont-parasitised cells may be found in impression smears from all tissues: lung, spleen, kidney and lymph node smears are particularly useful for demonstrating schizonts. However, schizonts can be easily detected in smears from lymph nodes, spleen and liver tissues obtained by needle biopsy of these organs.

**Equine Piroplasmosis:** Equine piroplasmosis is a tick-borne protozoal disease of horses, mules, donkeys and zebra. The aetiological agents are blood parasites named *Theileria equi* and Babesia caballi. The parasites are found inside the red blood cells of the infected animals. Infected horses may be identified by demonstrating the parasites in stained blood, optimally collected from superficial skin capillaries, or organ smears during the acute phase of the disease. Romanovsky-type staining methods, such as the Giemsa method, usually give the best results. Thick blood smear technique to detect very low parasitaemia. Thick films are made by placing a small drop (approximately 50 μl) of blood on to a clean glass slide which is then air-dried, heat fixed at 80°C for 5 minutes, and stained in 5% Giemsa for 20–30 minutes. For Serological tests, serum separated as described earlier and sent. For detection by molecular techniques, whole blood has to be sent in ice with proper care.

**Anaplasmosis:** **Samples from live cattle:** should include thin blood smears and blood collected into an anticoagulant. Air-dried thin blood smears will keep satisfactorily at room temperature for at least 1 week. The blood sample in anticoagulant should be held and transferred at 4°C, unless it can reach the laboratory within a few hours for preparing fresh smears. In addition, a low packed cell volume and/or erythrocyte count can help to substantiate the involvement of *A. marginale* when only small numbers of the parasites are detected in smears, such as may occur in the recovery stage of the disease. In contrast to *Babesia bovis*, *A. marginale* does not accumulate in capillaries, so blood drawn from the jugular or other large vessel is satisfactory. Because of the rather indistinctive morphology of *Anaplasma*, it is essential that smears be well prepared and free from foreign matter, as specks of debris can confuse diagnosis. **Samples from dead animals:** should include air-dried thin smears from the liver, kidney, heart and lungs and from a peripheral blood vessel. The latter is particularly recommended should there be a significant delay before post-mortem examination because, under these circumstances, bacterial contamination of organ smears often makes identification of *Anaplasma* equivocal. Blood from organs, rather than organ tissues per se, is required for smear preparation, as the aim is to be able to examine microscopically intact erythrocytes for the presence of *Anaplasma*. Organ-derived blood smears will store satisfactorily at room temperature for several days. Both blood and organ smears can be stained in 10% Giemsa stain.

**Helminth diseases in animals:**

**Collection of faecal samples:** It is important that the examinations are carried out on fresh faeces, as eggs of many helminths hatch within 24 hours (e.g. *Oesophagostomum* spp.) or even after 8 hrs (*Strongyloides*) at room temperature, and the young larvae will not be detected by the standard laboratory techniques. **Equipment required:** Plastic gloves (cheap plastic gloves are often preferable for the more expensive latex gloves) Marking pen (waterproof) Plastic bags Insulated cooling box (storage
temperature: 0-8°C), if the transport time to the laboratory exceeds 1-2 hrs 3% formalin and plastic containers with tight lids, if a long transport time is expected, and a cooling box is not available.

Procedure: Faecal samples are preferably collected directly from the rectum, to ensure that they are completely fresh. This will also allow for registration of the animals age, sex, reproductive status etc., and repeated samples from the same individual may be avoided. If rectal samples cannot be obtained, freshly deposited faeces may be collected from the pens/pastures. The samples may be stored in the plastic gloves by turning their inside out. Each sample should be unambiguously labelled with animal identification, date and localization in waterproof ink directly on the plastic glove. The amount of faecal matter required depends on the analyses, but at least 4 g is needed for most egg count procedures, and 20 g is preferred if more than one kind of analysis is needed, or if an unexpected result must be confirmed by repeated analyses. The samples are gathered in larger plastic bags. If the transport time to the laboratory is expected to exceed 1-2 hours (depending on temperature), the samples should be packed in a cooling box to avoid hatching of the eggs. The storage temperature should be 0-8°C, and care should be taken to avoid freezing, as this may damage the eggs and invalidate later results. If larval cultures are to be done, the faecal samples may not be cooled, as even 24 hours at 5°C may interfere with larval development. When a cooling box is not available, the samples may be placed in plastic containers with tight lids, and 3% formalin should be admixed to the faeces (approx. 1 ml formalin to 4 g faeces). This will preserve the sample and the parasite eggs, but it should be noted that quantitative egg counts will not be completely correct, and that formalin-fixed faeces cannot be used for faecal cultures. In the laboratory, the samples for egg counts should immediately be placed in a refrigerator (approx. 4°C) until they are processed. Samples may be stored at this temperature for more than 3 weeks without significant changes in egg counts. If faecal cultures are to be set up, a storage in a refrigerator cannot be recommended at all, but the cultures should be set up immediately. Note again that faecal samples should never be kept in a freezer.

Nasal schistosomiasis –Nasal discharge in normal saline, Nasal granuloma in normal saline.

Trichomoniasis :Vaginal or uterine discharges, Prepucial scraping/ washing

**Figure 1.** Example of a basic necropsy kit that can be packed into a small day pack. Clockwise, from top of photo: Data recording: field notebook, tags, pencils, markers. Protective apparel: rubber gloves, disposable shoe covers and coveralls, mask. Necropsy equipment: disinfectant for cleaning instruments, scrub brush, heavy shears, forceps, scissors, scalpel handle and blades. Measuring equipment: hanging scale and ruler. Sampling materials: microscope slides, syringes, needles, swabs, blood tubes, aluminum foil, plastic bags, wide mouth plastic jars. Preservatives: ethanol for parasites, formalin for tissue samples.

**Guidelines for submitting samples for parasitological examination in labs**

**Fresh Feces**

1. Fresh fecal samples less than 48 hours old are preferrable.
2. Samples should be placed in individual sealed containers labeled with the animal number/name and the date collected.
3. Plastic containers with lids, WhirlPaks, and ziplock bags are acceptable.
4. Refrigerate samples as soon as possible after collection, but do not freeze.
5. Submit samples as soon as possible in a Styrofoam container with frozen gel packs via any of the 24 to 48 hour transport services.
6. Most tests require 5 grams of feces for accurate results.
7. For larval examination, fecal samples must be sent within 1-2 hours unrefrigerated condition.
8. If an animal has been treated with anti-diarrhea preparations containing bismuth or kaolin, mineral oil, oral contrast material (barium) for radiology (all of these materials float) or antibiotics, then parasites may be difficult or impossible to find. Therefore, repeat the fecal exam 5-10 days after treatment withdrawal.

Whole Blood
1. 1-2 mL of whole blood submitted in a purple top tube (EDTA tube).

Serum
1. Serum samples need to be at least .5mL.

Skin Scrapings/ Hair
1. If possible submit samples in a glass tube. Red top blood collecting tubes work well as specimen containers.
2. If necessary or if sample size is small, samples can be submitted on a glass slide if the cover slip is sealed with nail polish or VasPar.
3. Please do not use scotch tape as a cover slip as it obscures vital details of the sample.

Parasites
1. Ectoparasites (Arthropods) can be transported in 70% alcohol in a sealed container. Red top blood collecting tubes work well.
2. Intestinal parasites need to be transported in water in a sealed container. Intestinal parasites received in formalin cannot be identified.
3. Please label container with host and location where the parasite was found.
4. Level of Parasite Identification is determined by lab personnel when the sample arrives in the laboratory to be identified.

Soil samples
1. Soil samples need to be at least 30 grams.
2. Collect soil from 2-3 inches below the surface from 3 places in the area to be tested.
3. Please send sample in a sealed plastic container or ziplock bag.

Direct Smear
1. Direct fecal smears are most useful for the diagnosis of protozoal parasites which have motile trophozoite stages that are passed in the feces. Cysts and oocysts of coccidia and Giardia sp. can be seen on direct smears; however, these non-motile stages are more likely to be recovered when concentrated using a flotation technique.
2. In order to be diagnostic, direct smears MUST be performed using fresh feces. Fresh feces means body temperature (usually less than five or ten minutes old!). As the specimen cools, trophozoites lose their motility and their diagnostic features become less recognizable. In preparing the smear, use saline. Water will rupture some trophozoites, rendering them unrecognizable.
3. Indications: Motile protozoa trophozoites (feces must be body temperature).
4. Limitations: Small sample size (sample size is so small that if you see nothing, it may not mean that the animal has no parasites; it just means there aren’t enough to show up in a direct smear).

References:
Laboratory Standards


Application of GIS in understanding the Spatial Epidemiology of Babesiosis in Karnataka

Md. Mudassar Chanda, Siju Susan Jacob and P.P. Sengupta

ICAR – National Institute of Veterinary Epidemiology and Disease Informatics
Yelahanka, Bengaluru, Karnataka 560064

Introduction to GIS

A geographic information system (GIS) is a system designed to capture, store, manipulate, analyse, manage, and present all types of spatial or geographical data in a computer. GIS is a system of computer software, hardware and data, and the personnel to enter, manipulate, analyse the data. The hardware component of computer is on which a GIS operates. The software components of GIS rely on the underlying Database Management System (DBMS). The data is the most important component of GIS comprising of geographic features and attribute data. There are two types of data stored in the database of GIS. The attribute data gives information about the data like for example the livestock population. The spatial feature gives the information about where the feature is located in spatial domain, for example babesiosis percent positivity in different districts of Karnataka. Spatial data can be either stored in raster or a vector format. A raster data is a continuous surface and the attribute data has rows and columns of number with a Digital Value/Number (DN) for each cell. Units are usually represented as square grid cells that are uniform in size. The satellite images, aerial photography or scanned images can all be stored in raster format. The vector data are discrete features of spatial data and they can be of three different forms - points, lines and polygons. Vector data are stored as x and y coordinates or a series of x & y coordinates.

Application of GIS in understanding the Spatial Epidemiology of Babesiosis in Karnataka

The GIS can be very helpful in mapping spatial distribution of Babesiosis in Karnataka or any other state in India depending on the availability of data. The GIS can help us to understand the spatial features of Babesiosis revealing hidden patterns, trends etc., which may not be apparent in spreadsheets. The application of GIS is not only restricted to better visualization but can also be used for many other purposes as discussed below.

1. Field surveys to collect the epidemiological data using GPS
   Whenever the samples are collected suspected of Babesiosis or any other parasitic disease, recording of latitude/longitude will be very helpful to map the location of affected places. The GPS can be integrated into GIS software and point maps can be developed to know the clustering of cases which will help in identifying source of infection and also to suggest appropriate control measures.

2. Mapping the point data and interpolation
   Point data can be used for interpolation of cases/percent positivity in the areas where the samples have not been collected. There are many interpolation techniques available in GIS software. The interpolated maps can be of great help in planning future surveillance activities and also in strategizing babesiosis control measures.

3. Choropleth mapping
A map that uses graded differences in shading or colour or the placing of symbols inside defined areas on the map in order to indicate the average values of some property or quantity in those areas. A choropleth map showing Percent positivity of Babesiosis is shown in Figure 1a (Muraleedharan 2015). The maximum percent positivity is observed in Kodagu district of Karnataka. Comparison of the babesiosis percent positivity and rainfall is as shown in Figure 1b.

Figure 1: Choropleth maps showing spatial distribution of percent Seropositivity in Karnataka (1a) and distribution of rainfall (1b).

4. Overlaying disease data with other layers
   The percent Seropositivity of babesiosis can be over layered with other probable risk factors. Over layering can be used to understand the epidemiology of babesiosis and the probable factors responsible for the outbreaks can be identified. The point map (exact location of the cases) can be overlaid with other layers such as river map, vegetation map, forest map etc. Likewise it can be over layered with other probable risk factors which can be identified by quantitative analysis.

5. Analysis of disease data
   The GIS can also be used to quantify the relationship between babesiosis and the environmental factors so those factors can be mitigated for effective prevention and control of the disease.

Further reading

Note: Babesiosis data used in this write-up was taken from the paper (Muraleedharan K, 2015) only as an example to use GIS for parasitic diseases.
Disease surveillance by Enzyme-linked Immunosorbent Assays: A powerful laboratory test

Rajeswari Shome
rajeswarishome@gmail.com
ICAR - National Institute of Veterinary Epidemiology and Disease Informatics
(ICAR- NIVEDI), Yelahanka, Bengaluru- 560 064

The Immuno enzymatic assay is the powerful laboratory technique for detection and measurement of variety of biological substance of clinical importance. This assay is a suitable alternative to the radioimmuno-assay due to its simplicity, requirement for relatively cheap and simple equipments, longer shelf life of its reagents and safety because of absence of radiation biohazards and disposable problems associated with radioactive waste. Enzyme-linked Immunosorbent Assays (ELISAs) is an immunoassay which combines the specificity of antibodies with the sensitivity of simple enzyme assays, by using antibodies or antigens coupled to an easily-assayed enzyme. The ELISA technique was conceptualized and developed by two Swedish scientists: Peter Perlmann (principal investigator) and Eva Engvall at Stockholm University in 1971.

ELISAs can provide a useful measurement of antigen or antibody concentration. There are two main variations on this method: The ELISA can be used to detect the presence of antigens that are recognized by an antibody or it can be used to test for antibodies that recognize an antigen. There are three necessary reagents: (a) antibody coupled to solid supports, “immunosorbent”; (b) antigen or antibody which is marked with enzyme and is called conjugate; (c) substrate.

According to the reagents, samples and the constitution of the detection, there are many different types of ELISAs, including the most common indirect ELISA, direct ELISA, sandwich ELISA, competitive ELISA, etc. According to different applications of ELISA, different types of ELISA can be chosen.

1. **Direct ELISA**

Direct ELISA, can be regarded as the simplest type of ELISA. In direct ELISA, an antigen is adsorbed to a plastic plate, then an excess of other protein (using bovine serum albumin) is added to block all the other binding sites. Then an enzyme is linked to an antibody in a separate reaction. The enzyme-antibody conjugate is allowed to adsorb to the antigen, then excess enzyme-antibody conjugate is washed off, leaving enzyme-antibody bound to antigen. By adding the enzyme’s substrate, the enzyme is detected and thus the antigen.
2. **Indirect ELISA**

Indirect ELISA, the steps are similar, but with important differences and an additional step. After the antigen is adsorbed to the plate, the next antibody to be added is the antibody that recognizes the antigen (this antibody does not have the enzyme attached to it). Then, an enzyme-antibody conjugate is prepared, which is added to the plate and detects the antibody that is adsorbed to the antigen, then the substrate is added which detects the presence of the enzyme and thus the antigen.

Eg: Detection of HIV antibodies, *Brucella* antibodies, Detection of antibodies against Bovine Herpes Virus type 4 in bovine serum or plasma, Detection of antibodies against *Besnoitia besnoiti* in cattle serum or plasma.

