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PROCEEDINGS OF
REGIONAL SEMINAR ON VETERINARY EPIDEMIOLOGY

SANTO DOMINGO, DOMINICAN REPUBLIC
JULY 16-18, 1981



INTER-AMERICAN INSTITUTE FOR COOPERATION ON AGRICULTURE

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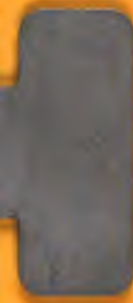
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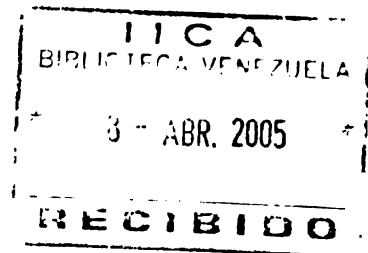
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C O N T E N T S

History

Objectives

Speakers

Timetable

Opening Session

Opening Address by the Secretary of State for Agriculture

List of Participants

Principles of Epidemiology

Epidemiological Methods

Disease Surveillance

Ticks: Distribution and Ecology by Species

Anaplasmosis

Babesiosis

Arthropod-Borne Diseases: African Swine Fever

Heartwater

Tick Control: Acaricides

Why Tick Control or Eradication is Considered?

Outbreaks of Equine Encephalitis in the Dominican Republic

Laboratory Diagnosis for Equine Encephalomyelitis

Different Stages of Anaplasmosis and Babesiosis in the Country (D.R.)

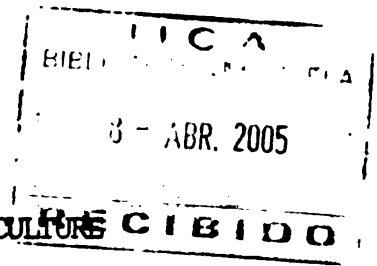
Recommendations on Equine Encephalitis

Recommendations on Tick Control

General Recommendations

Evaluation

Closing Session



STATE SECRETARIAT FOR AGRICULTURE

INTER-AMERICAN INSTITUTE FOR COOPERATION ON AGRICULTURE

Regional Seminar on Veterinary Epidemiology

July 6-10, 1981

Santo Domingo, Dominican Republic

History

The Directors of Animal Health at their Second Inter-American Meeting, REDISA II, held in San José, Costa Rica in September 1980, recommended that advanced training should be undertaken in Veterinary Epidemiology. Participants would then be assisted to put on similar training courses for their colleagues in their own country and further training might be identified for those of particular aptitude with respect to their country's needs.

Host Country

The government of the Dominican Republic has kindly agreed to host a bi-lingual seminar for the region on Veterinary Epidemiology from July 6th to 10th, 1981.

Objectives

1. To identify disease Surveillance Methods.
2. Data gathering and analyses.
3. To evaluate disease and essential techniques for epidemiological control, especially in relation to Ticks, Tick-Borne Diseases, Equine Encephalitis and African Swine Fever.

Guest Speakers

- | | |
|------------------------------|---|
| Dr. M.J. Burridge | Associate Professor, University of Florida. |
| Dr. Glen Garris | Entomologist, U.S. Department of Agriculture. |
| Dr. Robert E. Ormiston (DVM) | Tick Control, U.S. Department of Agriculture. |
| Dr. Gary S. Colgrove | African Swine Fever, USAID. |
| Dr. John Mason | |

Guest Speakers (contd.)

Dra. Rosario Cabrera

Dra. Lucia Duval

Venue

INDOTEC's Auditorium

Time-Table

JULY 6th

8.30 - 9.30 a.m.	Registration of Participants
9.30 - 10.00 a.m.	Opening Session
10.00 - 10.30 a.m.	Coffee Break
10.30 - 12 noon	Epidemiological Principles (Dr. Burridge)
2.00 - 3.30 p.m.	Epidemiological Methods (Dr. Burridge)
3.30 - 4.00 p.m.	Coffee Break
4.00 - 5.30 p.m.	Disease Surveillance (Dr. Burridge)

JULY 7th

8.30 - 10.00 a.m.	Ticks: Distribution and Ecology by Species (Dr. Garris)
10.00 - 10.30 a.m.	Coffee Break
10.30 - 12 noon	Ticks: Distribution and Ecology by Species (Dr. Garris)
2.00 - 2.45 p.m.	Tick-borne Diseases: Anaplasmosis (Dr. Ormiston)
2.45 - 3.30 p.m.	Tick-borne Diseases: Babesiosis (Dr. Ormiston)
3.30 - 4.00 p.m.	Coffee Break
4.00 - 4.45 p.m.	African Swine Fever (Dr. Colgrove)
4.45 - 5.30 p.m.	Heartwater (Dr. Burridge)

JULY 8th

- 8.30 - 10.00 a.m. Tick Control: Acaricides (Dr. Garris)
- 10.00 - 10.30 a.m. Coffee Break
- 10.30 - 12 noon Tick Control: Control Programmes (Dr. Ormiston)
- 2.00 - 3.30 p.m. Equine Encephalitis: Epidemiology
- 3.30 - 4.00 p.m. Coffee Break
- 4.00 - 5.30 p.m. Equine Encephalitis: Outbreaks in the Dominican Republic (Dra. Cabrera and Dra. Duval)

JULY 9th

- 8.30 - 10.00 a.m. Equine Encephalitis: Control and Surveillance (Dr. John Mason)
- 10.00 - 10.30 a.m. Coffee Break
- 10.30 - 12 noon Equine Encephalitis: Outbreaks and Diagnosis in the D.R. (Dra. R. Cabrera and Dra. L. Duval)
- 2.00 - 3.30 p.m. Review Session: Epidemiology and Equine Encephalitis
- 3.30 - 4.00 p.m. Coffee Break
- 4.00 - 5.30 p.m. Review Session: Tick-borne Diseases and Tick Control

JULY 10th

- 7.00 - 12 noon Practical Session, Central Veterinary Laboratory, San Cristóbal
- 2.00 - 4.00 p.m. Conclusions and Recommendations for Future Seminars
- 4.30 - 5.00 p.m. Closing Remarks and Presentation of Certificates to Participants

Coordinators

- Dr. F.C.M. Alexander - IICA, Georgetown, Guyana.
- Dr. Héctor Morales Jara - Director, IICA Office in the D.R.
- Dr. Reynaldo Peña de la Cruz - Director of Animal Health, SEA.
- Mr. Raúl A. Pineda - SEA/IICA.

Remarks

1. Transportation for foreign participants will be available twice a day.
2. Social activities will develop according to special programme.
3. Arrangements have been made to have lunch at the INDOTEC's auditorium. Each person will pay for his meals.

STATE SECRETARIAT FOR AGRICULTURE
and
INTER-AMERICAN INSTITUTE FOR COOPERATION ON AGRICULTURE

REGIONAL SEMINAR ON VETERINARY EPIDEMIOLOGY

INAUGURAL SESSION

1. Speech by the Secretary of State for Agriculture in the Dominican Republic, R. Hipólito Mejía D.
2. Speech by the Director of the IICA Office in the Dominican Republic, Dr. Héctor Morales Jara.

TABLE OF HONOUR:

From the left, the following were at the table:

Dr. Reynaldo Peña de la Cruz, Director of Animal Health
Dr. Héctor Morales Jara, Director of IICA Office in the D.R.
R. Hipólito Mejía D., Secretary of State for Agriculture
Dr. Marcelino Vargas y Vargas, Director General for Livestock
Dr. José Alberto Torres, Representative of the Director General of IICA
Dr. J.M. Burrige, Professor of Florida State University

1. The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that this is essential for ensuring transparency and accountability in the organization's operations.

2. The second part of the document outlines the various methods and tools used to collect and analyze data. It highlights the need for consistent and reliable data collection processes to support effective decision-making.

3. The third part of the document focuses on the role of technology in data management and analysis. It discusses how modern software solutions can streamline data collection, storage, and reporting, thereby improving efficiency and accuracy.

4. The fourth part of the document addresses the challenges associated with data management, such as data quality, security, and privacy. It provides strategies to mitigate these risks and ensure that data is used responsibly and ethically.

5. The fifth part of the document concludes by summarizing the key findings and recommendations. It stresses the importance of ongoing monitoring and evaluation to ensure that data management practices remain effective and aligned with the organization's goals.

6. The sixth part of the document provides a detailed overview of the data collection process, including the identification of data sources, the design of data collection instruments, and the implementation of data collection procedures.

7. The seventh part of the document discusses the various methods used for data analysis, such as descriptive statistics, inferential statistics, and regression analysis. It explains how these methods can be used to interpret the data and draw meaningful conclusions.