3. **Competitive ELISA:**

This is another variation for measurement of antigen. After addition of the primary antibody unlabelled sample containing antigen is added. Upon addition of conjugated antigen followed by substrate colour change is observed if the conjugated Ag bind to the sites of the primary antibody which are not occupied by unlabelled antigen. The concentration of antigen is inversely proportional to the colour produced so more the unlabelled antigen in the sample lesser the amount of conjugated antigen bound.

Eg: Antibody detection of Blue tongue Virus, Brucellosis, contagious bovine pleuropneumonia, Peste des Petits Ruminants Virus.

4. **Sandwich ELISA.**

The plate is coated with a capture antibody followed by the addition of sample. The antigen present will bind to capture antibody. Then the detecting antibody is added, which binds to the antigen. This is followed by the addition of enzyme-linked secondary antibody which will bind to detecting antibody, and then the substrate is added, and is converted by enzyme to detectable form.

Eg: Detection of Antibodies to Porcine Circovirus 2, detection of antigen of foot-and-mouth disease virus (FMDV), Equine encephalosis virus, Newcastle Disease Virus, Peste des petits ruminant in sheep and goats.
Use of monoclonal antibodies in Competitive and Sandwich ELISA systems

- **Higher sensitivity**: either by selection of antibodies with a extremely high affinity, or by reduction of the height and variability of the background reaction, which makes very low concentrations of analyte more readily detectable.
- **Higher specificity**: by avoiding the presence of any antibody in the assay system with specific reactivity against non-analyte epitopes, and by selecting combinations of monoclonal antibodies which may further increase specificity.
- **Higher practicality**: e.g. by introducing simultaneous incubation of label, solid phase and sample without risk of "prozone effect".

5. **Avidin-Biotin Complex (ABC) ELISA Method**:

This test includes a secondary antibody coupled to biotin molecule following which the Avidin Biotin peroxidase complex added. The substrate on being reacted by the enzyme produces the color. In the test the Avidin molecules serves as a bridge between the biotin molecules and since the Avidin has four biotin binding sites the signal observed by the technique is approximately four times that of normal ELISA. It can detect very low level of antibodies in serum.

Eg: Detection of *L. monocytogenes* antibodies in bovine milk samples, detection of antibodies to BHV-1 infection, Bovine brucellosis, detection of infectious bursal disease virus antigen.

**Enzymes and substrates used in ELISA**

Most of the assays employ horse-radish peroxidase, alkaline phosphatase, or B-D-galactosidase. The most interesting recent developments have been in new methods to detect these enzymes rather than the use of new enzymes. Fluorimeters were introduced in 1984 for the detection of alkaline phosphatase and B-D-galactosidase. Methods are available to detect horse radish peroxidase by means of chemiluminescence. Fluorimetric and luminometric methods offer higher sensitivity and a wider measuring range than conventional spectrometry. TMB is gradually replacing mutagenic substrates such as OPD, leading to increased sensitivity and safety.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

64
### Heresadish Peroxidase (HRP)

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>(TMB)</td>
<td>Blue(652nm)</td>
<td></td>
</tr>
<tr>
<td>(OPD)</td>
<td>Yellow (492nm)</td>
<td></td>
</tr>
<tr>
<td>(ABTS)</td>
<td>Green (410nm)</td>
<td></td>
</tr>
</tbody>
</table>

### β-Galactosidase

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>o- Nitrophenyle-β-D-galactopyranosite (ONPG)</td>
<td>Yellow (405nm)</td>
<td></td>
</tr>
</tbody>
</table>

### Alkaline Phosphatase

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-nitro phenyl phosphate (PNPP)</td>
<td>Yellow (405nm)</td>
<td></td>
</tr>
</tbody>
</table>

### Advantages of ELISA

- Fast - 90 samples tested in 2-3 hr
- High Sensitivity (up to 10 pg/mL)
- Strong Specificity
- Many samples can be processed at once
- Small sample size required (10 L - 100 L)
- Colorimetric results – easily observed and measured (spectrophotometer)
- Test for presence of Ag or Ab
- Flexible usage for research design
- Easy to learn, simple procedure

### Disadvantages of ELISA

- Negative controls may indicate positive results if blocking solution is ineffective [secondary antibody or antigen (unknown sample) can bind to open sites in well]
- Enzyme/substrate reaction is short term so microwells must be read as soon as possible
- Monoclonal antibodies can cost more than polyclonal antibodies
- Test requires sophisticated instruments like ELISA reader to read the results
- Cannot be used under field condition

### Application of ELISA

- ELISA is used as an alternative tool for epidemiological surveillance.
- Disease outbreaks - tracking the spread of disease.
- Serum Antibody Concentrations.
- Detections of antigens.
- Detection of antibodies in blood sample for past exposure to disease.

### Trouble shooting Tips in ELISA

- **Background color**
  Background color in the plate can be minimized by proper washing or by using higher dilution of conjugate and also by reducing the incubation temperature of the conjugate. Substrate incubation carried out in light and also plate left too long before reading on incubation time on the plate reader also leads to background color.
- **Positive results in negative control**
  May be contamination of reagents or samples, Insufficient washing of plates, too much antibody used leading to non-specific binding and also contaminants from
laboratory glassware like antibodies, peroxides can also gives the false positive results.

- Low absorbance values
  Insufficient antibody, substrate solutions not fresh or combined incorrectly, Reagents not fresh or not at the correct pH, Incubation time not long enough, Incubation temperature too low, stop solution not added all these conditions will lead to low absorbance values.

- High absorbance values for samples and/or positive control
  Reduce the concentration of samples and control by dilution before adding to the plate. Take the dilution into account when calculating the resulting concentrations.

- Color developing slowly
  Plates are not at the correct temperature or maybe because of too weak conjugate. Presence of contaminants, such as sodium azide and peroxidise can affect the substrate reaction.

- Improper antigen binding
  Prefer polysorp plates if the antigen nature is hydrophobic and Maxisorb/High binding p plates if the protein nature is unknown.

**Diagnosis of brucellosis by recombinant Protein G based indirect ELISA: (NIVEDI kit) (Patent Application No. 5031/CHE/2013)**

The enzyme-linked immunosorbent assay (ELISA) detects antibodies to smooth LPS (sLPS) against *B. melitensis, B. suis* and *B. abortus*. The sample antibodies bind to *Brucella* sLPS molecules attached to the polysorp micro-titre plate wells. Binding of these antibodies is detected by reaction with horseradish peroxidase (HRP) labelled affinity purified antibodies to ruminant immunoglobulins. Attached complex (antigen+ antibodies + conjugate) are detected by addition of enzyme substrate O-Phenylenediamine dihydrochloride (OPD). The degree of colour that develops (optical density measured at 492nm) is directly proportional to the amount of antibody present in the sample. The diagnostic interpretation is made by comparing the optical density (OD) of the sample with the OD of the high positive sera control.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Diagnostic Kit</th>
<th>Price Rs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Brucella Cattle IELISA Kit (Sufficient for 500 samples)</td>
<td>19,000/- &amp; 12% GST</td>
</tr>
<tr>
<td>2.</td>
<td>Brucella Sheep and Goat IELISA Kit (Sufficient for 500 samples)</td>
<td>19,000/- &amp; 12% GST</td>
</tr>
</tbody>
</table>

**Materials required:**

- Smooth lipopolysaccharide antigen(SLPs), ELISA plates, Protein-G conjugate,
- Hydrogen peroxide, Phenylene Diamine Dihydrochloride (OPD), H₂O₂ (3%) solution, 1 M sulphuric acid: Add 5.5 ml of acid to 94.5 ml of distilled water.
Micropipettes, ELISA reader with washer, Refrigerator, Incubator and plate shaker etc.,

**What is Protein-G HRP Conjugate?**

Recombinant Protein G is an immunoglobulin-binding protein derived from the cell wall of certain strains of β-hemolytic Streptococci. It binds with high affinity to the Fc portion of various classes and subclasses of immunoglobulins from a variety of species. DNA sequencing of native Protein G identifies two IgG-binding domains and sites for albumin and cell surface binding. The albumin and cell surface binding domains have been eliminated from Recombinant Protein G to reduce nonspecific binding. Optimal binding occurs at pH 5, although binding is also effective at pH 7.0 to 7.2. Protein-G conjugated with any detective enzyme can be useful for serodiagnosis by ELISA in nondomestic hoofstock, although different assay interpretation algorithms and assay protocols may need to be developed on a per species basis for maximum diagnostic effectiveness.

**Preparation of reagents:**

a) **Coating buffer:**

Solution - A: Sodium Carbonate: 1.06 gm
Distilled water: 50 ml

Solution - B: Sodium Bicarbonate: 0.84 gm
Distilled water: 50 ml

To prepare 25ml Coating buffer (sufficient for coating 2 plates) add

Solution A: 1.75 ml
Solution B: 4.25 ml
Distilled Water 19.0 ml

- The pH of the Coating buffer should be 9.6 and prepared fresh every time.

b) **Phosphate Buffer Saline (1X):**

NaCl 7.0 gm
KCl 0.2 gm
NaH₂PO₄ 0.353 gm
Na₂HPO₄ 1.09 gm
Distilled Water 1000 ml

Dissolve 1 sachet of PBS provided with the kit in

1000 ml of distilled water to make 1X PBS. Store at 4°C for longer period.

c) **Washing buffer:** prepare fresh every time

PBS (1X) 500ml
Tween-20 0.25ml

d) **Blocking buffer:** Sufficient for performing 2 plates

Bovine gelatin 2.0 gm
PBS (1X) 100 ml

- Keep in water bath at 37°C until bovine gelatin dissolves completely (15-20 min).
  (Note: After taking it out of the water bath add 50µl of Tween-20).

e) **Stopping solution (0.5M H₂SO₄)**
Conc. $\text{H}_2\text{SO}_4$  
Distilled water  
**Total**

5.5 ml  
94.5 ml  
100 ml

f) **Hydrogen peroxide (3%)**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>30% Hydrogen peroxide</td>
<td>10 l</td>
</tr>
<tr>
<td>Distilled water</td>
<td>90 l</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100 l</strong></td>
</tr>
</tbody>
</table>

**Procedure**

1. **Coating the micro-plate**

   Antigen from stock (stored at -20°C) is added @ 33 l/10ml of coating buffer (10 ml is sufficient to coat 1 plate). It is mixed properly and then dispensed @ 100 l into each well of the micro-plate. Tap the sides of the plates to ensure that the antigen is evenly distributed over the bottom of each well. The micro-plates are covered with aluminium foil/lid and kept for incubation overnight at 4°C in refrigerator. After incubation, plates are washed 3 times using 300 l wash buffer and tap on a towel or tissue paper to remove residual wash buffer. Avoid drying of the plates between washes and prior to the addition of the next reagent. (Note: Always use freshly prepared overnight coated plates or within one week of coating.)

2. **Addition of test and control sera**

   Control and test sera should be diluted in blocking buffer separately in perpx plate for which 5µl of test and control sera are diluted in 500µl blocking buffer. Serum and blocking buffer is mixed thoroughly 10 TIMES to ensure homogeneity before loading to the micro-titre plate. The diluted 100µl test sera samples in duplicate wells (in 2-wells) and three control sera (high, moderate and negative sera) samples in quadruplicate wells (in 4-wells) are transferred from the perpx plate to the micro-plates as per the layout provided and incubate at 37 °C for one hour on the ELISA plate shaker @300 rpm.

3. **Addition of the conjugate (sufficient for one plate)**

   The plates are taken out of the shaker and washed three times with washing buffer as mentioned earlier. The working dilution of the conjugate (Protein-G HRP conjugate, Sigma Cat No. P8170) is made by adding 1.5µl of the conjugate to 12ml of blocking buffer (1:8000 dilution). Then 100 l of the working dilution of conjugate is added to each well and incubate at 37 °C for one hour on the shaker @300 rpm as mentioned above.

4. **Addition of substrate/chromogen (sufficient for 1 plate)**

   The plates are taken out of the shaker and washed three times with washing buffer. Substrate/Chromogen solution is prepared by adding 1 OPD tablet (5 mg) to 12.5ml distilled water followed by the addition of 50µl hydrogen peroxide (3%). Chromogen solution @ 100 l is added to all the wells of the micro-plate, incubate at room temperature for 7 minutes or wait until a visible yellow colour develops in the strong positive sera wells till 10 minutes by covering with aluminium foil or towel in dark.

5. **Stopping Solution**

   After the colour develops, immediately stop the reaction by adding 50 l of stopping solution to all wells. The plates are read in the ELISA reader at 492 nm immediately. The
OD values of the test controls in each ELISA test performed should fall within the defined upper control limit (UCL) and lower control limits (LCL) for the acceptance and rejection of the test. The OD values of UCL for the positive and negative sera controls should be between 1.2 and 0.2, respectively. Similarly, OD values of the LCL should be of 0.6 and 0.09 for positive and negative controls, respectively. The test should be repeated if any deviations in the OD values of the defined UCL & LCL of the positive and negative control sera samples are observed.

6. Interpretation of the results

Percent positivity (PP) values which are used for the diagnostic interpretations are calculated as follows:

$$PP = \frac{Average\ OD\ value\ of\ test\ serum}{Median\ OD\ value\ of\ C++\ control} \times 100$$

Any sample that gives PP value between 55% to 65% is considered moderate positive and more than 65% as strong positive. If sample shows a PP value of below 55% is taken as negative and samples with PP value of only 55% should be either re-sampled or re-tested.

References

enzyme-linked immunosorbent assay for the detection of \textit{Brucella ovis} in sheep. Vet. Rec. 143, 390-394

ART AND SCIENCE OF PARASITIC DISEASE INVESTIGATION

Dr. P. Krishnamoorthy

ICAR - National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI),

Post Box No.6450, Ramagondanahalli, Yelahanka, Bengaluru-560064, Karnataka

Disease Investigation

The disease investigation is done for the following purpose

- Investigation of disease to identify the disease
- Can help in identify the source of ongoing outbreaks and prevent additional cases
- Increase our knowledge on a disease
- Prevent future outbreaks
- Foster cooperation between the clinical and public health communities
- Describe new diseases and learn more about the known diseases
- Evaluate the existing prevention strategies eg., vaccines
- Teach and learn epidemiology
- Address the public concern about the outbreak

Public Health

Role of disease investigation agencies

- Leads multijurisdictional investigations of disease clusters
- Consultation and assistance to local health departments and state partners regarding the investigation and control of infections and outbreaks of public health importance
- Consultation to local health jurisdictions regarding public health mandates to prevent and control communicable diseases
• Produces evidence-based guidelines to prevent infections and control outbreaks
• Collaborates with partners at the local, state and central levels to improve surveillance for infectious diseases, investigate outbreaks and develop preventive measures

**Ten steps in Disease investigation process**

1. Investigation team and resources
2. How disease outbreaks are recognized
3. Verifying the diagnosis
4. Construct case definition
5. Identifying cases
6. Descriptive epidemiology
7. Testing hypothesis
8. Control and preventive measures
9. Communication of findings
10. Maintain Surveillance

**Sample**

The term “sample” may either mean a specimen (animal, blood sample and others) or, used in the statistical sense, a sub-collection or sub-set of units. The aim in general is collection of samples representative of the study population. It is difficult to collect materials required for disease diagnosis from all the animals in a population. Hence the samples are collected to represent the population; usually samples are collected at random. Clinical samples means samples collected from an ailing or affected animals with particular diseases.

**Collection of samples**

Sample collection is done mainly for direct examination of the sample from the animals, isolation of microorganisms for definitive diagnosis and serological diagnosis which will help in the diagnosis of animal diseases.

**Precautions during sample collection**

• Care should be taken to avoid injury or distress to the animal or danger to the operator and attendants
• Risk of zoonotic disease should be kept in mind
• Post-mortem examinations should be carried out under aseptic conditions with protective clothing
• Care should be taken to avoid environmental contamination, or risk of spread of disease through insects or fomites
• Arrangements should be made for appropriate safe disposal of animals and tissues
• Samples collected should be representative of the condition being investigated and the lesions observed
• Samples collected should be appropriate for the intended purpose, and adequate in number and amount to provide statistically valid results
• Samples should be carefully packaged, labelled, and transmitted to the laboratory by the fastest practicable method, with the appropriate temperature control

**Parasitic diseases:**

<table>
<thead>
<tr>
<th></th>
<th>Parasitic Diseases</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Theileriosis</td>
<td>Blood smears, biopsy smears from swollen lymph nodes from early stage of disease fixed with methanol</td>
</tr>
<tr>
<td>2</td>
<td>Babesiosis/ Anaplasmosis</td>
<td>Thin blood smears fixed in methanol.</td>
</tr>
<tr>
<td>3</td>
<td>Surra/Trypanosomiasis</td>
<td>Wet film examination of blood by hanging drop, fixed blood smears, blood in anticoagulant on ice.</td>
</tr>
<tr>
<td>4</td>
<td>Schistosomiasis</td>
<td>Nasal schistosomias – Nasal discharge and nasal granuloma in normal saline.</td>
</tr>
<tr>
<td>5</td>
<td>Trichomoniasis</td>
<td>Vaginal or uterine discharges, preputial scraping/washing.</td>
</tr>
<tr>
<td>6</td>
<td>Gastro-Intestinal Parasitic Diseases</td>
<td>Dung sample and affected internal organs in 10% formalin.</td>
</tr>
</tbody>
</table>

Thus, the epidemiological investigations are essential to determine the source of outbreaks in field conditions. The steps of disease/outbreak investigation should be followed systematically for better understanding of the diseases. Determination of control and preventive measures may be identified dynamically for different livestock diseases. It is also important to maintain surveillance for the diseases so as to enable the effective disease control and prevention.

**References**

3. Sample collection procedure manual, diagnostic services of Manitoba Inc., USA.
Application of Geographical Information Systems (GIS) in Natural Resource Management

Dr. S. Srinivas, Principal Scientist,
National Bureau of Soil Survey and Land Use Planning (NBSS & LUP), Regional Centre, Bangalore – 560 024

Geographic Information System (GIS)

GIS stands for Geographical Information System. The development GIS is considered to be a technological advancement in the area of computer modeling. During the recent years GIS proved to be an efficient tool in Agricultural Research and Development. Some of its well-known applications are

- Thematic mapping
- Spatial database management
- Precision farming
- Land use planning
- Watershed modeling and management
- Pest and disease modeling and management
- Irrigation modeling and management
- Resource inventory, mapping and modeling
- Characterization studies related to resources and also socio-economic aspects
- Yield forecasting
- Targeting new technologies

An information system can be defined as the chain of operations that takes us from planning the observation and collection of data to the use of the derived information in some decision making process. Better information helps in making better decisions. Map is also a kind of information system. For example maps showing the distribution of soil series or land use, which are made for more limited purposes are often called as thematic maps. In the late twentieth century, demands for data on the topography and specific themes of the earth’s surface, such as natural resources, have accelerated greatly. The growing demands for more spatial data and for better means to analyze them can only be met by using computer software such as GIS. GIS stores the spatial information without losing its geographical reference. GIS is designed to accept large volumes of spatial data that are derived from different sources and to efficiently store, manipulate, retrieve, analyze and display these data according to some specifications. GIS is a computer hardware and software system designed to collect, manage, analyse, and display spatially referenced data (USDA)

A map is a kind of spatial information system and it is a collection of stored, analyzed data and information to be used in making decisions. If the source of information is an existing paper map then that is considered to be the weakest part of the information system. The map (spatial) information should be available in computer readable digital form. GIS is a computer software which reads and stores digital maps and helps in integrating, analyzing with other tabular (numerical) data. This opens powerful new ways of looking at and analyzing data in which one can access information in the tabular database through the spatial database (map).

There are three levels at which GIS approach can be used for resource management (Harrison and Sharma 1992). The first level provides a simple inventory of current resources and their characteristics in a region. The second level deals with data
management from a wide variety of sources housed within a single framework. At this level integration of different types of data such as these maps of a region showing soils, ratio of actual to potential evapotranspiration, and topography, might be used to examine potential production of a crop in that region. The third and more advanced level involve a combination of spatial analysis and simulation models.

**Data Model**

The data model represents a set of guidelines to convert the real world (called entity) to the digitally and logically represented spatial objects consisting of the attributes and geometry. The attributes are managed by thematic or semantic structure while the geometry is represented by geometric-topological structure.

There are two major types of geometric data model; vector and raster model, as shown in

**a. Vector Model**

Vector model uses discrete points, lines and/or areas corresponding to discrete objects with name or code number of attributes.

![Vector Model Diagram](image)

**b. Raster Model**

Raster model uses regularly spaced grid cells in specific sequence. An element of the grid cell is called a pixel (picture cell). The conventional sequence is row by row from the left to the right and then line-by-line from the top to bottom. Every location is given in two dimensional image coordinates; pixel number and line number, which contain a single value of attributes.

![Raster Model Diagram](image)
Uses of GIS

Why is a GIS needed?

- These are the following reasons why a GIS is needed.
  - geospatial data are poorly maintained
  - maps and statistics are out of date
  - data and information are inaccurate
  - there is no data retrieval service
  - there is no data sharing
- Once a GIS is implemented, the following benefits are expected.
  - geospatial data are better maintained in a standard format
  - revision and updating are easier
  - geospatial data and information are easier to search, analyze and represent
  - more value added product
- geospatial data can be shared and exchanged freely
- productivity of the staff is improved and more efficient
- time and money are saved
- better decisions can be made

- Different layers of spatial information such as, soils, land use, weather, hydrology, socio-economic etc., can be stored with its spatial identity;
- Thematic maps of various resources can be prepared for specific purpose quickly;
- It allows experimentation with different ways of representing the same data on maps for easy understanding and interpretation;
- It facilitates analysis of data that demand interaction between statistical analysis and mapping;
- It is a powerful spatial data-base system and allows to link various types of crop, hydrological and statistical models;
- Maps that are difficult to make by hand can be created and updated easily.

GIS is not simply a computer system for making maps, it is a powerful analytical tool for analyzing spatial data.

For any GIS application, there are five general questions that a GIS can answer.

- Where is the location?
- What is at that location?
- What are the trends and what has changed since at the given location?
- What spatial pattern exists?
- What if ………………….? (modeling)

The first question seeks to find out the identification of the location. For example a location can be described in terms of latitude and longitude. The second questions require spatial analysis to answer. For example, soil type, type of land use, means annual precipitation etc. The third question seeks to find the changes on a specified area over time. The fourth question is more sophisticated. For example why the productivity of a particular crop yield is higher at one place compared to another? What spatial patterns are affecting the yield? To answer the fifth question the GIS has to be linked to other modeling software. For example, how the productivity at different locations vary when different land use treatments are mapped. This information will be useful to the administrators in arriving at decision in optimizing the use of resources in the point of sustainability and economic viability.

Various activities involved in executing a GIS project is as follows:

- Getting spatial data into GIS
- Making spatial data usable
Steps involved in executing a GIS project can be summarized as follows:

**Determine the objectives of the project**: The important issues to consider are: a) what is the problem to solve? b) How is it solved now? c) What are the final products of the project? d) How frequently might these products be generated? e) Who is the intended audience for the final products? f) Are there any other uses for this same data? These issues determine the scope and implementation of the project.

**Building the database**: This is the most critical and often most time-consuming part of the project. The completeness and accuracy of the spatial database determines the quality of the analysis and final products.

**Analysis of the data**: Analysis is where the true value of GIS becomes important. A GIS efficiently performs analytical tasks that are extremely time-consuming, an even impossible to do manually. With GIS, one can test alternative scenarios simply by making minor changes in the input values. The analytical capability of GIS can be increased by linking with various computer simulation and analytical models.

**Present the Result of the Analysis**: A GIS offers many options for creating map and reports. It is the individuals skills at summarizing and presenting spatial (map and graphic) and tabular results of analysis which effects the decision making process.

**Conclusion**

GIS has a significant role to play in the decision making process of natural resource management. At various levels i.e., field, regional, national and global levels. This is one of the important tools of Information Technology (I.T), which is very useful to Natural resource Scientists/ Managers to arrive at better decisions by integrating research results with intensive location specific information on various biophysical and socioeconomic variables. This technology allows use to examine and handle a wider range of spatial data bases such as soils, hydrology, weather etc and integrate with socio economic variable as also the new technologies that are being developed from time to time. Simultaneous examination of these variables leads to a better understanding of various land resource management related process and their interactions over space and time. Linking of simulation models such as crop simulation models, hydrological models, and statistical models to GIS databases is one of the current active areas of research. By linking these models the tools GIS is emerging as a powerful spatial decision support system.

**References for further reading**

2. ESRI (1990) Understanding GIS

Detection of microbe genome by real time quantitative polymerase chain reaction (qRT-PCR)

G.B. Manjunatha Reddy, Yogisharadhya R and Apsana R

ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Bengaluru, Karnataka 560064

1. Introduction

The advent of real-time PCR and real-time reverse transcription PCR (real-time RT-PCR) has dramatically changed the field of detection and quantifying the pathogen and its use has to the point where it is now accepted as the gold standard for detecting nucleic acids from a number of origins. Real-time PCR is the technique of collecting data throughout the PCR process as it occurs, thus combining amplification and detection into a single step. This is achieved using a variety of different fluorescent chemistries that correlate PCR product concentration to fluorescence intensity. Reactions are characterized by the point in time (or PCR cycle) where the target amplification is first detected. This value is usually referred to as cycle threshold (Ct), the time at which fluorescence intensity is greater than background fluorescence. Consequently, the greater the quantity of target template/DNA in the starting material, the faster a significant increase in fluorescent signal will appear, yielding a lower Ct. There are two flavors of real-time PCR, probe-based and intercalator-based. In fluorecent dye based or Intercalator-based method (SYBR Green method), SYBR Green fluorescence is enormously increased upon binding to double-stranded DNA. During the extension phase, more and more SYBR Green I will bind to the PCR product, resulting in an increased fluorescence (Fig 2). Consequently, during each subsequent PCR cycle more fluorescence signal will be detected. In probe-based real-time PCR (TaqMan PCR) requires in addition to primers, a fluorogenic probe which is an oligonucleotide with a reporter fluorescent dye at 5’ end and a quencher dye at 3’ end. FRET or Florescent Resonance Energy Transfer technology is utilized in the 5’ nuclease assay. The principle is that when a high-energy dye is in close proximity to a low-energy dye, there will be a transfer of energy from high to low. When this probe is intact and excited by a light source, the Reporter dye’s emission is suppressed by the Quencher dye as a result of the close proximity of the dyes. When the probe is cleaved by the 5’ nuclease activity of the enzyme, the distance between the Reporter and the Quencher increases causing the transfer of energy to stop. The fluorescent emissions of the reporter increase and the quencher decrease. The increase in amount of reporter is proportional to the amount of product being produced for a given sample. The amount of template is measured by absolute or relative quantitation. Relative quantitation describes changes in the amount of target sequence compared with its level in related matrix, while absolute quantitation states the exact number of nucleic acid present in the sample in relation to a specific unit. There are many benefits of using real-time PCR over other methods to detect and quantify viral genome. The ability to quantify nucleic acids over an extraordinarily wide dynamic range (at least 5 log units) coupled with extreme sensitivity, allowing the detection of less than five copies (perhaps only one copy in some cases) of a target sequence, making it possible to analyze small samples like clinical biopsies or miniscule
lysates from laser capture microdissection. In addition, all real-time platforms are relatively quick, with some affording high-throughput automation. Finally, real-time PCR requires no post-PCR manipulations, thereby minimizing the chances for cross contamination in the laboratory.

2. Methods

2.1 There are different methods of DNA isolation and different kits are available in the market.

2.2 Real time PCR reaction

The reaction mix will be prepared by adding following reagents (according to manufacturer protocol)

1. 10.0 μl of 2X real time PCR master mix.
2. 1.0 μl of forward primer (10 pM of final concentration).
3. 1.0 μl of reverse primer (10 pM of final concentration).
4. 1.0 μl of DNA (~ 50 ng final concentration).
5. Add nuclease free PCR grade water to adjust the final volume to 20 μl.

The known positive and negative controls (NTC) should be included. Place the tubes on real time PCR platform then setup the reactions conditions and run programme.

In case of absolute quantification, the known template is diluted to create a range of standard concentrations. The unknown test samples are amplified in parallel with the standards in the same experimental run. The standard curve constructed from the diluted standard template can then be used to determine the target quantity in the unknown sample by interpolation, similarly to the way molecular size standards are used to determine the molecular size of an unknown DNA band on an agarose gel.

<table>
<thead>
<tr>
<th>Cycles</th>
<th>Time</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5 min</td>
<td>94°</td>
</tr>
<tr>
<td></td>
<td>30 s</td>
<td>94°</td>
</tr>
<tr>
<td>40</td>
<td>30 s</td>
<td>55-62°</td>
</tr>
<tr>
<td></td>
<td>30 s</td>
<td>72°</td>
</tr>
</tbody>
</table>

2. Expected results

Many companies supplies inbuilt software system for analysis of data by either absolute or relative quantification method. The amplification and melting curves will be displayed on screen will provide weather there is specific amplification or not.

1. **Positive**: There will be amplification curve with Ct values usually below 30 and the melting curve (sharp one peak) will reveal Tm of usually above 78°C depending on the size of the target amplicon (Fig).
2. **Negative:** The positive control sample will only show amplification curve with specific Tm. But, if test sample and negative control shows amplification curve with Tm less than 72°C is considered negative because of primer dimer.

3. **Non specific amplification:** If there is no amplification curve in NTC but test samples shows amplification curve with different Tm than positive control then it is considered as nonspecific amplification. Multiple melting curve peaks in a single sample is also considered as the non specific amplification.