8. The eighth part of the document focuses on the presentation of data, including the use of tables, charts, and graphs. It provides guidelines for creating clear and concise reports that effectively communicate the results of the data analysis.

9. The ninth part of the document discusses the importance of data security and privacy. It outlines the measures that should be taken to protect sensitive data from unauthorized access and ensure compliance with relevant regulations.

10. The tenth part of the document provides a final summary and concludes the report. It reiterates the key findings and offers final recommendations for improving data management practices.

OPENING ADDRESS

by
R. Hipólito Mejía D.

LADIES AND GENTLEMEN:

Until a few years ago our countries have had to face cyclical onslaughts of typical livestock diseases on their own and, in the majority of cases, our technical, human and economic resources were so limited that diseases which can easily be controlled today decimated entire livestock populations.

It is due to this that we have considered international solidarity to be of major importance for the livestock development of our peoples, and we have encouraged the celebration of international meetings in whose forums we can pose our common problems and possible solutions.

Tangible proof of this affirmation is offered by the presence of distinguished representatives of Caribbean nations at this Regional Seminar on Veterinary Epidemiology, and the various bilateral and multilateral programmes for cooperation which these nations have signed to face, together, the risks of diseases among their respective livestock.

The Dominican Republic, which has often had to combat serious epidemics which have also decimated livestock populations, as is the case with African Swine Fever, admires the willingness of Caribbean nations, represented here, to help in specific cases threatening their livestock resources.

Veterinary Epidemiology is of special importance in the fight against livestock diseases as, through that science, we are able to adopt control and eradication measures for those diseases.

Applied to the case of the Dominican Republic its importance is even more evident since our livestock enterprises suffer the effects of low profitability each time a serious disease breaks out, or it is necessary to invest substantial resources to create basic infrastructure to prevent greater damages.

There are about 87 thousand meat and dairy livestock enterprises in the country on which almost half a million people depend. More than 80% of those enterprises belong to small and average producers and it is calculated that these, individually, possess less than 100 heads per herd.

These producers do not have amply available production resources and lack, on occasion, the technical assistance necessary to make their enterprises more profitable and to contribute, in this way, to an increase in livestock products and by-products offered.

Our country finds itself cyclically affected by exotic diseases, as is the case with African Swine Fever which forced us to exterminate all swine. It is only now, after more than three years, that we have been able to begin repopulation in the eastern region of the country.

Other diseases have become endemic in our environment producing great losses which reflect negatively on the national economy.

The complex Piroplasmosis and Anaplasmosis, transmitted by tick bites, stand out among diseases which affect our cattle, provoking annual losses which exceed 10 million pesos, as is the case with Equine Encephalitis which appeared for the first time in our country in the year 1948. Since then six outbreaks have been reported in the Montecristy areas in the southern peninsula of the country and finally, in 1978, in the María Trinidad Sánchez and Samaná area.

The Dominican government, headed and led by our agriculturist President, Mr. Antonio Guzmán Fernández, recognizes that the health situation of livestock in the Dominican Republic has a long way to go to reach acceptable security levels for the country and producers.

We see, with satisfaction, how international organizations, and among them it is fitting to mention the Inter-American Institute for Cooperation on Agriculture in which the Dominican Republic has been participating actively for years, are seeking practical solutions and efficient operative mechanisms at country level to face production and productivity problems according to their individual intrinsic characteristics.

Ticks, African Swine Fever and Equine Encephalitis are very prevalent themes in our country, and in the entire Caribbean area, and actions and programmes presently in execution need more epidemiologists and qualified personnel.

It is important to develop human talent so that each professional or technician involved in the animal health programmes may try to get to the root of the problems which afflict us, in order to look for definite solutions or develop an awareness of their dimensions.

It is hardly nine months ago that, under the auspices of IICA, the Second Meeting of Animal Health Directors in the hemisphere took place emphasizing the need for veterinary professionals to receive training in veterinary epidemiology so as to fill the gaps produced by apparent deficiencies in the studies of our universities.

Since last January the IICA Animal Health Programme, headed in the Antilles Zone by Dr. F.C.M. Alexander, began to implement activities through our Animal Health Department.

Ticks, Equine Encephalitis and African Swine Fever have been chosen as topics for this Seminar. They are diseases which are a threat for our livestock and, in the case of Encephalitis, for our men and women too.

This Regional Epidemiology Seminar is of great importance, as much for the quality of international experts who will share their knowledge and experiences with us, as for the alternatives which will be offered for facing the health problems which afflict our livestock.

We need our field veterinarians to approach animal health problems in a global, logical manner, capable of predicting the immediate effects and knowing which are the weak points through which the diseases can be attacked for control or eradication.

We cordially welcome veterinarians from Jamaica, Haiti, Puerto Rico, Trinidad and Tobago, Barbados, Guyana, Suriname and Dominica who are today joining with their Dominican colleagues in search of universal solutions to problems which we unfortunately have to share.

Allow us to express acknowledgement to the Inter-American Institute for Cooperation on Agriculture (IICA) which, in a new hemispheric and humanistic

dimension, visualized and implemented this Animal Health Programme which covers a great need within this field of livestock.

Likewise we wish to acknowledge the support which Florida State University and the United States Department of Agriculture offers us for this activity and its follow-up.

On behalf of the Dominican government we formally declare this Seminar open and we wish that all participants, during this week of exchange and learning, may analyse and discuss the technical aspects which are to be dealt with from the point of view of the particular problems of the countries represented here.

THANK YOU.

SEA / IICA

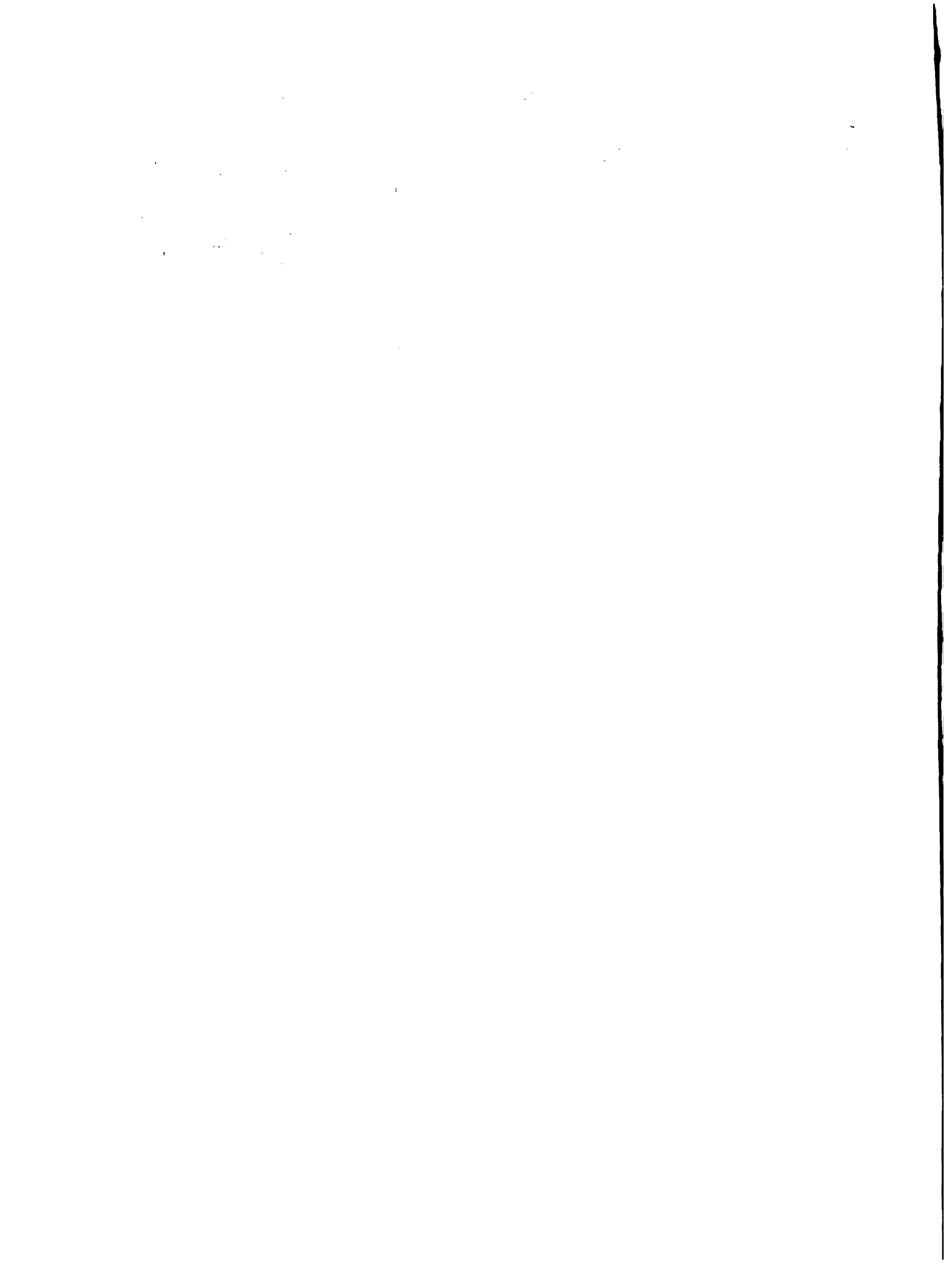
REGIONAL SEMINAR ON VETERINARY EPIDEMIOLOGY

List of Participants

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PRINCIPLES OF EPIDEMIOLOGY

by
Dr. M.J. Burridge

Definition of Epidemiology

Epidemiology (or epizootiology) is the study of the relationships between the various factors that determine the frequency and distribution of diseases in animal populations.