![Fig. A &B: All samples showing amplification curves expect the NTC (yellow color line) in which there is no amplification (Fig A). Melting curve analysis all samples showing single peak with Tm above 82°C, but the NTC showing no peak and Tm (FigB).](image)

3. **Notes**

1. Ensure good primer and probe design: Use of latest software to design primers and is highly recommended

2. DNA preparation is crucial for quantification. All samples should be treated according to identical protocols and – in the optimal case – in parallel. Check the quality and concentration of DNA by conventional electrophoresis and spectrophotometry or with devices such as bioanalyzer.

3. Include NTCs, negative and positive controls where ever applicable.

4. Pay attention to the technical analysis of data. In case of a SYBR Green I assay, a melting (dissociation) curve analysis should be performed yielding only one sharp peak in the first derivative plot. Ensure accurate baseline and threshold setting.
Establishment and Maintenance of National Livestock Serum Bank

Divakar Hemadri

Principal Scientist, NIVEDI, Bengaluru

Serum Bank

Serum bank is a repository of serum which are catalogued, stored and preserved for any future use. In the serum bank, care is taken to preserve the immunological and other biochemical properties of the sera stored. Thus both the catalogue and the storage conditions are essential components of a successful serum bank.

Uses of a serum bank

• Retrospective screening of antibodies against a particular pathogen.
• Retrospective detection of a particular pathogen.
• For serological survey
• For evaluation and validation of a diagnostic assay
• For disease survey
• For screening of trace elements, toxins, hormones and enzymes.

Cataloguing

It is essential to fully document and identify each individual sample. For this it is compulsory to develop a database which contains all relevant information about the origin of the sample and test results obtained. Additional data that may be of interest, such as the animal’s productivity, vaccination status may also be included. The serum bank catalogue should be well organised and maintained on a computer database with appropriate backup.

Storage

The terms of usage determine storage of the serum. For example, sera which are intended for periodic use need to be stored in multiple aliquots, while those intended for long-term storage may only need to be stored in two or three aliquots. Therefore it is essential to separate these two functions. Storage conditions determine quality of the serum over a period. Therefore care should be taken to minimise loss of immunological and other biochemical properties during the storage. There are mainly three methods of storage: Deep freezing, dry storage on paper disks at ambient temperature and lyophilisation (freeze-drying).

Short term storage

Dry storage on paper disks at ambient temperature
In this kind of storage system serum samples are spotted on filter paper, air-dried, and stored in airtight polyethylene bags. The fact that samples can be stored on filter paper at room temperature for a few weeks before being assayed may make this assay useful in remote areas of developing countries where access to refrigerators or freezers is limited. Upon arrival on a reference laboratory, the serum is eluted from the filter paper and assayed. Unfortunately, this kind of storage system is useful only for storing minute quantity of serum and not useful for antibody screening. Paper disk storage is a simple and inexpensive method, but it allows only a small quantity of serum to be stored and the eluted serum is only suitable for a limited number of tests. The disks should be kept in a cool, dry atmosphere. They can probably provide satisfactory results for up to about 5 years.

**Long term storage**

For long term storage either lyophilization or deep freezing is practiced. The serum may deteriorate and may not be suitable for detection of some properties, especially if stored for long periods at a temperature of −20°C. Therefore lower the temperature the better the shelf life. Deep freezing, below a core temperature of −60°C is ideal for long term storage. Unfortunately, lower temperatures are more expensive to maintain. Storing of serum in either liquid or vapour phase of N₂ is not practicable.

Lyophilisation or freeze drying is generally regarded as the best method for long-term storage of sera or any other biological material. Besides the expensive equipment, it requires optimisation of freeze-drying conditions for minimising the loss of serum characteristics. Lyophilised vials should always be stored at 4°C.

Above all serum bank requires a stand-by generator and also alternative cold storage space in case the contents of a freezer must be transferred.

**Collection of serum for disease survey**

How the sera are collected determine the outcome of the survey results; therefore sampling methods are needed to be scientifically based. It is important that the specimens collected should be representative of the condition being investigated and the lesions observed. When developing a programme of surveillance and monitoring for animal health in the absence of clinically evident disease, as AICRP centers are currently doing, some general statistical sampling methods should be used. As a thumb of rule, those many animals are needed to be sampled from herd/flock of a certain size, to achieve a 95% probability of detecting infection or previous exposure. Hence it is always recommended to seek statistician’s help in determining the sample size.

**Information to be sent with samples**

It is highly essential that individual samples be clearly identified before dispatching to a reference laboratory. It is not only important that which kind of marking instruments are used for labeling, but also it is important that what kind of labeling materials are used. Both the marking and labels should withstand the condition of use, i.e. being wet or frozen. Information and case history should always accompany the samples to the laboratory, and
should be placed in a self sealing plastic envelope on the outside of the shipping container. It would be advisable to contact the receiving laboratory to determine if it has a submission form that it would like to have submitted with the samples or if it needs other information. The standard operating procedure for collection and dispatch of serum are given in the appendix.

**Transportation of specimens**

The specimens should be forwarded to the laboratory by the fastest method available. If they can reach the laboratory within 48 hours, samples should be sent refrigerated. If dry ice is used, the additional packaging requirements must be met.

**Packaging**

The shipper should ensure that the specimens are packaged so they arrive at the laboratory in good condition and there is no leakage during shipment. The SOP for shipping serum to NIVEDI is given in the appendix.

**Serum Bank at NIVEDI**

**Genesis**

It may be recalled that the NIVEDI was nodal center for sero-monitoring of rinderpest in the country (somewhere during 1996-2002). At that time, it was mandatory for the all animal husbandry department of the country to collect and deposit the serum samples to ADMAS as per the guidelines issued by the Institute. As a result, the institute received large number of serum samples from various parts of the country. Considering efforts made to collect these samples and that these samples are random in nature, it was decided to hold on to these samples for any future use, besides screening for anti-rinderpest antibodies.

**Functioning**

Since the institute has been mandated with animal disease monitoring and surveillance, the serum samples are currently screened for Brucellosis (in sheep, goat, cattle, buffalo and pigs, humans), Infectious Bovine Rhinotracheitis (cattle), Classical swine fever (pigs), peste des petits ruminants (goats/cattle), Neospora caninum (cattle) and bluetongue (sheep/goat). The serum bank has created an all India level village (some six lakh forty three thousand eight hundred three villages) directory and is updated after every human census. Using this directory national random sampling frame for the entire nation has been developed for foot and mouth disease control programme. The same registry is also used for designing sampling frame for AICRP collaborating units. Besides this the serum bank also houses serum samples collected by ADMAS scientists during disease investigation and also as part of any other survey. The random samples received from collaborating at the serum bank are given NIVEDI accession number and then aliquoted and distributed to various above mentioned laboratories for screening. One aliquote from above samples is barcoded and kept in the freezer for any future use.
Cataloguing

Presently cataloguing is done using MS office access data base. A handful of forms viz., entry of receipt of samples, dispatch of samples to various labs, results, storage have been developed and currently being used. There exists a plan to barcode these samples. Screen shots of access database developed at NIVEDI for cataloguing of sera are shown below.

**Receipt entry form**

This is used to enter information accompanied with sample.

**Dispatch entry form**

This is used to enter dispatch information to various labs.

**Results form**

This is used to enter screening results of various labs.
Results communication form

This is used to enter screening results of various labs.

Storage form

This is used to enter storage info of various serum samples.

Appendix I

Standard Operating Procedure (SOP) for collection and dispatch of Serum

General requirements: Aseptic precautions should be observed right from collection of blood to dispatch of serum. It is advisable to wear gloves at all times (be it removal of stopper from vacutainer, centrifugation, pipetting, disposal of contaminated tubes, and clean up of any spills) when handling the specimens. Used vacutainers, needles, and pipets must be properly disposed in accordance with biosafety/institutional requirements.
Livestock disease impact assessment – An introduction

G. Govindaraj
National Institute of Veterinary Epidemiology and Disease Informatics, Bangalore – 560 024

Introduction

The disease in animals reduces the efficiency with which inputs are converted into outputs as well as mortality and morbidity effects. Further, there are several indirect effects on the farmer-producer and to the society, of which some can be valued or quantified easily and some are difficult to quantify. Besides quantification using the reliable data collected from primary or secondary sources, the effect of a disease can be modeled by implying certain assumptions like increased death rate, lower yield, decreased body weight, increased calving/kidding interval etc. It is also sometimes referred as simulation assessment of the impact of the disease.

Disease impact

Any disease in animals has direct and indirect impacts on the performance of animals and on animal keepers. The direct impact can be further classified into visible and invisible impacts. The visible impacts can be easily quantified. Eg. mortality loss, reduction in milk, wool reduction in draught power, money expended on vaccines and veterinarians fee. The invisible impacts due to a disease are reduced fertility and changed herd structure. The quantification of invisible losses can be made using certain models and assumptions since the exact information/data is not usually available and are difficult to obtain.

The indirect impact includes societal and financial impact. The non-disposal of dead animals in the scientific manner has impact on environment through release of harmful gases or spread of disease to disease free herds/animals in the neighborhood. The death of animals in large numbers in general or death of certain breeds/species due to outbreaks in a certain geographical region has variable societal impact. The indirect impact like change in methane gas emission levels, change in dung availability and its impact on agriculture and allied activities productivity, change in carrying capacity etc., also affects the society at large due to disease in animals. The death of animal also affects the availability of animal products like milk to the farm family especially in subsistence agriculture families. It also affects the human nutrition and thereby reduces the longevity. It also ends up in various social problems in the long run. The indirect impact of zoonotic diseases affects humans and has larger societal ramifications.

The indirect financial impact includes lesser price for the diseased animals in the market or lower value for the normal animals also in a particular locality due to an outbreak. The price of the complement and substitute goods also changes due a disease outbreak and hence the price effect should be a part of impact analysis.

All the above impacts have to be quantified in a scientific manner so as to arrive the overall impact of the disease. Quantifying the direct impact of any disease is relatively easy compared to quantifying the indirect impact. However, with use of implicit assumptions and indirect valuation methods the overall impact can be quantified.

Information required for developing a disease economic module

i) The foremost information required is about the disease itself, i.e.
a) whether the disease is bacterial or viral or parasitic or combination thereof or any other agent?
b) incidence rate of the disease
c) duration of infection

ii) Diseased animal information
   a) The species information (Eg. bovine, small ruminants, poultry, pig, horse, etc.)
   b) The breed details (Eg. indigenous, crossbred, exotic)
   c) The age and sex of the diseased animal
   d) The susceptibility information agewise, sexwise, breedwise

iii) The physiological parameters at normal and diseased state

iv) Productive and economic traits at normal and diseased state

**Indicators required for assessing the losses**

The indicators required for assessing the losses due to animal diseases may vary for macro (national) or micro (farm) level estimation. The important indicators required for macro level disease impact estimates are as follows:

a) The number of animals died in different species and across different ages
b) The number of animals culled due to disease in different species and across different ages
c) The number of animals disabled due to disease in different species and across different ages
d) The productivity loss in animals like milk loss/day/animal
e) The number of days of milking loss in a lactation etc.
f) The number of days of draught power loss due to a disease
g) Information on decreased levels of fertility
h) Information on incidence of abortion
i) Changes in herd structure due to a disease
j) Loss due to non- access to markets
k) Treatment cost on drugs and vaccines
l) The cost of manpower/ labour

Besides, the basic requirements listed above the economic information are also necessary to assess the impact of the disease. For time series disease impact assessment, the data on different time periods are necessary whereas for the cross section analysis the information during a particular time on different variables are required.

Some of the very important economic information required are given below:

a) Price of milk, wool, meat, etc.
b) Price of live animal for different breeds, for different age groups and for different sex
c) Price of the culled animal for different breeds, for different age groups and for different sex
d) Price of disabled animal across breeds, age and sex
e) Rates of bullock labour per day
f) Rates of hired labour per day for male and female
g) Own price variation and change in prices of complements and substitutes
h) Veterinarian or para-veterinarians fee

The information listed above is necessary to assess the losses due to a disease. However, depending on the disease and level of the study the data requirements vary.

**Conclusion**

The analysis of disease losses from different angles helps the farmers as well as policy makers and planners. The estimation of losses species-wise tells farmers which species are more susceptible to diseases and by how much and thus they can manage to reduce losses in livestock. Similarly, age or herd size-wise estimate of losses suggest them at what age the animal is more susceptible. The disease loss information also help policy makers and planners to decide which disease ranks first in terms of losses so that they can get due importance in policy planning.
CONCEPT OF ANTIMICROBIAL RESISTANCE

Dr. B R Shome, MVSc, Ph.D, Principal Scientist,
ICAR-NIVEDI, Bangalore

Antimicrobial Resistance

Micro-organisms are termed ‘antimicrobial-resistant’ or ‘drug-resistant’ when they are no longer inhibited by an antimicrobial to which they were previously sensitive. Such resistance is called ‘acquired resistance’ and is encoded by resistance genes in the DNA of the microbe. Resistance genes can arise through spontaneous mutations in the microbial DNA, but some have evolved over many years due to natural selection by natural antimicrobials in the environment. These genes can also transfer from drug-resistant microbes to drug-sensitive ones. Today, almost all important bacterial infection in the India and throughout the world are becoming resistant to antibiotics. Antibiotic resistance has been called one of the world’s most pressing public health concerns. The rational use of antibiotics is the key to controlling the spread of resistance.\(^1\)

The first drug-resistant bacterium in a clinical setting was identified in the late 1940s; only 4 years after mass treatment with penicillin had been introduced.\(^2\) Since then the emergence and spread of drug-resistant microbes has continued to grow. Highly resistant bacteria, such as methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci, account for a high percentage of hospital-acquired infections.\(^3\)

Rational Use of Antibiotics

Treating with antimicrobials causes micro-organisms to adapt or die: this is known as ‘Selective pressure’. Incorrect use of antimicrobials increases the likelihood that bacteria and other microbes will adapt and replicate. Development and spread of antimicrobial resistance (AMR) is commonly due to overuse, misuse, and indiscriminate use of antimicrobials by doctors, nurses and pharmacists, non-compliance and self medication by patients and use in animal husbandry and agriculture. It is estimated that 70-80% of prescriptions for antimicrobials are probably advised unnecessarily by the health professionals. In-spite of the fact that most common colds and diarrheal episodes are viral in origin, yet, antimicrobials are used indiscriminately. Reasons for over prescribing are often lack of confidence, peer pressure, patient pressure and pharmaceutical company pressure. Antimicrobial use is a key driver of the resistance. Poverty and inadequate access to antibiotics constitute a major factor in the development of resistance. Another common cause of developing resistance is
improper diagnosis. In many instances death of an adequately equipped diagnostic laboratory in the vicinity compels the physician to prescribe antibiotics empirically, thus, increasing the likelihood of the patient receiving a wrong antibiotic. Furthermore, ready availability of antibiotics over-the-counter and sales promotion schemes by the pharmaceutical manufacturers also leads to the promotion of indiscriminate use, thus, increasing the likelihood of development of resistance. Counterfeit drugs are also a problem contributing to development of resistance. These contain either the wrong ingredient, or lesser amount of the active ingredient. The impact of the media has also contributed to the development of resistance. Patients often demand antibiotics for their ailment on the basis of advertisements read or seen. Unwitting use of more active drugs at sub therapeutic doses leads directly to the development of multi drug resistance. Irrational use of antimicrobials is widespread throughout the world. This is harmful in terms of increased cost of therapy, unnecessary adverse drug reactions, therapeutic failure, reduced quality of care and worst of it is AMR.