Principle Uses of Epidemiology

1. Diagnosis: elucidation of the origins and causes of specific disease outbreaks (in this instance, epidemiology acts as a diagnostic discipline for population or herd medicine).
2. Research: determination of causal mechanisms of diseases and description of their natural histories.
3. Disease control, prevention, and eradication: epidemiology is the basic discipline for a rational approach to the control, prevention and eradication of diseases and, as such, it is used to determine and to evaluate methods for directed action against diseases.

Multiple Causality of Disease

Epidemiology is concerned primarily with the relationships between the following:

1. populations of animal hosts;
2. potentially harmful agents which they or the environment may harbor;
3. the environment in which the hosts and agents occur and interact;
4. time.

The visible products of these relationships are health and disease.

Determinants of Disease

Many variables directly or indirectly influence the frequency of occurrence and the distribution of any given disease. Such variables are known as determinants of disease, and they include specific agents and their properties,

host characteristics, and environmental factors. These disease determinants, together with some of their important interrelationships such as modes of transmission of infectious agents will determine the patterns of disease in animal populations.

1. Agent determinants: Disease agents are of three kinds: biological (bacteria, viruses, fungi, protozoa, and metazoa), chemical (natural poisons and synthetic compounds), and physical (e.g. temperature, sunlight, trauma, radiation). Biological agents possess important properties that can have profound effects on the patterns of diseases in animal populations. These properties include the following:
 - a. infectivity (ability to infect animal hosts which is often dose-related);
 - b. pathogenicity (ability to cause disease);
 - c. virulence (property that determines the severity of the infection);
 - d. antigenicity (ability to induce the production of antibodies by the host);
 - e. immunogenicity (ability to induce immunity in the host);
 - f. antigenic variability (e.g. evolution of serotypes, antigenic drift and shift, antigenic variation);
 - g. development of resistant free-living forms;
 - h. variability in tissue-tropism.
2. Host determinants: The occurrence of many diseases is strongly associated with particular host variables that influence an animal's susceptibility or resistance to disease. These host variables include species, breed, sex, age, genetic characteristics, immunological state, physiological state (e.g. pregnancy, lactation), and functional use of animal (analog of occupation for man).
3. Environmental determinants: Many environmental factors have major influences on determination of patterns of diseases in animal populations. Physical factors include climate, topography, and soil. Various meteorological factors affect host and vector populations and the free-living stages of infectious agents. Biological factors include the influence

of man through management and cultural practices and the influence of other animals, especially those that serve as reservoirs or as vectors of infectious agents.

Modes of Transmission of Infectious Agents

1. Vertical vs. horizontal transmission

Vertical transmission is transmission of an agent from an individual to its offspring (i.e. from one generation to the next, such as prenatal infection, infection via colostrum, or transovarian transmission of an agent from an infected individual to a susceptible contemporary.

2. Direct vs. indirect transmission

Direct: a. physical contact (e.g. bite-transmitted rabies);

- b. airborne droplets, which are large infectious particles that are propelled only short distances, such as during coughing or sneezing, to the mucous membranes of other animals (e.g. canine distemper).

Indirect: a. vectors, which are invertebrates that transmit infectious agents from an infected animal or its excreta to a susceptible animal or some immediate source of infection such as water or food. Vector transmission may be mechanical, involving survival of agent without its multiplication or development (e.g. equine infectious anemia virus by tabanids), or biological, involving essential multiplication and/or development of the agent in the vector (e.g. *Babesia* spp. in ticks).

- b. vehicles, which are inanimate substances (e.g. food, milk, dust, fomites such as blankets or instruments, biologics such as serum, blood or plasma) by which or upon which infectious agents pass from infected to susceptible animals. Vehicles, therefore, act as important sources of infection, and examples include water for leptospires and contaminated needles for bovine leukemia virus.

- c. droplet nuclei, which are solid residues of evaporated droplets that are formed when expiratory droplets from infected hosts evaporate quickly in unsaturated atmospheres; these small particles remain suspended in the air as a cloud of droplet nuclei and they can travel considerable distance in wind (e.g. foot-and-mouth disease, newcastle disease).

Carrier Status

Hosts that harbor potentially transmissible inapparent infections are called carriers. Three types of carrier animals are recognized:

- a. incubatory carriers, which are animals capable of transmitting infectious agents before clinical disease becomes manifest (e.g. rabies virus in saliva of dogs and cats);
- b. convalescent carriers, which are animals capable of transmitting infectious agents after clinical recovery has taken place (e.g. *Leptospira canicola* in urine of dogs);
- c. healthy carriers, which are animals capable of transmitting infectious agents throughout the entire duration of infection (e.g. bovine leukemia virus infection).

Reservoirs of Infection

Reservoirs of infection are either animals or inanimate materials in which the infectious agent multiplies and develops and upon which the agent is dependent for survival in nature. Reservoirs may be vertebrate animals (e.g. mongooses for rabies virus), invertebrates (e.g. ticks for Rocky Mountain spotted fever rickettsiae), or inanimate materials (e.g. soil for *Histoplasma capsulatum*, the fungus causing histoplasmosis). Reservoirs are essential for the maintenance of infections during times when active transmission is not occurring.

Amplifier Hosts

Amplifier hosts are animals in which infectious agents multiply rapidly to high levels. For example, Venezuelan equine encephalitis (VEE) virus rapidly produces a high-titer viremia in horses, and a single viremic horse can be the source of infection for literally thousands of mosquitoes in a single night.

Horses, therefore, are the most important vertebrate host in the amplification and spread of virus during an epidemic of VEE. In contrast, the horse is a dead-end host for both the Eastern and Western equine encephalitis viruses since the levels of viremia are too low to regularly infect mosquitoes.

Temporal Patterns of Disease Occurrence

There are three commonly recognized patterns of disease occurrence in populations:

1. Sporadic: disease occurs rarely and without regularity in a population.
2. Endemic (enzootic): disease occurs with predictable regularity in a population with only relatively minor fluctuations in its frequency pattern over time; a disease may be endemic at any level of occurrence.
3. Epidemic (epizootic): disease occurs with a frequency clearly in excess of the expected frequency in that population during a given time interval. The epidemic occurrence of disease is purely a relative term for unexpectedly high frequencies of disease occurrence, and in no way does it have anything to do with absolute numbers of cases. For example, one case of African Swine Fever in Jamaica would represent an epidemic because the expected frequency of occurrence of that disease in that country is zero. A pandemic is a very large-scale epidemic, usually involving several countries or even continents.

Sporadic, endemic and epidemic patterns of disease are statements of the frequency of disease events against time. Their graphical representation in the form of a frequency histogram or polygon is known as an epidemic curve (See Fig. 1). Epidemic curves are very useful means of visualizing the dynamics of particular disease events in a population. One particular type of epidemic curve is known as a point epidemic where a pronounced clustering of cases in time occurs due to exposure of animals to a common source of infection, such as food or water (See Fig. 2).