The bacterial infections which contribute most to human mortality and morbidity are also those in which emerging antimicrobial resistance is most obvious: diarrhoeal diseases, respiratory infections, meningitis, sexually transmitted diseases, and hospital-acquired infections. Some important examples include penicillin-resistant *Streptococcus pneumoniae*, vancomycin-resistant *enterococci*, methicillin-resistant *Staphylococcus aureus*, multi-resistant *Salmonella typhi*, *Shigelladysenteriae*, *Neisseria gonorrhea*, *Pseudomonas aeruginosa* and multi-resistant *Mycobacterium tuberculosis*.

**Established mechanisms of AMR**

For an antibiotic to be effective, it must reach the target site in an active form, bind to the target, and interfere with its function. Thus, bacterial resistance to an antimicrobial agent can occur due to three general mechanisms:

**a) The drug does not reach its target.**

In Gram negative bacteria, many antibiotics enter the cell through protein channels called porins. Mutations or loss of these channels can prevent/slow the rate of antibiotic entry into a cell, effectively reducing drug concentration at the target site. If the drug target is intracellular and the drug requires active transport across the cell membrane, a mutation that interferes with the transport mechanism can confer resistance e.g. aminoglycosides. Bacteria
can also transport antimicrobial drugs out of the cell through efflux pumps. Resistance to numerous drugs, including fluoroquinolones, macrolides, tetracyclines and beta lactam antibiotics, is mediated by this mechanism.\[7\]

b) **The drug is inactivated.**

Bacterial resistance to aminoglycosides can be due to a plasmid encoded aminoglycoside-modifying enzymes. Similarly, β-lactamase production is the most common mechanism of resistance to penicillins and other β-lactam drugs. Many hundreds of different β-lactamases have now been identified. A variation of this mechanism is failure of the bacterial cell to activate a prodrug e.g. loss of ability of M. tuberculosis to activate isoniazid (INH).\[8\]

c) **The target site is altered.**

This may be due to mutations in drug binding region of target enzyme e.g. fluoroquinolones, target modification e.g. ribosomal protection type of resistance to macrolides and acquirement of a resistant form of the susceptible target e.g., methicillin resistance in Staphylococcus Spp. due to production of a low-affinity penicillin-binding protein (PBP).\[9\]

**Strategies to prevent AMR\[10,11,12\]**

- Establish a national alliance against antimicrobial resistance with all key stakeholders as its members. There should be an integrated approach between provider and consumer sides to effectively prevent the antimicrobial resistance. The implementation of national efforts to prevent and contain antimicrobial resistance should be through a multi-sectorial national steering committee headed by the senior-most health executive and facilitated through advisory or expert groups.
- Implement appropriate surveillance mechanisms in the health and veterinary sectors to generate reliable epidemiological information, baseline data, trends on antimicrobial resistance, utilization of antimicrobial agents and impact on the economy and health through designated national and regional reference centre's.
- Discourage non-therapeutic use of antimicrobial agents in veterinary, agriculture and fishery practices as growth-promoting agents.
- Develop national standard treatment and infection control guidelines and ensure their application at all levels of health care and veterinary services through training,
continuous educational activities, establishment of functional drugs and therapeutic committees and hospital infection control committees in health facilities with the focus on proven cost-effective interventions such as isolation, hand washing.

- To regulate and promote rational use of medicines and ensure proper patient care at all levels, there is a need to take necessary steps to stop across the counter sale of antibiotics without physician’s prescription and ensure uninterrupted access to essential medicines of assured quality at hospital and community. Also, vaccination strategies should be improved to further reduce the burden of infections.

- Conduct of operational research for better understanding of the technical and behavioral aspects of prevention and control of antimicrobial resistance. Utilize the outcomes of these research studies or interventions in policy and program development improvement in the national context.

- Constructive interactions with the pharmaceutical industry for ensuring appropriate licensure, promotion and marketing of existing antimicrobials and for encouraging the development of new drugs and vaccines.

- Educational and awareness program for communities and different categories of health care professionals.

- Strengthen communicable diseases control program to reduce disease burden and accord priority to the discipline of infectious diseases in medical education and health services.

**References**


--------------------------------------------------------------------------
Emergence of Anthelmintic Resistance- constraint to livestock sector

Dr. Siju Susan Jacob and Dr. P. P. Sengupta

ICAR- National Institute of Veterinary Epidemiology and Disease Informatics, Yelahanka, Bengaluru-560064, Karnataka

Introduction:

Helminth infestations are common in most animals but, particularly in adult healthy animals, the immune system keeps the burden of helminths at such levels that clinical symptoms do not occur. However, if this balance is not obtained, which could be the case for young or diseased animals, or when infestation pressure is very high, helminth infestation may impact severely on the health status of the animals. This may in turn affect performance and production (e.g. reduced milk and weight gain) and could also lead to increased mortality. It is, therefore, important to ensure the availability of effective anthelmintics to treat animals. However, anthelmintic resistance (AR) in parasitic nematodes is a threat to sustainable livestock production worldwide and a growing concern for the control of parasites especially in the developing world. Our limited understanding of how resistance mutations arise and spread in parasitic nematode populations limits our ability to develop evidence-based mitigation strategies. Moreover, there is little information on the changes that occur in the genome as anthelmintic resistance mutations increase in frequency in parasite populations; the so called “genetic signature” of selection. Such knowledge is critical if we have to apply genome-wide population genomic approaches to identify new anthelmintic resistance mutations.

Anthelmintic resistance (AR) is defined as a decrease in the efficacy of an anthelmintic against a population of parasites that is generally susceptible to that drug. According to Sangster, 1999, the development of AR in general is a very simple phenomenon and whatever the better way is used to control worms with drugs the more likely resistance develops. This is because drug treatment leads to the survival of resistant worms, which, if allowed to reproduce, contribute resistance genes to the next generation. If the resistance genes have become fixed in the parasite genome (lack of genetic reversion) then AR becomes a prolonged phenomenon and the susceptibility of parasites to the drugs to resume takes a long time. Less progress and lack of awareness to farmers to understand GIN infections and development of AR has been witnessed particularly in the developing countries.
Current status of AR

Although there is a lack of systematic monitoring data, the occurrence of resistance in many helminth species against various classes of anthelmintics is evident through scattered reports concerning almost all domestic species. Several scientific reports indicate an increase in helminth resistance to the older classes of anthelmintics (benzimidazoles, tetrahydropyrimidines, imidazothiazoles and macrocyclic lactones) (Borgsteede et al., 2007). Isolated reports on cases of helminth resistance to the newest classes of anthelmintics have also been published (e.g. resistance of *Haemonchus contortus* to monepantel, an amino-acetonitrile derivative, Van den Brom et al., 2015). Benzimidazoles are the oldest class of authorised anthelmintics; thiabendazole was introduced in the 1960s. The first report of decreased efficacy of thiabendazole against *Haemonchus contortus* strains dates from 1964, just 3 years after its introduction to the market (Van den Bossche et al., 1982).

Similarly, resistance has developed rapidly to other anthelmintic classes, particularly those used in sheep and horses, after their introduction to the market. For example, resistance to imidazothiazolotetrahydropyrimidine and avermectin-milbemycin classes developed within 3-9 years after introduction to the market in sheep (Kaplan, 2004). A major concern which is emerging is the decreased efficacy of triclabendazole against liverflukes (*Fasciola hepatica*) in sheep and cattle (Moll et al., 2000). Furthermore, resistant populations of *Cooperia* spp. to ivermectin are reported in cattle (El-Abdellati et al., 2010). In horses, resistance to benzimidazoles, pyrantel and macrocyclic lactones, has been reported for Cyathostominae and *Parascaris equorum* (Geurden et al., 2014). In addition, *Oesophagostomum* spp. in pigs have been reported to be resistant to pyrantel (Roepstorff et al., 1987), levamisole and benzimidazoles (Gerwert et al., 2002), and resistance to pyrantel in ascarids and hookworm has been observed in dogs and cats (Riggio et al., 2013).

4. Mechanisms of resistance

Due to advances in molecular technology, mechanisms of resistance in worms are becoming increasingly understood. The resistance in worms can be the result of a variety of mechanisms like genetic changes in the drug target, changes in the drug transport (e.g. ATP-binding Cassette (ABC) transporters), or changes in the metabolism of the drug within the parasite. Benzimidazole resistance in nematodes can be due to a mutation in the gene coding for the target site, the same mutation does not seem to cause resistance to triclabendazole in the trematode *Fasciola hepatica*. In addition, within a single worm species, different
mutations can lead to resistance to the same anthelmintic. For instance, benzimidazole resistance in *Haemonchus contortus* can be caused by the phenylalanine to tyrosine mutation at amino acid position 200 of the isotype 1β-tubulin gene (Kwa et al., 1994). However, the frequency of this resistance point mutation (single nucleotide polymorphism, SNP) varies considerably and it can be low in benzimidazole (BZ)-resistant populations which carry other mutations (e.g. codon 167). Although genetic selection contributes to resistance, changes in drug transport mechanisms or in the metabolism of the drug within a worm species also account for different resistance mechanisms to the same anthelmintic. The P-glycoprotein, a cell membrane transport protein able to transport many different drugs (including ivermectin, benzimidazoles and imidazothiazole derivatives), may lead to multi-drug resistance by increasing the active transport of drugs. Therefore, it can be concluded that more research is needed in order to understand the mechanisms and to develop suitable assays for detection of resistance.

5. Methods of detecting resistance

There are various *in vivo* and *in vitro* methods available to assess the efficacy of anthelmintics. Furthermore, specific laboratory methods can be applied to confirm a suspicion of resistance in the field, e.g. as described in the WAAVP study recommendations and guidelines.

**Nematodes**

**Faecal egg count reduction test**

Reduced efficacy, that may reflect the development of resistance, can be detected by using the Faecal Egg Count Reduction Test (FECRT). This test estimates the anthelmintic efficacy by comparing the number of worm eggs in faeces of infected animals before and after treatment. This test can be used for all anthelmintic classes, which is a great advantage compared to other tests. However, the sensitivity of the FECRT may be low. In addition, the egg output of some helminth species varies depending on the density of the adult worm population. This is the case for *Ancylostoma caninum* in dogs or *Oesophagostomum dentatum* in pigs. In cattle, there is also no clear correlation between egg output and worm number. This illustrates that FECRT has limitations as a tool for detecting resistance.

In general, FECRT can be used in horses, ruminants and pigs to detect nematodes which shed their eggs in the faeces. When evaluating the treatment effect, the interval between treatment and second sampling should be shorter than the pre-patent period of the specific worm. The correct sampling interval also depends on the type of anthelmintic, e.g.
for persistent anthelmintics like macrocyclic lactones the interval between treatment and faeces sampling is recommended to be 14–17 days whereas for levamisole an interval of 3–7 days is advised. According to the WAAVP guideline on anthelmintic resistance egg count reduction of less than 90% (arithmetic mean) indicates resistance in pigs, provided that a minimum pre-treatment individual egg count was confirmed.

In horses, a reduction in FEC of less than 90% is suggested to indicate resistance but there is some disagreement regarding this threshold. In some reports a mean of 95% is regarded to be an appropriate cut-off level (Larsen et al., 2011) whereas in other reports different cut-off values for different classes of anthelmintics are proposed; e.g. 90% for pyrantel and 95% for benzimidazoles and macrocyclic lactones (Dargatz et al., 2000). Further research is needed to conclude on the thresholds that indicate resistance when the FECRT is used in horses.

For small ruminants, according to the WAAVP guideline, resistance is confirmed when the percentage of reduction in egg count (arithmetic mean) is less than 95% and when the 95% confidence level is less than 90%. If only one of the two criteria is met, resistance is only suspected.

As previously mentioned, the usefulness of the FECRT as a tool to identify resistance is limited by its lack of sensitivity. Another disadvantage is that it is not species-specific; eggs of different nematode species cannot be differentiated within the test. Moreover, the interpretation of the test depends upon various factors including the detection limit of the method, the number of animals per group, the host species, and the level of egg excretion by the helminthes.

**Egg reappearance period test**

Egg reappearance period (ERP) is defined as the time interval between the last anthelmintic treatment and the resumption of significant helminth egg shedding. To evaluate potential occurrence of resistance, the ERP after dosing should be compared with the historical ERP of the veterinary medicinal product.

**Molecular assays**

Molecular techniques, such as polymerase chain reaction (PCR) or pyrosequencing, can reveal mutations in helminth genes responsible for resistance to a certain anthelmintic class. Currently, only resistance to benzimidazoles can be detected by PCR which is useful when resistance is caused by a single gene mutation (i.e. SNP), or by a small number of such mutations.

**Other methods**
Other methods potentially useful for the detection of resistance are the egg hatch assay (EHA) and the microagar larval development assay (LDA). These methods have been developed for detection of resistance to benzimidazoles or levamisole in horses, pigs or small ruminants. Coles et al. (2006) have described these assays and how to interpret the results. Yet another novel method for detection of drug resistant helminths is based on a digitalised evaluation of worm motility. Maintained motility of nematodes after administration of an anthelmintic that should lead to paralysis of the parasite could indicate a lack of efficacy. This method has been described for larval *Haemonchus contortus*, *Strongyloides ratti*, adult hookworms and blood flukes (Smout et al., 2010).

**Trematodes and cestodes**

Coles and Stafford (2001) proposed a “dose and slaughter” trial to substantiate suspected resistance of trematodes in the field; after artificial infestation followed by treatment with a flukicide (e.g. triclabendazole), the animals are killed and the number of flukes in the liver are counted. The usefulness of the FECRT to reflect resistance has not been evaluated for tapeworms or flukes. An egg hatch assay (EHA), recently developed for the detection of resistance against albendazole in *Fasciola hepatica*, needs to be validated (Robles-Perez et al., 2013). Fairweather et al. (2012) have developed an EHA test for the detection of triclabendazole (TCBZ) resistance in *Fasciola hepatica* which could be useful.

**Management strategies to delay the development of resistance**

Different management strategies are used with the purpose of preventing infestation and/or keeping infestation pressure low. This includes pasture management, refugia, and quarantine for animals which are newly introduced into a flock or herd. The overall aim is to reduce the need for anthelmintics and consequently to delay the development of resistance. In addition, when anthelmintic treatment is applied, certain treatment practices are recommended with the purpose of reducing the risk for resistance development.

**Correct use of anthelmintics**

The prudent use recommendations currently established have the overall aim to target treatment in the best possible way so as to reduce unnecessary exposure and thus limit the risk for resistance. Recommendations for prudent use of anthelmintics are generally based on an in-depth understanding of the helminth epidemiology. It is stressed that deworming is based on the confirmation of worm burden and that treatment with a relevant product is
applied at the right time in relation to the life cycle of the parasite so as to obtain sufficient effect without unnecessary exposure. It has been demonstrated that underdosing and/or a too frequent use of anthelmintics belonging to the same class will increase the risk for selection of resistance. It is recommended by experts that long-acting anthelmintics are applied only in situations when the grazing season is considerably longer than the duration of the effect (i.e. that these formulations are applied at the start of the grazing season).

The limited number of anthelmintics authorised for minor species leads to significant off-label use. Dosing strategies may then have insufficient scientific support which could cause, for example, unintentional under dosing. Although there is a lack of scientific evidence, rotation of anthelmintic classes is often recommended to delay the development of resistance. Rotation of drugs was originally suggested based on the hypothesis that reversion to susceptibility might occur if resistant worms were less fit than susceptible worms, and counter selection was applied via treatment with a drug from a distinct chemical class.

Routine deworming which is still often practised, leads to unnecessary treatment and, thus, an increase of the selection pressure. This is of particular concern when used in farm animals in situations when environmental refugia (i.e. susceptible helminth population) is low. One example of routine use of anthelmintics on farms is the “dose -and- move” practice, which may provide a survival advantage for resistant parasites.

It is generally agreed that the maintenance of refugia through the implementation of appropriate treatment and pasture management routines is important to decrease the selection pressure and reduce the risk for resistance development (Graef et al., 2013).

**Refugia**

Resistance spread is promoted if parasites carrying mutations that bring about reduced susceptibility to anthelmintics are provided with a survival advantage in the population. The refugia concept aims to keep the proportion of resistant worms within the population at a low level and it is thus advocated as a tool to slow the progress of anthelmintic resistance. Parasites in refugia are those that have not been exposed to an anthelmintic, including those present as free-living stages in the environment, those in untreated individuals, and those in any lifecycle stages in the host that are not affected by the anthelmintic treatment. Appropriate treatment strategies and pasture management need to be implemented to maintain refugia (Graef et al., 2013).
Use of multiactive anthelmintic products

It is currently under discussion whether combination products that contain two or more active substances targeting the same helminth but through different mode of actions could be advantageous with respect to delaying the emergence of resistance. Modelling studies and some field data have indicated that such products may delay the development of resistance to new active substances (Leathwick et al., 2012), or delay development of anthelmintic resistance to existing anthelmintic classes. However, the use of multiactive anthelmintics might select for multiple resistance to different anthelmintic classes. Whether multi-actives offer a benefit with regard to resistance development that would outweigh any risk for promoting multiple resistance needs to be further substantiated. This is important to determine before formulating any recommendation on the use of such products.

Other options

Other measures to control helminth infestation in animals are different pasture management routines, e.g. removal of faeces from pasture to reduce the level of infective larvae, reducing stocking densities, preventing high degree of infestation or improving drainage of pastures to decrease the risk of liver fluke infestations. To be effective, such measures would have to be tailored according to the specific epidemiology situation on the individual farm. In addition to this, other biological control methods are currently under development, e.g. vaccines and the selection for livestock that is genetically less susceptible to helminth infestation.

Conclusions

Scattered information from different sources makes it clear that anthelmintic resistance is present all across the region, mostly in small ruminants. Currently, there are no programmes that systematically monitor the occurrence of resistance in helminths of relevant animal species. A few local monitoring programmes for specific target species are running, but trends regarding the development of anthelmintic resistance are difficult to follow on the basis of such local programmes. Knowledge regarding the extent of resistance to different active substances in different helminth species in various geographical areas is useful for decision-making regarding the implementation of activities aimed at controlling further spread. Therefore, a systematic monitoring programme would be of great value.
Demonstration of anthelmintic resistance is, however, difficult. For many helminth species, there is currently a lack of standardised/validated test systems for confirming resistance. More research is necessary in order to understand the mechanisms and to develop validated methods that are affordable and easy to use. The establishment of reference laboratories with the tasks of maintaining a reference strain library and evaluating and validating tools for monitoring anthelmintic resistance could be very useful.

There is some scarcity of scientific data regarding important risk factors for resistance development of helminths in the different target species. Nevertheless, there is a common understanding among experts that measures to reduce the need of anthelmintics and promoting an appropriate use of these drugs are important to delay resistance development. Examples of prudent use advice are: to base treatment on confirmation of worm burden or solid epidemiological information, to employ targeted selective treatment approaches at farm level and to avoid routine and frequent use, to dose correctly and particularly avoid under-dosing, to use combination products only when all substances are necessary for effective treatment, to manage pastures properly and to maintain an appropriate level of refugia, in particular by keeping a part of the herd untreated. Furthermore, although scientific support is currently lacking, it is often recommended to rotate between different anthelmintic classes over time. Particular care is necessary when pour-on formulations and prolonged release formulations are used with regard to the timing of administration and the management of animals after administration of the product, i.e. to ensure sufficient drug exposure in all treated animals and to maintain a sufficient refugia population.

A lack of narrow-spectrum products might result in unnecessary use of active substances, e.g. inappropriate use of fixed combination products. In addition, there is a current shortage of authorized anthelmintic products for minor species, which may unintentionally lead to inappropriate off-label use.

References:


In biology, sequencing refers to determine the primary structure of an unbranched biopolymer. Sequencing results in a symbolic linear depiction known as a sequence which succinctly summarizes much of the atomic-level structure of the sequenced molecule. DNA sequencing is the process of determining the nucleotide order of a given DNA fragment. Initial efforts focused on sequencing the most readily available populations of relatively pure RNA species, such as microbial ribosomal or transfer RNA, or the genomes of single-stranded RNA bacteriophages. In 1965 Robert Holley and colleagues were able to produce the first whole nucleic acid sequence, that of alanine tRNA from Saccharomyces cerevisiae. 2-D fractionation (which often consisted of both electrophoresis and chromatography) with a single separation by polynucleotide length via electrophoresis through polyacrylamide gels, provided much greater resolving power and this technique was used in two influential yet complex protocols from the mid-1970s by Alan Coulson and Sanger's plus and minus system in 1975 and Allan Maxam and Walter Gilbert's chemical cleavage technique. The major breakthrough that forever altered the progress of DNA sequencing technology came in 1977, with the development of Sanger's chain-termination or dideoxy technique. A number of improvements were made to Sanger sequencing in the following years which contributed to the development of increasingly automated DNA sequencing machines. These first-generation DNA sequencing machines produce reads slightly less than one kilobase (kb) in length. In the second-generation DNA sequencing, luminescent method for measuring pyrophosphate synthesis called as pyro sequencing technique. Third generation technologies are those capable of sequencing single molecules, negating the requirement for DNA amplification shared by all previous technologies. It is hard to overstate the importance of DNA sequencing to biological research; at the most fundamental level it is how we measure one of the major properties by which terrestrial life forms can be defined and differentiated from each other. Therefore over the last half century many researchers from around the globe have invested a great deal of time and resources to developing and improving the technologies that underpin DNA sequencing.

From Sanger’s sequencing to next generation sequencing, the sequences obtained will be helpful epidemiologically to identify clones and their relation with the outbreaks. Hence sequence analysis becomes foremost important not only to characterize but also in identification of motif and in deducing phylogeny. To carry out the sequence analysis either by pairwise alignment or multiple alignment, sequence editing is important. Whenever sequencing is carried out commercially, we will be receiving the sequence file along with chromatogram. A chromatogram (sometimes also called electropherogram) is the visual
representative of a DNA sample produced by a sequencing machine. The obtained sequences should be edited properly to subsequently submit to GenBank or to do alignment.

**Sequence editing**

Various software are available for sequence editing and one among them is BioEdit.

Steps in editing the sequences obtained after sequencing

Step 1. The file is opened in bioedit and in the edit menu, press copy sequence as raw text and paste the sequence in a note pad file

Step2: Blastn the sequences at the URL http://blast.ncbi.nlm.nih.gov/Blast.cgi
Step 3: On Blastn, the alignment should be observed carefully, (in a subject sequence of our interest) (with maximum similarity) and analysed the sequence for actual indels (Insertions and deletions)

Step 4: the absence of nucleotide should be checked for sequencing artifact, by referring to the sequence chromatogram in Bioedit

Step 5: The sequence should be edited accordingly only after critical analysis of the chromatograms obtained from at least two sequencing reactions using the same primer.

Step 6: Similar way the reverse sequence also should be subjected.

Step 7: Then the reverse sequence should be reverse complemented by pasting in DNAclub (from http://molbiol-tools.ca/molecular_biology_freeware.htm)

Step 8: Taking the query sequence number from blast, the extra nucleotides from the sequence should be removed in DNAclub

Step 9: Joining the sequence will give a query sequence which is the complete sequence amplified. The complete sequence obtained is then submitted to the Genbank or used for further analysis.

References


DNA Sequencing methods and its application

V. Balamurugan,

Principal Scientist, ICAR-NIVEDI, Yelahanka, Bengaluru

DNA sequencing is one of the most important platforms for the study of biological systems today. The term DNA sequencing encompasses biochemical methods for determining the order of the nucleotide bases, adenine, guanine, cytosine, and thymine, in a DNA oligonucleotide. The sequence of DNA constitutes the heritable genetic information in nuclei, plasmids, mitochondria, and chloroplasts that forms the basis for the developmental programs of all living organisms. Determining the DNA sequence is therefore useful in basic research studying fundamental biological processes, as well as in applied fields such as diagnostic or forensic research. The advent of DNA sequencing has significantly accelerated biological research and discovery. The rapid speed of sequencing attainable with modern DNA sequencing technology has been instrumental in the large-scale sequencing of the human genome. Genome related projects, often by scientific collaboration across continents, have generated the complete DNA sequences of many animal, plant, and microbial genomes.

For thirty years, a large proportion of DNA sequencing has been carried out with the chain-termination method, developed by Frederick Sanger and co-workers in 1975. Prior to the development of rapid DNA sequencing methods in the early 1970s by Sanger in England and Gilbert et al. at Harvard, a number of laborious methods were used. For instance, in 1973 [Gilbert and Maxam reported the sequence of 24 basepairs using a method known as wandering-spot analysis.

In 1976-1977, Allan Maxam and Wafer Gilbert developed a DNA sequencing method based on chemical modification of DNA and subsequent cleavage at specific bases. Maxam-Gilbert sequencing (Chemical sequencing) rapidly became more popular, since purified DNA could be used directly, while the initial Sanger method required that each read start be cloned for production of single-stranded DNA. However, with the development and improvement of the chain-termination method, this method has fallen out of favour due to its technical complexity, extensive use of hazardous chemicals, and difficulties with scale-up. In addition, unlike the chain-termination method, chemicals used in the Maxam-Gilbert method cannot easily be customized for use in a standard molecular biology kit.

This method requires radioactive labelling at one end and purification of the DNA fragment to be sequenced. Chemical treatment generates breaks at a small proportion of one or two of the four nucleotide bases in each of four reactions (G, A+G, C, C+T). Thus, a series of labeled fragments is generated, from the radio labeled end to the first 'cut' site in each molecule. The fragments are then size-separated by gel electrophoresis, with the four reaction arranged side by side. To visualize the fragments generated in each reaction, the gel is exposed to X-ray film for autoradiography, yielding an image of a series of dark 'bands' corresponding to the radiolabeled DNA fragments, from which the sequence may be inferred. Chemical and the plus-minus method of Sanger and Coulson were orders of magnitude faster than previous methods, the chain-termination method developed by Sanger was even more efficient, and rapidly became the method of choice.

The Maxam-Gilbert technique requires the use of highly toxic chemicals, and large amounts of radio labeled DNA, whereas the chain-terminator method uses fewer toxic chemicals and lower amounts of radioactivity. The key principle of the Sanger method was the use of dideoxynucleotides triphosphates (ddNTPs) as DNA chain terminators. The classical chain-termination or Sanger method requires a single-stranded DNA template, a DNA primer, a DNA polymerase, radioactively or fluorescently labeled nucleotides, and
modified nucleotides that terminate DNA strand elongation. The DNA sample is divided into four separate sequencing reactions, containing the four standard deoxynucleotides (dATP, dGTP, dCTP and dTTP) and the DNA polymerase. To each reaction is added only one of the four dideoxynucleotides (ddATP, ddGTP, ddCTP, or ddTTP). These dideoxynucleotides are the chain-terminating nucleotides, lacking a 3'-OH group required for the formation of a phosphodiester bond between two nucleotides during DNA strand elongation. Incorporation of a dideoxynucleotide into the nascent (elongating) DNA strand therefore terminates DNA strand extension, resulting in various DNA fragments of varying length. The dideoxynucleotides are added at lower concentration than the standard deoxynucleotides to allow strand elongation sufficient for sequence analysis.

The newly synthesized and labeled DNA fragments are heat denatured, and separated by size (with a resolution of just one nucleotide) by gel electrophoresis on a denaturing polyacrylamide-urea gel. Each of the four DNA synthesis reactions is run in one of four individual lanes (lanes A, T, G, C); the DNA bands are then visualized by autoradiography or UV light, and the DNA sequence can be directly read off the X-ray film or gel image. The relative positions of the different bands among the four lanes are then used to read (from bottom to top) the DNA sequence as indicated. In one method, the DNA fragments are tagged with nucleotides containing radioactive phosphorus for radiolabelling. Alternatively, a primer labeled at the 5' end with a fluorescent dye is used for the tagging. Four separate reactions are still required, but DNA fragments with dye labels can be read using an optical system, facilitating faster and more economical analysis and automation. This approach is known as 'dye-primer sequencing'. The later development by L Hood and co-workers of fluorescently labeled ddNTPs and primers set the stage for automated, high-throughput DNA sequencing.

The major advantage of this Dye-terminator sequencing method is that the sequencing can be performed in a single reaction, rather than four reactions as in the labelled-primer method. In this method, each of the four dideoxynucleotide chain terminators is labelled with a different fluorescent dye, each fluorescing at a different wavelength. This method is attractive because of its greater expediency and speed and is now the mainstay in automated sequencing with computer-controlled sequence analyzers. Its potential limitations include dye effects due to differences in the incorporation of the dye-labelled chain terminators into the DNA fragment, resulting in unequal peak heights and shapes in the electronic DNA sequence trace chromatogram after capillary electrophoresis. This problem has largely been overcome with the introduction of new DNA polymerase enzyme systems and dyes that minimize incorporation variability. This method, along with automated high-throughput DNA sequence analyzers, is now being used for the vast majority of sequencing projects, as it is both easier to perform and lower in cost than most previous sequencing methods.