Measures of Disease Frequency

There are two common measures of morbidity, prevalence and incidence:

1. Prevalence: Prevalence is a static measure of the total number of affected individuals in a population at a particular moment (point prevalence) or during a period of time (period prevalence). It is a measure of the amount of disease present. Prevalence, expressed as a proportion (usually as a percentage), is defined as follows:

$$\text{Prevalence} = \frac{\text{total number of cases of a disease existing in a population at a point in time (or during a given period of time)}}{\text{total number of animals in that population at the same point in time (or at middle of time period)}}$$

2. Incidence: Incidence is a dynamic measure of new cases occurring in a population within any defined interval of time. It is a measure of the risk of acquiring the disease. Incidence, expressed either as a proportion or as a rate, is defined as follows:

$$\text{Incidence} = \frac{\text{number of new cases of a disease occurring in a population during a given period of time}}{\text{average number of animals alive in that population during same time period}}$$

FIGURE 1
EPIDEMIC CURVES

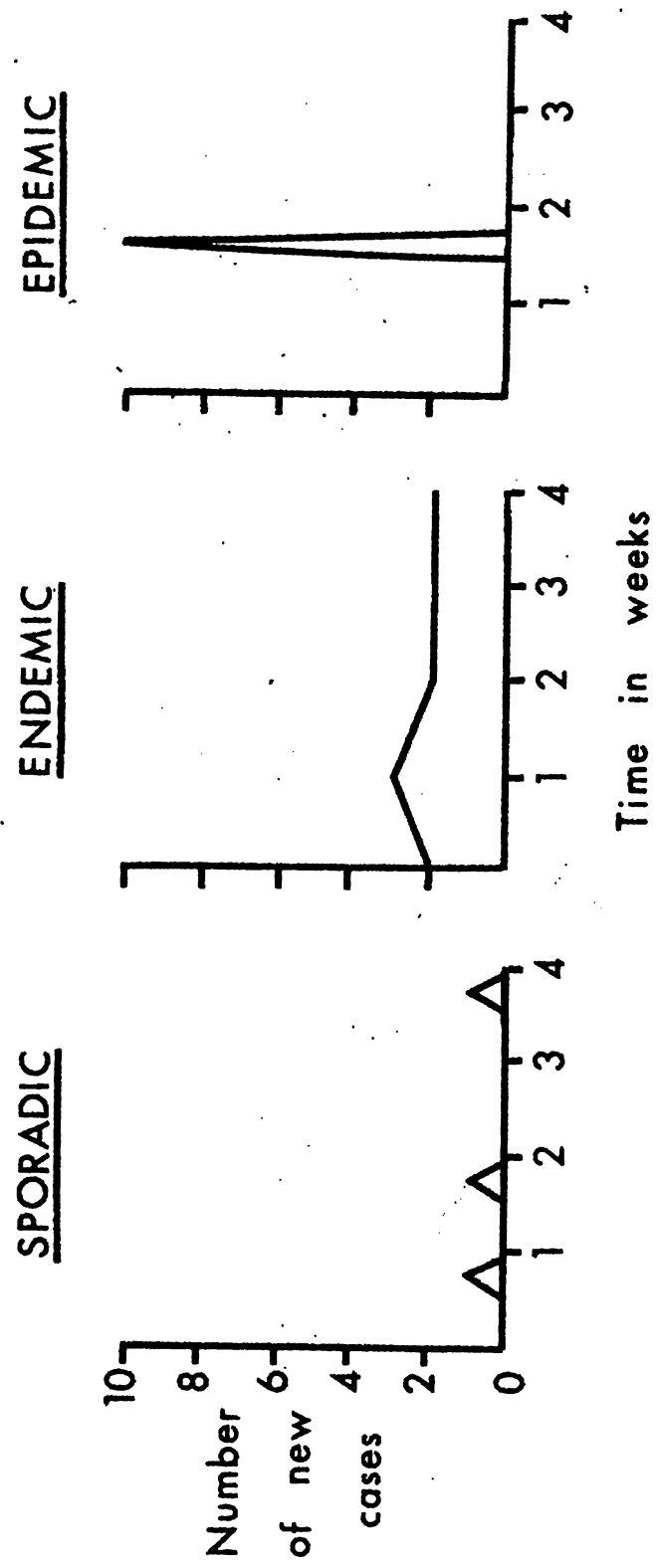
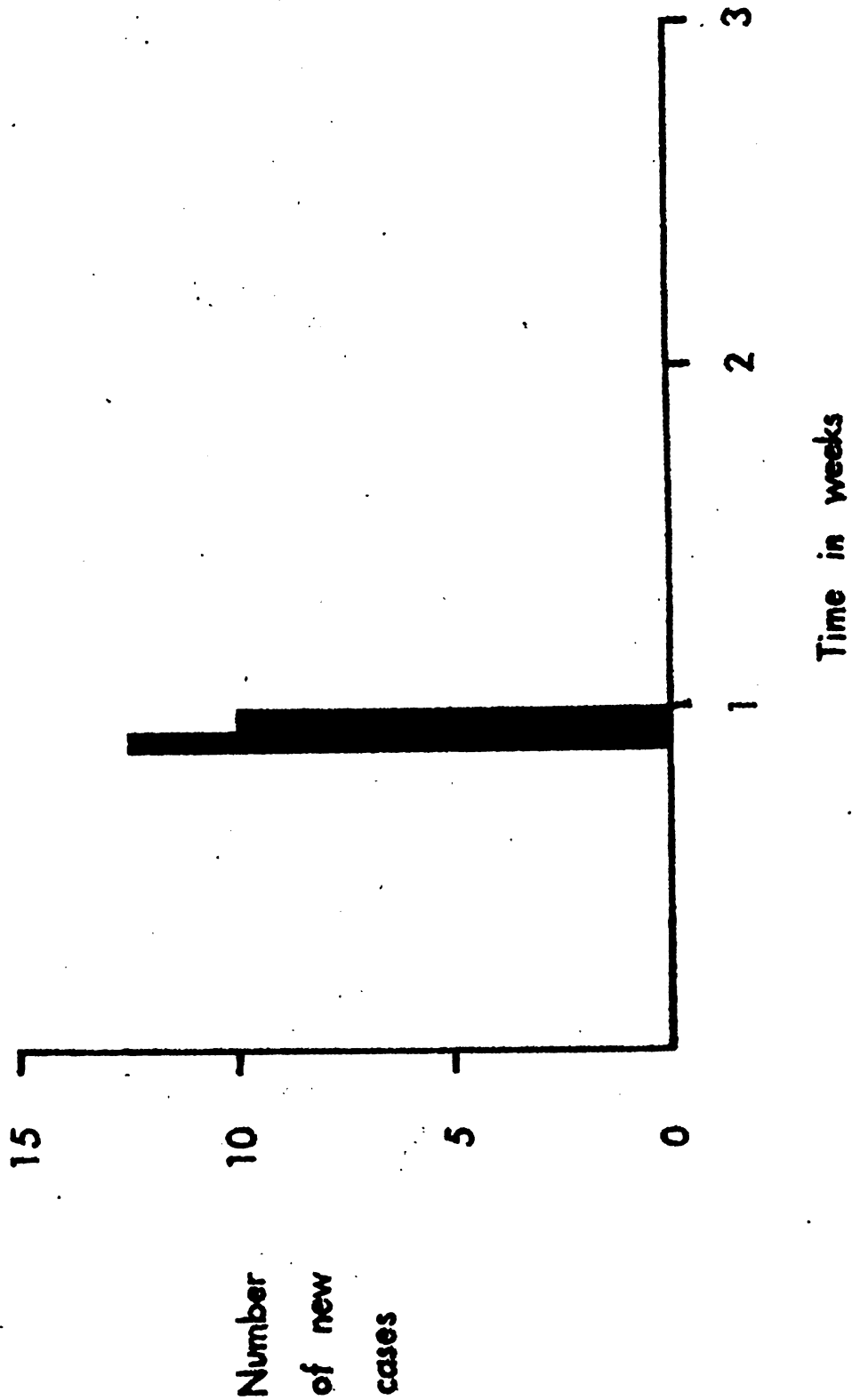


FIGURE 2
POINT EPIDEMIC



EPIDEMIOLOGICAL METHODS

by
Dr. M.J. Burridge

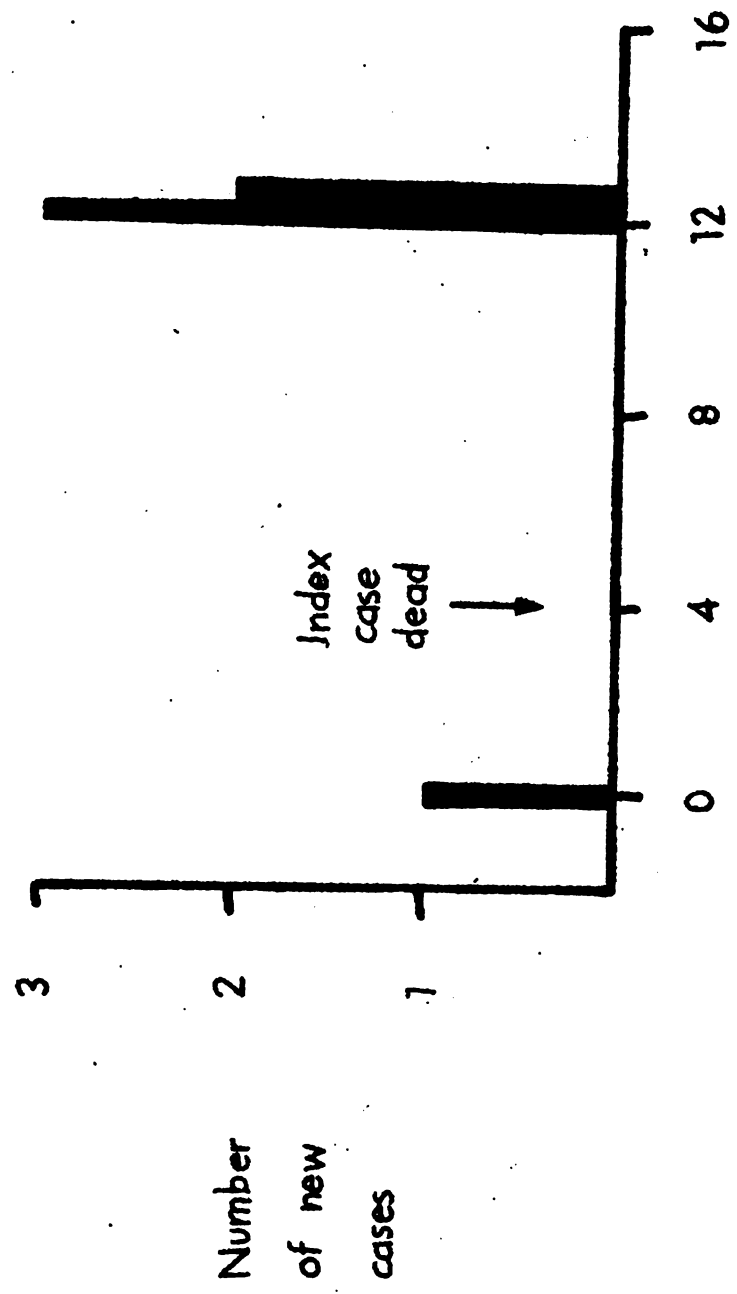
INVESTIGATION OF DISEASE OUTBREAKS

Disease outbreaks are investigated by asking the following types of questions:

1. What is the incidence of the disease? Clinical and pathological observations may suggest possible etiologic agents and will give guidance to specimen collection for laboratory testing.
2. Which animals are involved? Description of the characteristics of both affected and unaffected animals may suggest possible host determinants of the disease.
3. Where is the disease occurring? Description of the distribution of cases on maps may suggest possible environmental determinants of the disease and also may provide information on the cause, source, and method of spread of the disease. For example, if the distribution of the disease matches the distribution of some factor (e.g. a poisonous plant, water source, or disease vector), it indicates that the factor and the disease may be related.
4. What are the temporal relationships between the cases? Description of the temporal sequence of events in the form of epidemic curves may indicate the incubation period of the disease (see Fig. 3), and may suggest the source and method of spread of the disease. If the epidemic curve shows a point epidemic (see Fig. 4) with a pronounced clustering of cases in time, it indicates a common source of infection such as food or water. Since the shape of epidemic curves are influenced by the mode of transmission of infectious agents, examination of epidemic curves will also provide information on the method of spread of the disease:
 - a) via vehicles: those infections acquired through ingestion of water or food exhibit an explosive pattern (point epidemic), reflecting simultaneous exposure to a common source of infection, whereas the pattern of spread is usually sporadic when dust or fomites are the vehicle (see Fig. 5);

- b) via airborne droplets or droplet nuclei: spread usually is rapid with well-defined outbreaks if a sufficient population of susceptible animals is available (see Fig. 6);
 - c) via vector: commonly a gradual build-up of cases (see Fig. 7);
 - d) via physical contact: pattern of spread is sporadic except when there is a common source of infection (see Fig. 8).
5. How and why has the disease problem occurred? Identification of special or unusual events may suggest possible sources of infection or environmental determinants of the disease. Examples of such events would include the introduction of new animals onto the premises, visits by persons from other infected premises, and periods of unusually heavy rainfall.
6. What progress is being made? Calculation of incidence rates during the course of the disease will indicate the efficacy of the therapeutic and control measures taken, and may suggest possible etiologic agents. Increasing incidence indicates a deteriorating disease situation in a population, while decreasing incidence reflects an improvement.

FIGURE 3
DISEASE OUTBREAK IN DAIRY HERD



Days since index case became sick

$$\begin{aligned} \text{Incubation period} &= (12 - 4) \text{ to } (13 - 0) \text{ days} \\ &= 8 \text{ to } 13 \text{ days} \end{aligned}$$