Pyrosequencing is a method of DNA sequencing (determining the order of nucleotides in DNA) based on the "sequencing by synthesis". This principle developed initially by Pal Nyrenand co-workers during a period from 1985 to the late 1990s, then further by Biotage. The first major improvement was substitution of dATPcx.S for dATP in the polymerization reaction, which enabled the pyrosequencing reaction to be performed in homogeneous phase in real time. This technique is a widely applicable, alternative technology for the detailed characterization of nucleic acids. The technique dispenses with the need for labeled primers, labeled nucleotides, and gel-electrophoresis. The fastest of all the sequencing methods. It can sequence 10 million bases/hour. It is a method primarily used for sequencing of short stretches of DNA, SNP (small genetic variations) detection and methylation analysis. Such analyses are crucial for biological research, genetics and some medical and forensic applications. Pyrosequencing is fully automated, reliable and accurate, flexibility, parallel processing, and large numbers of samples can be analysed in a short time. These methods
have been pursued to reduce costs relative to other automated sequencing methods.

The technique has been further developed by the company 454 into a technology known as 454 Pyrosequencing. To date this is the fastest sequencing method. However, a limitation of the method is that read lengths are currently somewhat shorter than those obtained by di-deoxy nucleotide based chain termination methods, which, makes the process of genome assembly more complicated, particularly for genomes which contain a large amount of repetitive DNA. Pyrosequencing is most commonly used for resequencing or sequencing of genomes for which the sequence of a close relative is already available. The templates for pyrosequencing can be made both by solid phase template preparation (Streptavidin coated magnetic beads) and enzymatic template preparation (Apyrase+Exonuclease). A single run can generate around 30Mb of sequence, with an estimated cost per kilobase of raw sequence that is IO-fold less than conventional sequencing. The disadvantage of this technique is the short read length, which is currently 26bp per amplicon (13bp per tag). The colony method has now been taken by Applied Biosystems (Foster City, CA, USA). They have adapted the method so it is capable of 50bp reads and generating>1Mb of sequence in a single run. The technology now named as SOLiD.

Many of the problems, and inherent errors, of DNA sequencing result from the fact that thousands or millions of amplified templates are assessed in a single-molecule sequencing reaction. It would be far better to read DNA in the same way as cells do; as single molecules. This method involves hybridizing target DNA to complimentary primers that are streptavidin-biotin bound to a silica surface. The primers are then extended by the addition of Cy3- and Cy5-labeled nucleotides; as each base is added, the incorporation is captured using a camera mounted on a microscope. A limitation of this technology is that it generates short reads, which at the time of publication was 5bp; however, this technology has been taken up by Helicos Biosciences Corporation, Cambridge, MA, USA, who are reporting much longer reads. This method is highly parallel, and on a 25mm square it would be possible to sequence 12 million templates simultaneously, so, even with 5bp reads, each 'run' would generate 60 million bases of information.

Nanopore sequencing is other method of single-molecule sequencing that is in the very early stages of development involves 'reading' DNA as it is passed through a nanopore. This would not involve an enzymatic extension reaction of any kind but instead the physical properties of the molecule would be read as the bases wind through a tiny pore. In theory, this method would have no limit on read length and, hence, if the technical hurdles are overcome it could revolutionize how genome sequencing is achieved. The physicists used mathematical calculations and computer modeling of the motions and electrical fluctuations of DNA molecules to determine how to distinguish each of the four different bases (A, G, C, T) that constitute a strand of DNA. They based their calculations on a pore about a nanometer in diameter made from silicon nitride—a material that is easy to work with and commonly used in nanostructures—surrounded by two pairs of tiny gold electrodes. The electrodes would record the electrical current perpendicular to the DNA strand as the DNA passed through the pore. Because each DNA base is structurally and chemically different, each base creates its own distinct electronic signature.

Modern automated DNA sequencing instruments (DNA sequencers) can sequence up to 384 fluorescently labelled samples in a single batch (run) and perform as many as 24 runs a day. However, automated DNA sequencers carry out only DNA size separation by capillary electrophoresis, detection and recording of dye fluorescence, and data output as fluorescent peak trace chromatograms. Sequencing reactions by thermocycling, cleanup and re-suspension in a buffer solution before loading onto the sequencer are performed separately. The field of DNA sequencing technology development has a rich and diverse history. However, the overwhelming majority of DNA sequence production to date has relied on
some version of the Sanger biochemistry. In high-throughput production pipelines, DNA to be sequenced is prepared by one of two approaches: first, for shotgun de novo sequencing, randomly fragmented DNA is cloned into a high-copy-number plasmid, which is then used to transform  *Escherichia coli*; or second, for targeted resequencing. PCR amplification is carried out with primers that flank the target. With high-throughput shotgun Sanger sequencing, genomic DNA is fragmented, then cloned to a plasmid vector and used to transform *E. coli*. For each sequencing reaction, a single bacterial colony is picked and plasmid DNA isolated. Each cycle sequencing reaction takes place within a microliter-scale volume, generating a ladder of ddNTP-terminated, dye-labelled products, which are subjected to high-resolution electrophoretic separation within one of 96 or 384 capillaries in one run of a sequencing instrument. As fluorescently labelled fragments of discrete sizes pass a detector, the four-channel emission spectrum is used to generate a sequencing trace.

The shotgun method is the most practical for sequencing large genomes, but its assembly process is complex and potentially error-prone—particularly in the presence of repeating sequences. In shotgun sequencing with cyclic-array methods, common adaptors are ligated to fragmented genomic DNA, which is then subjected to one of several protocols that results in an array of millions of spatially immobilized PCR colonies or ‘polonies’. Each colony consists of many copies of a single shotgun library fragment. As all polonies are tethered to a planar array, a single microliter-scale reagent volume (e.g. for primer hybridization and then for enzymatic extension reactions) can be applied to manipulate all array features in parallel. Similarly, imaging-based detection of fluorescent labels incorporated with each extension can be used to acquire sequencing data on all features in parallel. Successive iterations of enzymatic interrogation and imaging are used to build up a contiguous sequencing read for each array feature.

The 454 system was the first next-generation pyrosequencing platform available as a commercial product. In this approach, libraries may be constructed by any method that gives rise to a mixture of short, adaptor-flanked fragments. Clonal sequencing features are generated by emulsion PCR, with amplicons captured, to the surface of beads. After breaking the emulsion, beads are treated with denaturant to remove untethered strands, and then subjected to a hybridization-based enrichment for amplicon-bearing beads (that is, those that were present in an emulsion compartment supporting a productive PCR reaction). A sequencing primer is hybridized to the universal adaptor at the appropriate position and orientation, that is, immediately adjacent to the start of unknown sequence.

Illumina Genome Analyzer: Commonly referred to as 'the Solexa', this platform has its origins in work by Turcatti and colleagues and the merger of four companies—Solexa (Essex, UK), Lynx Therapeutics (Hayward, CA, USA), Manteia Predictive Medicine (Coinsins, Switzerland) and Illumina. Libraries can be constructed by any method that gives rise to a mixture of adaptor-flanked fragments up to several hundred base-pairs (bp) in length. Amplified sequencing features are generated by bridge PCR. In this approach, both forward and reverse PCR primers are tethered to a solid substrate by a flexible linker, such that all amplicons arising from any single template molecule during the amplification remain immobilized and clustered to a single physical location on an array. AB SOLiD platform has its origins in the system described by J.S. and colleagues in 2005 and in work by McKernan and colleagues at Agencourt Personal Genomics (Beverly, MA, USA) (acquired by Applied Biosystems (Foster City, CA, USA) in 2006). Libraries may be constructed by any method that gives rise to a mixture of short, adaptor-flanked fragments, though much effort with this system has been put into protocols for mate-paired tag libraries with controllable and highly flexible distance distributions. Clonal sequencing features are generated by emulsion PCR, with amplicons captured to
the surface of I-M paramagnetic beads. After breaking the emulsion, beads bearing amplification products are selectively recovered, and then immobilized to a solid planar substrate to generate a dense, disordered array. Sequencing by synthesis is driven by a DNA ligase, rather than a polymerase.

The Helicos sequencer, based on work by Quake’s group, also relies on cyclic interrogation of a dense array of sequencing features. However, a unique aspect of this platform is that no clonal amplification is required. Instead, a highly sensitive fluorescence detection system is used to directly interrogate single DNA molecules via sequencing by synthesis. Template libraries, prepared by random fragmentation and poly-A tailing (no PCR amplification), are captured by hybridization to surface-tethered poly-T oligomers to yield a disordered array of primed single-molecule sequencing templates. At each cycle, DNA polymerase and a single species of fluorescently labeled nucleotide are added, resulting in template-dependent extension of the surface-immobilized primer-template duplexes. After acquisition of images tiling the full array, chemical cleavage and release of the fluorescent label permits the subsequent cycle of extension and imaging.

The diversity and rapid evolution of next-generation sequencing technology is post challenges for bioinformatics in areas including sequence quality scoring, alignment: assembly and data release. The topic of sequence quality scoring has become an area of intent interest, given the relatively low quality of raw data from the new sequencing platform and the various contextdependent error distributions associated with different sequencing by synthesis biochemistries. Even at this early stage of commercial availability, a variety of software tools are available for analysing next-generation sequencing data. Their functions fit into several general categories, including: (i) alignment of sequence reads to a reference; (ii) base-calling and/or polymorphism detection; (iii) de novo assembly, from paired or unpaired reads; and (iv) genome browsing and annotation. Alignment and assembly represent particularly interesting problems.

Applications of new-generation sequencing include (i) full-genome resequencing or more targeted discovery of mutations or polymorphisms (ii) mapping of structural rearrangements, which may include copy number variation, balanced translocation breakpoints and chromosomal inversions; (iii) RNA-Seq, analogous to expressed sequence tags (EST) or serial analysis of gene expression (SAGE), where shotgun libraries derived from mRNA or small RNAs are deeply sequenced; the counts corresponding to individual species can be used for quantification over a broad dynamic range, and the sequences themselves can be used for annotation (e.g., splice junctions and transcript boundaries); (iv) large-scale analysis of DNA methylation, by deep sequencing of bisulfite-treated DNA; (v) 'ChIP-Seq', or genome-wide mapping of DNA-protein interactions, by deep sequencing of DNA fragments pulled down by chromatin immunoprecipitation. Over the next few years, the list of applications will undoubtedly grow, as will the sophistication with which existing applications are carried out.

Suggested Readings


Molecular Epidemiology of Parasitic Diseases  
Dr. Siju Susan Jacob and Dr. P. P. Sengupta  
ICAR- National Institute of Veterinary Epidemiology and Disease Informatics, Yelahanka, Bengaluru-560064, Karnataka

Introduction

Molecular epidemiology, broadly defined, is the application of molecular genetic techniques to the dynamics of disease in a population. Epidemiology is the study of the causation and dynamics of disease in a population. For parasitic diseases, this is determined by the transmission of the parasite between hosts, and how this transmission affects the dispersal of the parasite within and among host populations. To control parasitic disease, therefore, we need to understand parasite ecology, particularly transmission dynamics, how life cycles may interact and the nature of interactions within the host. This requires an input from both population and evolutionary biology, to determine the genetic structure and evolution of infectious agents, their population biology, and the evolutionary consequences of medical and public health interventions. Traditionally, both epidemiology and parasite ecology have concentrated on an empirical approach. Epidemiological studies typically begin with a description of the frequency and distribution of disease and then attempt to associate these patterns with the frequency and distribution of independent variables or risk factors. Identifying risk factors is important because it allows for targeted control programs, but the efficacy of such control programs hinges upon knowing how the risk factors interact with the parasite’s life cycle to increase exposure. Ecological studies of parasite life cycles usually start with a description of parasite prevalence, and sometimes also intensity of infection, within different host species. These data, often accompanied by in vitro and experimental infection studies, can then be used to infer the major pathways of parasite transmission. In the last 20 years, these empirical studies of the ecology of parasite life cycles and the epidemiology of parasitic disease have been complemented by a more theoretical approach, which uses mathematical models of parasite and host population sizes to guide epidemiological interpretation.

The application of molecular and analytical tools, derived largely from the fields of population genetics and systematics, can contribute enormously to both empirical and theoretical studies of the epidemiology of parasitic disease. Molecular epidemiological approaches enable the reconstruction of evolutionary relationships between parasites over a wide range of temporal and spatial scales, improving our ability to identify parasites, track
their movements, relate their spread to environmental factors and understand the role they play in disease causation.

Molecular epidemiological studies of parasitic diseases could be classified into two different types, depending on whether they were concerned purely with identification of the causative agents of disease, or whether they considered the impact of genetic variation on “downstream functions”, such as transmission, infectivity, virulence or drug resistance. To date, the main applications of molecular techniques have been in parasite identification rather than to study patterns of disease progression or transmission. This has been due partly to the inability of molecular tools to distinguish genetic variation at the appropriate level of resolution for addressing downstream function and partly to the inadequacy of analytical methods to interpret genetic variation in an ecologically meaningful fashion. Rapid advances in both these areas mean that an increasing number of molecular epidemiological studies are addressing questions of function, although parasite identification remains a critical issue. From a practical perspective, genotyping the agents should not become a dominant aim of molecular epidemiological investigations, since the existence of different genotypes does not imply they necessarily have some phenotypic importance. The scope and potential of molecular epidemiology is much greater, and in this regard the search for genetic markers for ‘medically’ important traits such as infectivity, drug resistance and virulence present important challenges for molecular epidemiological investigations.

**Molecular tools**

Our ability, using molecular techniques, to detect and characterize the genetic variability of infectious agents, particularly at the intraspecific level, can be seen as the foundation for most molecular epidemiological studies. The application of appropriate molecular tools will aid in the identification and surveillance of infectious agents and in determining sources of infection. The availability of such tools, particularly those based on the polymerase chain reaction (PCR), which allow direct examination of clinical or environmental isolates, has had an enormous impact on the genetic characterisation, diagnosis and taxonomy of parasites. They also obviate the need for laboratory amplification of parasite isolates, which was a major limiting factor in characterising parasites refractory to in vitro culture, and may lead to biased sampling of natural diversity by the selective amplification of those parasites amenable to culture. Using PCR, defined gene sequences of infectious agents can be detected from small quantities of material and the resultant data can
be used not only for diagnosis, but also to assess the effect of interventions on the population structure of infectious agents, assessment of intraspecies diversity, and transmission studies. The value of such tools is greatest if they can be applied directly to faecal or tissue specimens, as well as environmental samples, and if there is the potential to automate such procedures. Emphasis will be given in the future to establishing high throughput molecular assays such as pyrosequencing, as well as their field applicability. Pyrosequencing techniques have the added advantage of allowing the simultaneous detection of multiple species/genotypes in a single sample. Multiplex PCR (mPCR) also enables the amplification of more than one target of interest in a PCR by using multiple primer pairs and producing amplicons of different size. Loop-mediated isothermal DNA amplification (LAMP) is a newly developed, rapid, quantitative, highly sensitive and specific nucleic acid-based, non-PCR diagnostic tool, applicable to ‘low-cost’ laboratory settings. This simple molecular test can be carried out on a bench with a heating block instead of a thermal cycler and may prove to be an invaluable ‘field friendly’ tool for screening and quantifying infections in host populations while providing important genotypic information.