FIGURE 4
POINT EPIDEMIC

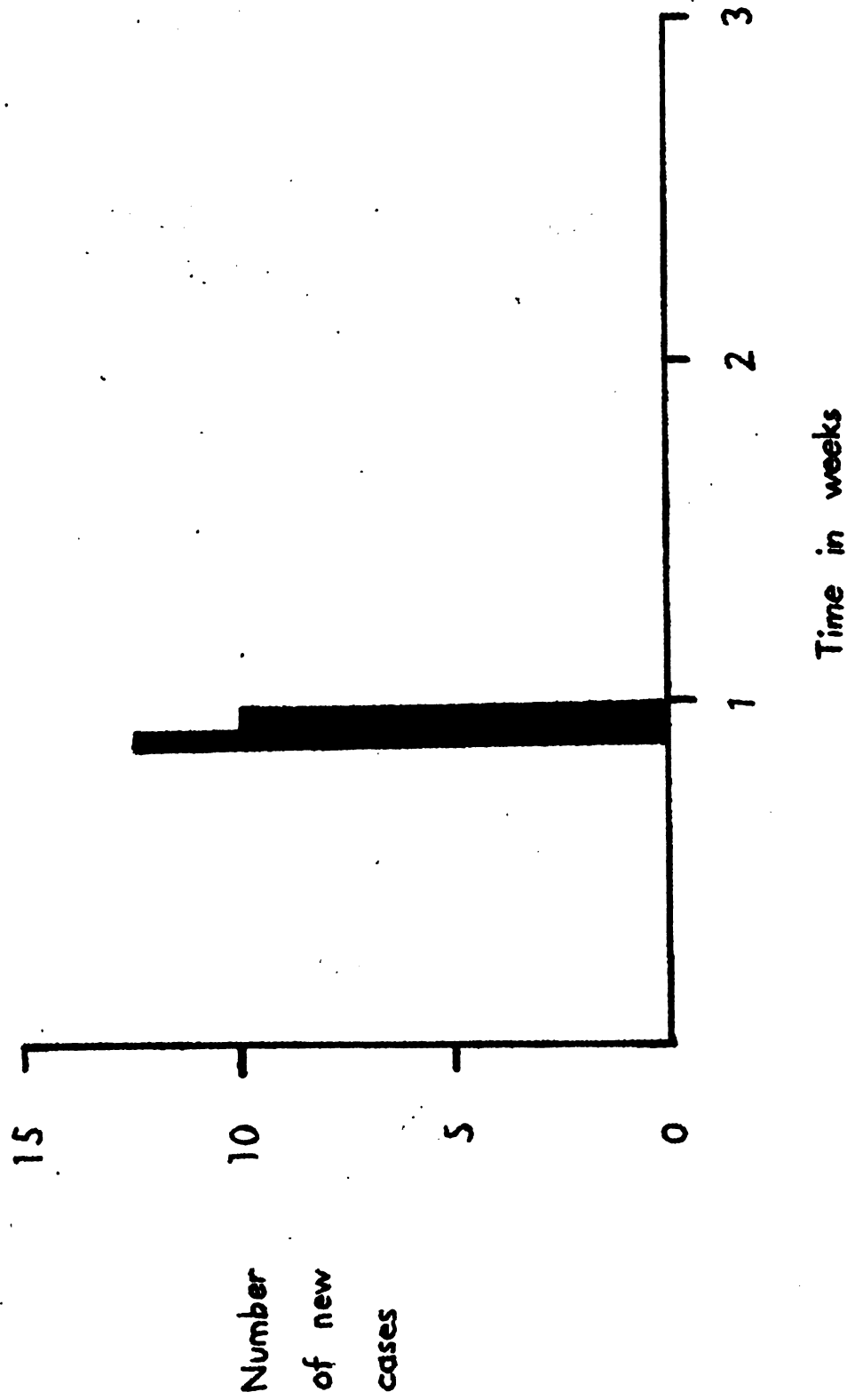


FIGURE 5
VEHICLE TRANSMISSION

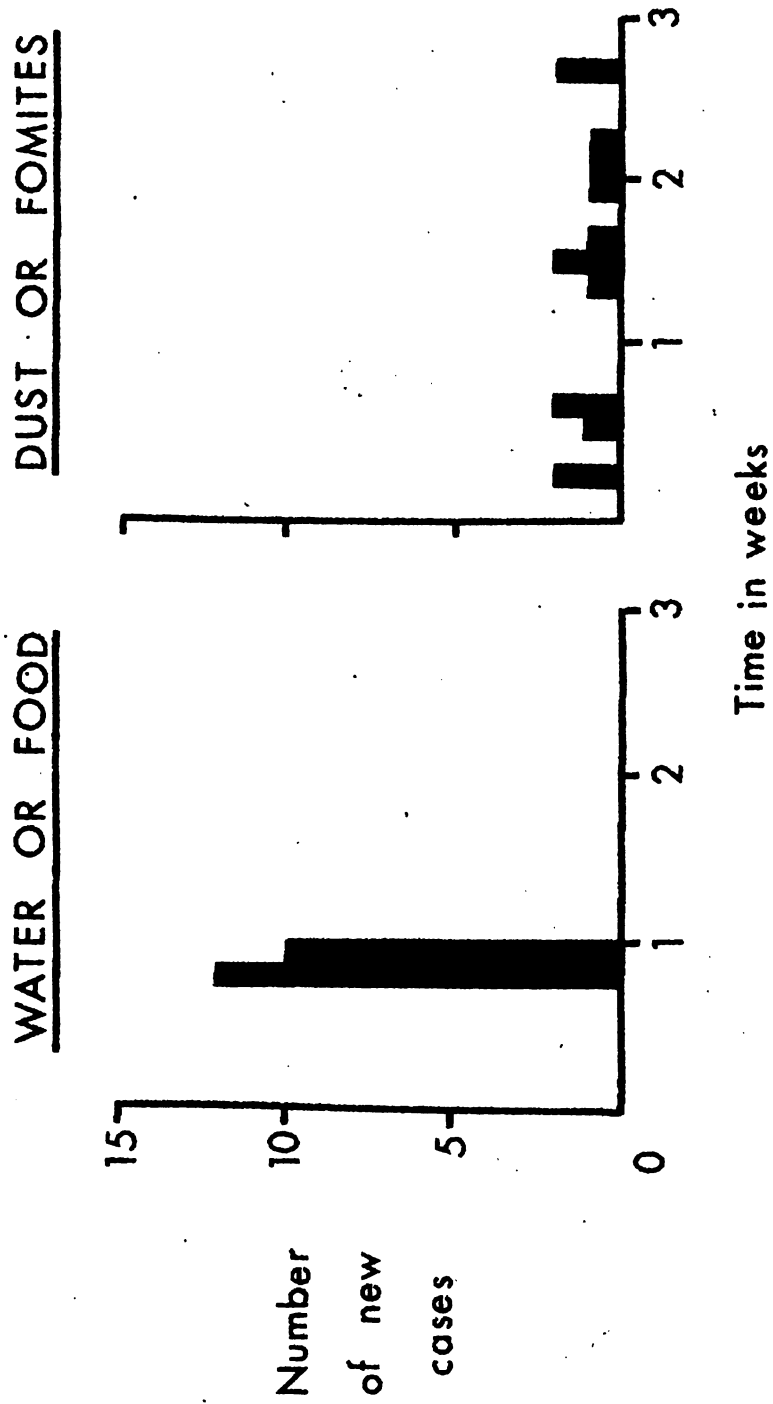


FIGURE 6
AIRBORNE TRANSMISSION

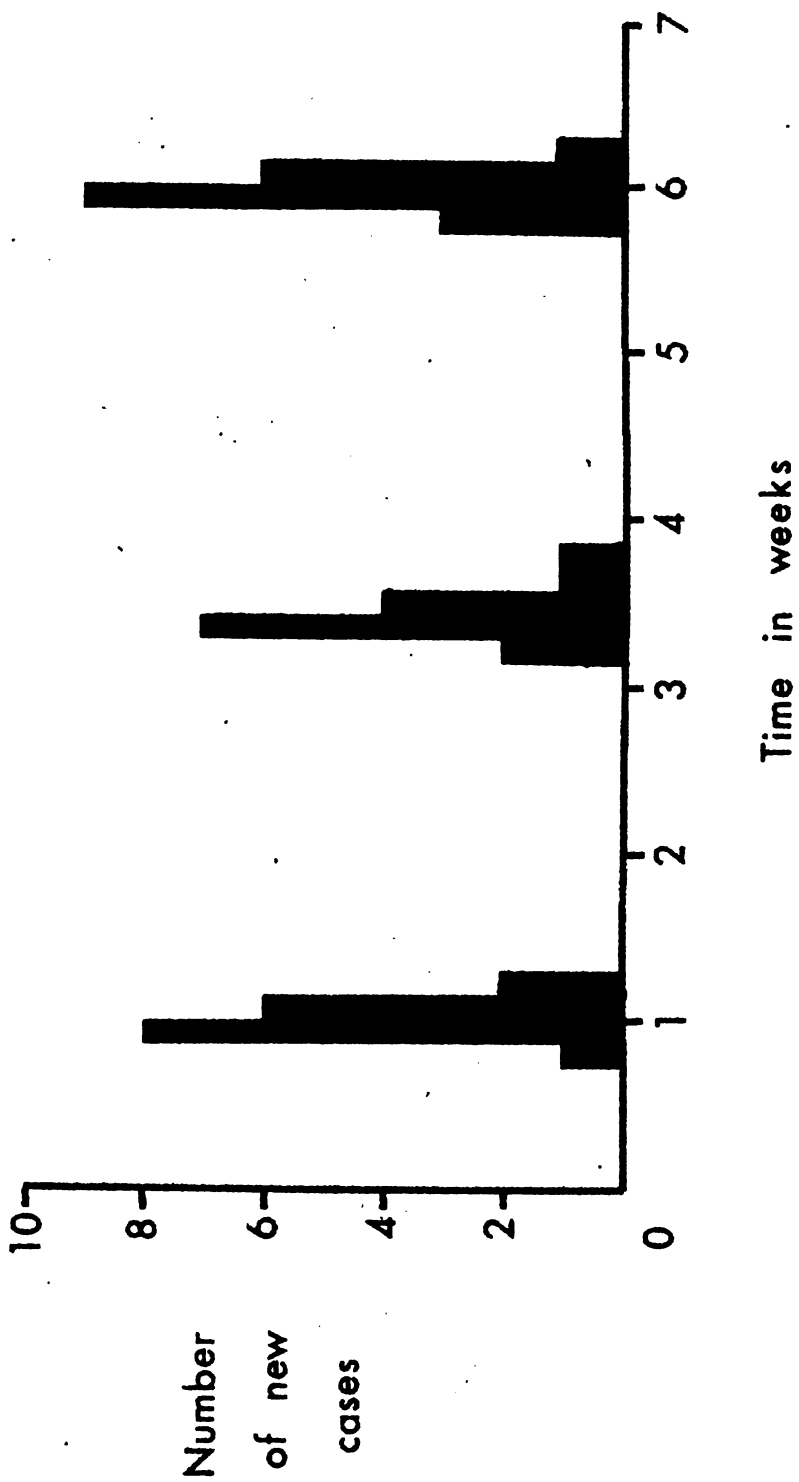


FIGURE 7
VECTOR TRANSMISSION

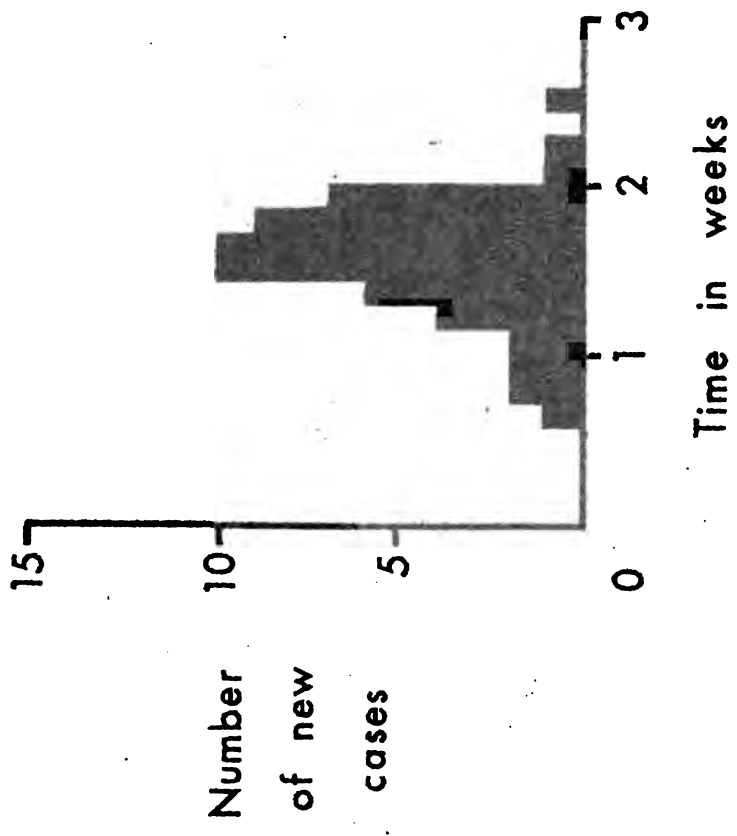
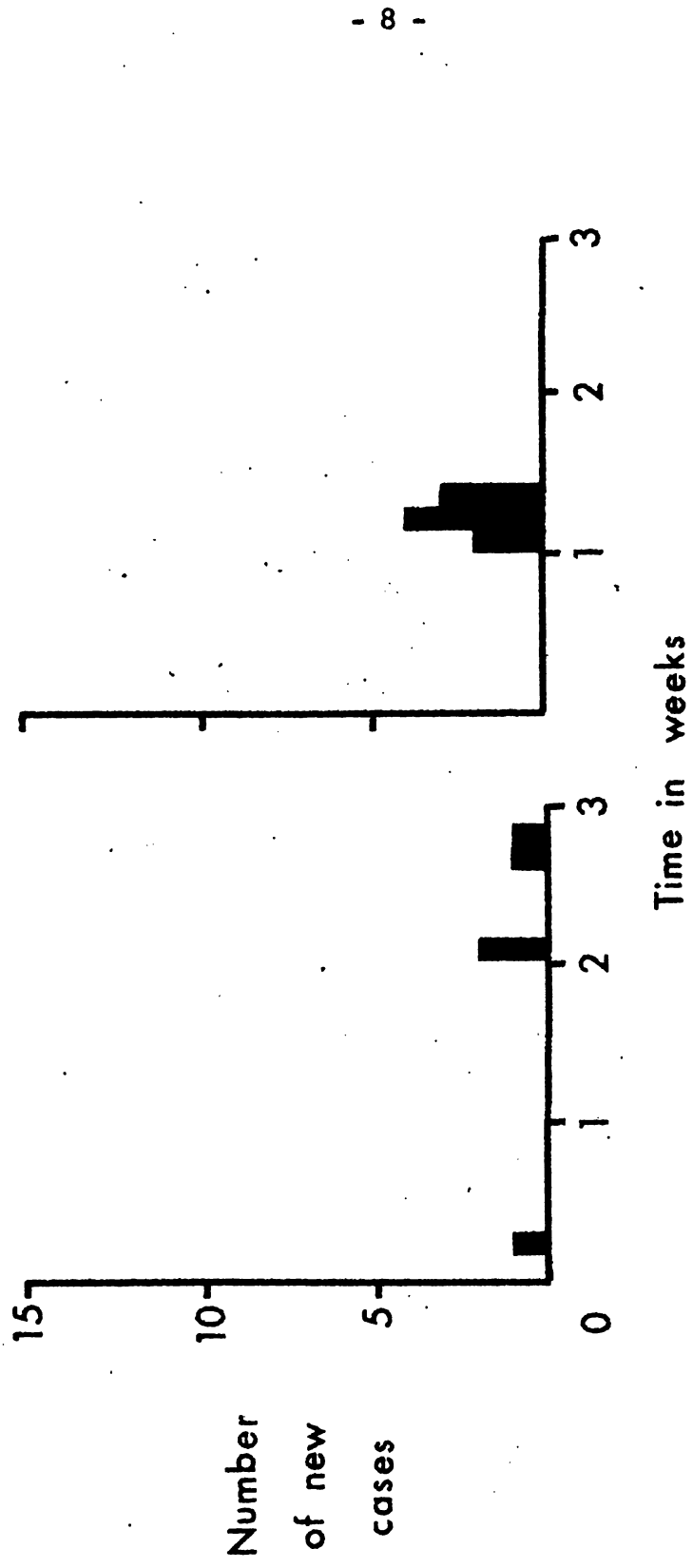


FIGURE 8

TRANSMISSION BY PHYSICAL CONTACT

TYPICAL COMMON SOURCE



SEROEPIDEMIOLOGY

Sensitivity and Specificity of Serological Tests

Consider the following population of N animals categorized by their true infection status and by their reactions to a serological test (positive or negative).

		Infection status		
		+	-	
Test Result	+	a	b	a+b
	-	c	d	c+d
		a+c	b+d	N

- a = infected animals detected by the test (true positives)
- b = uninfected animals positive to the test (false positives)
- c = infected animals not detected by the test (false negatives)
- d = uninfected animals negative to the test (true negatives)

1. Sensitivity of a serological test is the ability of the test to give positive results when animals tested truly have been infected with the disease agent under study. Sensitivity measures the proportion of infected animals which are test positive and it is estimated by:

$$\text{sensitivity} = \frac{a}{a+c} \times 100$$

Lack of sensitivity leads to false negative results.

2. Specificity of a serological test is the ability of the test to give negative results when animals tested are free of the infection under study. Specificity measures the proportion of uninfected animals which are test negative and it is estimated by:

$$\text{specificity} = \frac{d}{b+d} \times 100$$

Lack of specificity leads to false positive results.

Relationship between Sensitivity and Specificity

For almost all serological tests, sensitivity and specificity are inversely related. Hence, if the screening level (i.e. the lowest titer considered positive) is altered to increase sensitivity, then the specificity of that test

decreases. This is a consequence of an overlapping distribution of serum titers between animals with the specified infection and those without that infection.

Determination of Sensitivity and Specificity

The first prerequisites are clearly defined groups of infected and uninfected animals. The infection status should be determined by non-serological methods, such as microbial culture, which approach 100% diagnostic efficiency. In other words, the methods to define infection status should be biologically independent of the serological test that is being evaluated. Sensitivity is determined by calculating the proportion of animals positive on, say, microbial culture which react to the serological test, and specificity is determined by calculating the proportion of animals negative on culture which do not react to the test. When determining specificity, consideration must be given to other infections, especially those caused by closely-related organisms, that might cross-react serologically.