Choosing an appropriate marker for molecular epidemiological studies requires consideration of the required level of resolution for the study, the precision of the genetic data collected and the historical information content of the data.

Genetic markers, although identified in individuals, are influenced by processes which are more readily measured at the level of populations, such as mode of reproduction, breeding system, mutation, migration and selection. Population level processes eventually influence speciation and thus all cladogenetic events in the history of a lineage. Therefore, by using genetic markers with appropriate rates of change, we should be able to examine evolutionary patterns and processes at all levels throughout the hierarchy of life, from individuals to kingdoms. In this context, emphasis has been given to the importance of appropriate analysis and the value of characterising the genetic diversity of infectious agents at different levels of specificity. The latter requires choosing molecular tools which are capable of discriminating genetic variants at different hierarchical levels and the region of DNA examined must be appropriate to the level of questions being asked. e.g. taxonomy, diagnosis, population genetics, evolutionary relationships, isolate tracking etc. This is primarily a question of choosing a genomic region with an appropriate signal-to-noise ratio; too little variation will provide a signal which is too weak to discriminate among groups, whereas too much variation will swamp the signal with uninformative noise. The choice of genetic marker
typically involves a trade off between technical convenience and precision. Markers such as RAPDs and AFLPs do not require specific sequence information from the target genome, and hence can be utilized more readily for less well studied species. The variation they detect, however, may be non-heritable and even when heritable is dominant rather than codominant. This means that alternative alleles at a locus cannot be distinguished, greatly reducing the range and power of analytical techniques which can be applied to the resultant data. It also means that the data cannot be compared effectively over different studies, and are therefore limited in usefulness to the particular time and place where they were collected. Traditional genetic markers, such as allozymes and RFLPs have little historical information content. That is, we do not usually know the phylogenetic relationship between alternative alleles or haplotypes and the data are therefore analysed as allele or haplotype frequency differences among groups. Sequence data, however, do provide historical information because the phylogeny of sequences can usually be inferred. This enables sequence data to be analysed in ways which are not possible for allele frequency data.

**Conclusions:**

Molecular epidemiological studies have had a major impact on the identification and classification of parasites, which has in turn improved our ability to unravel transmission patterns and identify risk factors for parasite infection. The development of new molecular tools allowing relatively cheap, high throughput molecular assays and of new analytical approaches for population genetic studies will continue to drive a deeper understanding of parasite ecology and the epidemiology of parasitic disease. A major challenge for the immediate future will be to correlate genetic and phenotypic variation to identify markers for complex epidemiological traits, such as drug resistance, zoonotic potential and virulence.

**Further reading:**


Numerous studies demonstrate the importance of the inherent losses from infectious animal diseases and from emerging zoonotic risks threatening humanity. For example, Foot and Mouth Disease cause losses of approximately USD 25 billion per year (J. Rushton, 2012). Ticks and tick-borne diseases cost approximately USD 17 billion per year (De Castro, 1997). The World Bank has estimated losses of USD 200 billion (direct and indirect) caused by zoonoses during 2000 and 2010. According to an OECD report (2011), a severe avian influenza pandemic could cause the death of 70 million people and decrease global GBP by 4.8%. However, there is little information on the impact of endemic livestock disease and non-infectious diseases, among which include bacterial and parasitic diseases, for example, and diseases affecting the musculoskeletal, reproduction and production systems.

The OIE estimates that morbidity and mortality due to animal diseases cause the loss of at least 20% of livestock production globally. This represents at least 60 million tonnes of meat and 150 million tonnes of milk with a value of approximately USD 300 billion per year (http://www.rr-africa.oie.int/en/news/index.html)

The purpose and scope of setting up of Quarantine Stations is to prevent the ingress of dangerous exotic diseases into the country through imported livestock and livestock products. The increased and faster international trade and travel exposed every country to the danger of infiltration of known and unknown transmissible diseases which have the potential of very serious and rapid spread, adverse socio-economic and human/animal health consequences. There are many infectious diseases of livestock which are prevalent in other countries but luckily not present in India. It is, therefore, necessary that such exotic diseases do not gain entry into our country through movement of livestock and livestock product from across the borders. The entire procedure of keeping a watch on livestock disease is the responsibility of the Office of International Epizooties (O.I.E.) through its International Zoo Sanitary Code. For this purpose this organization has classified the prevalent disease as OIE listed diseases. The speedy travel by air has reduced the travel time which used to be an effective barrier when the transport was done by sea. Even sea travel has become quicker now-a-days and so gone all the days when the travel used to be for many days which generally exceeded the incubation period of many acute diseases thus exposing the presence of an infection in the livestock during such travel. Hence, an efficient Animal Quarantine Organization is necessary for conducting checks at the International Airports/Seaports and land routes. Because livestock may covertly carry pathogens without showing overt signs of clinical disease, they must be held in quarantine for observation and testing to establish their pathogen free status before release. Keeping this in view, Government of India initiated a central sector scheme namely “Animal Quarantine & Certification Services” (AQCS) during the Fourth Five Year Plan (1969-74) under which four Animal Quarantine stations were set up at Delhi, Chennai, Kolkata & Mumbai and now two more quarantine station at Hyderabad and Bangalore in 11th plan. The responsibility of preventing ingress of exotic diseases including zoonotic diseases and thereby safeguarding the health of country’s livestock
population lies solely with the Animal Quarantine and Certification services. This programme envisages provision of an Internationally acceptable certification service for the livestock & livestock product exported to other countries from India confirming to the health requirements of the importing country or the health regulations prescribed in the International Zoo Sanitary code.

**OBJECTIVES**

1. To prevent the ingress of any Exotic Livestock Diseases into India through importation of livestock & livestock products as per the provisions of Livestock Importation Act (Act No. IX. of 1898) as amended by the Livestock Importation (Amendment) Act, 2001 (5.7.2001) and the regulations orders and SPS standards of the country issued thereunder.

2. To provide an internationally accepted certification service for augmenting export and to play important role to increase International trade of livestock and livestock products. 3. To inspect and register the plants/mills exporting the animal by-products.


3. To inspect and register the plants/mills exporting the animal by-products.


**ECONOMIC IMPORTANCE OF ANIMAL QUARANTINE**

IMPORT: Saving the huge money, labour and time required in disease eradication programmes (sometimes eradication not sure) by preventing the entry of exotic diseases.

EXPORT: • Important role in Increasing the demand of LS/LSP in the international market by quality certification of livestock and livestock products as per the requirement of importing country.

CATEGORIES OF Livestock (LS)/Livestock products (LSP) for IMPORT PURPOSE:

• Requiring import license from DGFT. (Restricted items)
• Requiring Sanitary Import Permit (SIP) from DAHDF. (Free items)
• LS/LSP prohibited in view of Disease status .

**IMPORT PROCEDURES**

Livestock and Livestock Products are imported as a baggage and cargo through Airport/Seaport and ICDs. The said consignments are referred by the customs for Animal Quarantine Clearance in compliance to import Quarantine health rules of the Government of India. The consignments are examined along with the accompanied health certificates and other relevant papers before allowing the entry into India. In case the consignment is not fulfilling the health protocol laid down by Government of India then appropriate action is taken as per the rules in National Interest. The imported livestock are kept under quarantine for observation and testing as per the prescribed health protocol. The products are also checked and tested accordingly in compliance to the health protocol prescribed by Government of India.
Documents required cum check list with Application Form for IMPORT OF LIVESTOCK (under DGFT Licence/ Sanitary Import Permit)- ALL LIVESTOCK EXCEPT PET (Dog/Cat upto two number with owner under baggage rule)

1. Copy of valid DGFT Licence/Sanitary Import Permit (SIP) 
2. Bill of entry with Custom reference. 
3. Official health certificate from the country of origin fulfilling all import health guidelines of India as per the Notification/supplied protocol with license or SIP/AQCS requirement as the case may be. The Identification detail/sheet no./passport no./origin detail shall be mentioned in health certificate/certified officially. 
4. Laboratory Reports (not mandatory in each case). 
5. The name and address of owner in the official health certificate must match with the Licence/SIP to establish ownership. 
6. No additional feed, bedding etc. be allowed during the journey without permission. 
7. Undertaking and declarations as per requirement. 
9. Any other document if required during examination of application. 
10. Original Health documents are mandatory on arrival for Provisional clearance. 
11. Authorization letter if owner is not approaching directly. Advance NOC will be issued within 7 days of arrival based on the self certified advance copies of all above documents. Original Health certificate will be retained by AQCS at the time of Provisional Clearance on arrival. Final Clearance will be issued as per the applicable post import Quarantine rule/regulation.

EXPORT PROCEDURES
This programme envisages provision of an Internationally acceptable certification service for the export of livestock & livestock product to other countries from India confirming to the health requirements of the importing country and the health regulations prescribed in the International Zoo Sanitary code.

Documents required cum check list with Application Form for EXPORT OF LIVESTOCK
1. Copy of valid Import/Export Licence or Permit as the case may be. If no permit/licence is required than undertaking from the exporter/owner in this regard.
2. Official health requirement/format of the importing country. If no prescribed health requirement/format than undertaking from the exporter/owner in this regard.
3. Fulfilled health requirement of importing country including testing, treatment, vaccination etc. (if applicable).
4. Self certified copies of present health documents including vaccination record of the animal. 
5. Undertaking and declarations as per requirement. 
6. Documents of origin, if applicable/asked. 
7. Copy of airway bill/journey details of animal. 
8. Any other document if required during examination of application. 
9. Authorization letter if owner is not approaching directly.
(http://dahd.nic.in/sites/default/files/Animal%20and%20quarantine%20certification%20services%20%205.pdf)
INTRODUCTION

Many times in vivo and in vitro maintenance, isolation and identification are very much useful for accurate diagnosis and treatment of the parasite. In this study we will demonstrate the maintenance, isolation and identification of Trypanosoma evansi protozoan parasite.

Trypanosoma evansi causes a trypanosomosis known as ‘surra’. It affects a large number of wild and domesticated animal species in Africa, Asia, and Central and South America. The principal host species varies geographically, but camels, horses, buffalos and cattle are particularly affected, although other animals, including wildlife, are also susceptible. It is an arthropod-borne disease; several species of haematophagous tabanid flies are implicated in transferring infection from host to host, acting as mechanical vectors. The disease in susceptible animals is manifested by pyrexia, directly associated with parasitaemia, together with a progressive anaemia, loss of condition and lassitude. Such recurrent episodes lead to intermittent fever (as high as 44°C in horses [Gill, 1977]) and parasitaemia during the course of the disease. Oedema, particularly of the lower parts of the body, rough coat in camels, urticarial plaques and petechial haemorrhages of the serous membranes are sometimes observed. In advanced cases, parasites invade the central nervous system (CNS), which can lead to nervous signs (progressive paralysis of the hind quarters and, exceptionally, paraplegia), especially in horses, but also in other host species before complete recumbency and death. Abortions have been reported in buffalos and camels (Gutierrez et al., 2005; Lohr et al., 1986) and there are indications that the disease causes immunodeficiency (Dargantes et al., 2005b; Onah et al., 1998).

OBSERVATION
• **REVIVAL OF T.evansi STABILATES**

a) Different isolates of *T.evansi* isolates (buffalo, dog, lion and leopard) are maintained in our laboratory, *in-vitro* in liquid nitrogen.

b) The stabilates maintained in the laboratory (*in-vitro*) are revived by infecting into rats/mice.

c) For infecting the rat, the vials taken from the LN$_2$ are thawed immediately and blood from capillary tubes is transferred to small beakers.

d) Depending upon the number of rats/mice, blood is equally distributed and administered intraperitoneally/intradermally using 1ml disposable syringe

• **SCREENING OF INFECTED ANIMAL**

1. Parasite level in rat/mice can be checked by collecting the blood (10-20µl) from the tail end.

2. Wipe tail end of the animal with 70% alcohol and pierce a sterile needle.

3. Draw the blood on to a glass slide and make a clear smear.

4. Allow the smear to dry and fix it by methanol for 1 minute.

5. Add Geimsa stain (1:4 diluted) (1ml of stain +8ml of phosphate buffer) and allow it for 45min.

6. Wash it with tap water & allow it to dry.

7. Observe under the microscope (100X) for the presence of parasite.

8. At very high parasitaemia (usually 30-40 tryps/field) sacrifice the animal and collect the blood from the heart with 2%EDTA.

**DISSECTION:**

**Materials required;**

Disposable syringe (5ml), 2% EDTA as anticoagulant, normal Saline, beakers with EDTA, chloroform & dessicator, glass slides (grease free)
Procedure:

1. Euthanize the infected animal and transfer to the dissecting tray
2. Dissect the animal and cut open the chest muscle to draw blood from the heart
3. Rinse 5 ml disposable syringe with 2% EDTA & draw blood from the heart.
4. Transfer the collected blood to the glass beakers slowly containing 2% EDTA.
5. Continuously and slowly stir the beaker to avoid clotting of blood.
6. Once the entire blood is collected from the heart, separate the plasma/buffy coat to purify the trypanosomes.

Purification of Trypanosomes

Trypanosomes from the blood/plasma can be purified by diethylaminoethyl (DEAE) anion-exchange column chromatography.

Equilibration of DEAE-cellulose

1. Suspend fifty gram of DEAE-cellulose in 2 liters of distilled water and mix for 20 minutes with a magnetic stirrer at low speed.
2. Adjust the pH to 8 with ortho-phosphoric acid and allow to settle for 30 minutes.
3. Discard the supernatant fluid containing the finest granules and repeat the procedure three times.
4. Store the equilibrated, concentrated slurry (DEAE-cellulose) in small aliquots at –20°C for till further use.

Packing of the column with equilibrated DEAE-cellulose

1. For packing of chromatographic glass column (10 ml), place a disc of Whatman No. 41 filter paper at the bottom of the syringe (column) and moisten by adding a few drops of PSG.
2. Fit the column to burette stand and add equilibrated cellulose slurry (~4 ml) into the syringe and allow packing for few minutes before elution of the buffer.

3. Wash the column later and equilibrate with 2 ml of PSG without disturbing the surface (the height of the sediment can be approximately 3cm).

**Adsorption of trypanosomes**

1. Centrifuge the heparinized blood at 3000 rpm for 3 minutes to collect the buffy coat.

2. Add the buffy coat on to the surface of the cellulose column and allow it penetrate the cellulose. The cellulose column was kept moistened throughout the procedure.

3. Add PSG (~2 ml) on to the column to elute the trypanosomes.

4. Collect the elution in 15 ml centrifuge tube and centrifuge at 1000g for 10 minutes.

5. Observe the elution under the microscope by placing a drop of elute on the glass slide for trypanosomes.