Often, in an attempt to establish sensitivity and specificity, the results of one serological test are compared to those of another serological test. This procedure does not establish sensitivity or specificity, but only relative sensitivity and relative specificity. Such comparisons should be made only when the sensitivity and specificity of a standard test are known and when they approach 100%. Otherwise, comparisons may lead to false conclusions and may delay the identification of tests that are superior to the standard test.

Use of Serological Tests in Epidemiology

Serology provides a convenient and relatively inexpensive method to estimate and monitor disease morbidity. Serology has found wide application in epidemiology, for example, in the estimation of disease prevalence by region or by animal species, in disease surveillance, and in disease control and eradication programmes.

1. Use in prevalence surveys

In serological surveys designed to estimate disease prevalence, it is important to know how closely apparent prevalence (i.e. prevalence as measured by the serological test) approaches true prevalence. Using the symbols in the 2x2 table already presented,

$$\text{Apparent prevalence} = \frac{a + b}{N} \times 100$$

$$\text{True prevalence} = \frac{a + c}{N} \times 100$$

Consideration of three levels of true prevalence (50%, 10% and 1%) will show how markedly sensitivity and specificity of a test can affect the apparent prevalence shown by the test results:

Apparent prevalence when true
prevalence is:

	<u>Sensitivity</u>	<u>Specificity</u>	<u>50%</u>	<u>10%</u>	<u>1%</u>
Tests of low specificity	50%	50%	50%	50%	50%
	75%	50%	63%	53%	50%
	95%	50%	73%	55%	50%
	100%	50%	75%	55%	51%
Tests of low sensitivity	50%	50%	50%	50%	50%
	50%	75%	38%	28%	25%
	50%	95%	28%	10%	5%
	50%	100%	25%	5%	1%
Tests of varying sensitivity and specificity	50%	50%	50%	50%	50%
	75%	75%	50%	30%	26%
	95%	95%	50%	14%	6%
	100%	100%	50%	10%	1%

Tests of low specificity markedly overestimate prevalence, especially when the infection is relatively rare. Tests of low sensitivity underestimate prevalence of common infections, particularly as specificity increases, whereas these tests overestimate prevalence of rare infections except when specificity is high. These data demonstrate the importance of having some idea of the probable prevalence of the infection under study before selecting a serological test for a prevalence survey. Optimally, a test of high sensitivity and high specificity would be selected for prevalence surveys. When one is not available, a test of comparable sensitivity and specificity would be chosen for an infection of high prevalence, and a test of high specificity for an infection of

low prevalence. Ideally, screening tests should have the following qualities:

- a. be of high sensitivity;
- b. be of high specificity;
- c. be quick to perform;
- d. be easy to perform;
- e. be inexpensive.

2. Use in disease control and eradication programmes

When utilizing serology to identify infected animals in control and eradication programmes, it is important to know both the sensitivity of the test and the predictive value of a positive test result. The predictive value is the proportion of test positive animals which are infected and, using the symbols in the 2x2 table already presented, it is calculated by:

$$\text{Predictive value} = \frac{a}{a + b} \times 100$$

When animals are condemned to slaughter on the basis of serology, as in the U.S. brucellosis eradication programme, it is essential that a high proportion of infected animals are detected by the test (i.e. test is of high sensitivity) and that the vast majority of seropositive animals are infected (i.e. test has high predictive value). Consideration of the same three levels of prevalence will demonstrate the effect of true prevalence and of sensitivity and specificity of the test on predictive value:

	<u>Predictive value when true prevalence is:</u>				
	<u>Sensitivity</u>	<u>Specificity</u>	<u>50%</u>	<u>10%</u>	<u>1%</u>
Tests of low specificity	50%	50%	50%	10%	1%
	75%	50%	60%	14%	1%
	95%	50%	66%	17%	2%
	100%	50%	68%	18%	2%
Tests of low sensitivity	50%	50%	50%	10%	1%
	50%	75%	67%	18%	2%
	50%	95%	91%	53%	9%
	50%	100%	100%	100%	100%

	<u>Predictive value when true prevalence is:</u>				
	<u>Sensitivity</u>	<u>Specificity</u>	<u>50%</u>	<u>10%</u>	<u>1%</u>
Tests of varying sensitivity and specificity	50%	50%	50%	10%	1%
	75%	75%	75%	25%	3%
	95%	95%	95%	68%	16%
	100%	100%	100%	100%	100%

It is evident that a test of high sensitivity and high specificity (i.e. both approaching 100%) is required for use in disease control and eradication programmes, especially with infections of low prevalence or during the latter stages of eradication programmes when prevalence has been reduced to a low level.

The above table shows that as a disease becomes rarer, such as during an eradication programme, the predictive value of a positive test result for a given test is dramatically reduced, causing the number of uninfected animals that are condemned to rise. This is almost certain to cause dissatisfaction in agricultural and economic circles. In such situations, a second sensitive but more specific test should be introduced into the programme to retest all positive sera, with only animals reacting to both tests being condemned. An example of this approach would be the use of a modification of the card test to screen all cattle for brucellosis, with only reacting sera retested using the Rivanol and/or complement fixation (CF) tests. In this example, the Rivanol and CF tests are too expensive and complex to use for routine screening but they are valuable confirmatory tests for the serodiagnosis of bovine brucellosis.

PREVALENCE SURVEY DESIGN

Let us consider as an example a survey to estimate the prevalence of bluetongue virus infection in the Caribbean. There are two prerequisites to the design of such a survey. They are: (1) an understanding of the epidemiology of bluetongue; and (2) a knowledge of the herd and flock structures for susceptible species (i.e. cattle, sheep and goats for bluetongue) by Caribbean island or country.

From our knowledge of the epidemiology of bluetongue, only two host factors appear to be important determinants of infection; they are species and

age. In addition, consideration must be given to ecological factors that determine the distribution and frequency of occurrence of the different Culicoides spp. vectors of bluetongue virus. Consequently, in any prevalence survey, three factors must be considered in its design: species of host, age of host, and ecological zones influencing Culicoides distribution.

If possible, the sampling unit by region (island or country) should be all animals in the herd or flock that are one year of age or older. Younger animals less than one year old should be excluded because available serological tests cannot differentiate between colostral antibodies and antibodies indicative of infection. The herds should be selected for study using stratified random sampling procedures. The strata that must be considered include species of host, age of host, and ecological zones. This type of herd-sampling method will help to provide prevalence data that are representative of all susceptible livestock in each region. The addition of other strata to the survey design will be dependent upon examination of data on herd and flock structures by region.

In summary, each region should be divided into ecological zones influencing Culicoides distribution. Within each zone, cattle, sheep and goat herds will be chosen for study, ensuring that both age-groupings (1-5 years and 5 years for cattle, and 1-3 years and 3 years for sheep and goats) that influence bluetongue-virus prevalence are represented in each herd. All animals in each herd will be bled for serological testing for antibodies to bluetongue virus. The number of herds and of animals that need to be studied will be dependent upon knowledge of the herd and flock structures by region.

One final point of importance is the selection of a serological test for the prevalence survey. Optimally, a test of high sensitivity and high specificity which is inexpensive and easy and quick to perform should be chosen. Please refer to the previous section on seroepidemiology for further discussion on this subject.

DISEASE SURVEILLANCE

by
Dr. M.J. Burridge

Disease surveillance is an active process designed to continuously monitor and report the occurrence and distribution of diseases and infections in animal populations. In other words, disease surveillance is organized epidemiological intelligence on a large scale. Surveillance provides a relative appreciation of dynamic disease processes in animal populations and, therefore, enables those agencies responsible for disease control to apply appropriate control measures effectively and economically.

Surveillance is a continuous and systematic process consisting of four major components:

1. collection of data;
2. collation of data into meaningful arrangements;
3. analysis and interpretation of data;
4. prompt dissemination of disease information to those involved with animal disease control and to other interested parties.

Potential Data Sources

- a) Abattoir records: Condemnation data can provide useful surveillance data. Blood and other tissue specimens are readily obtainable from animals passing through abattoirs, providing a valuable opportunity to monitor the prevalence of many livestock diseases or infections on a regional basis. Also, with an efficient traceback system, potential or sub-clinical disease problems can be identified for a given herd. However, abattoir data are biased since they typically consider primarily the older animals in any given population.
- b) Diagnostic laboratory records: It is often difficult to relate these data to the actual population at risk.
- c) Seroepidemiological programmes: Periodic surveys of entire animal populations have formed the backbone of some disease control and eradication programmes. During these programmes, large numbers of serum samples are collected at great effort and cost, and are then discarded following a single specific use (e.g. serological testing for bovine brucellosis).

These samples provide invaluable material for the establishment of a serum bank that could be utilized for the continuous surveillance of many other animal diseases, as well as biological monitoring of the environment for pesticides and other pollutants. Furthermore, serum banks provide excellent reference material for retrospective investigation of the distribution of newly identified infections.

- d) Morbidity reports: Mandatory reporting of some important diseases (e.g. rabies in some countries) can provide valuable surveillance data.
- e) Herd records: They are often collected primarily or totally for production purposes (e.g. milk and egg production records). However, production records are potentially sensitive indicators of clinical or sub-clinical disease. Improved herd records which include disease data form a valuable basis for the surveillance of livestock diseases within individual herds.
- f) Veterinary hospital records: It is often difficult to relate these data to the actual population at risk. Also, the referral bias of the hospital must be taken into consideration.
- g) Veterinary practice records: They can provide some useful data on conditions of high prevalence that are not seen by diagnostic laboratories (e.g. hypocalcemia).

Collation of Data

Raw surveillance data must be reduced and presented in some usable form, commonly by the preparation of tables. The tabulated data can then be graphed or charted for easier identification of disease or infection trends. Such collation is achieved most efficiently by use of computer technology.

Analysis of Data

Surveillance data should be converted to the appropriate rates, ratios or proportions whenever possible before analysis. The resulting figures will provide a more accurate and meaningful measure of the occurrence of disease or infection since they take into account the actual or approximate size of the populations at risk. However, in a few instances, numbers of cases alone may be

adequate, such as when a disease is detected for the first time in an area or in an animal species. Again, computer technology is invaluable for analyzing voluminous surveillance data.

Dissemination of Information

Essential to the success of surveillance is the prompt dissemination of the collated and analyzed data, together with appropriately interpreted evaluations of current problems. This need for prompt dissemination of information can be satisfied only by a general disease surveillance document issued frequently and regularly, preferably on a weekly or biweekly or, at the very least, a monthly basis. Such a document should contain:

- a) current, cumulative and comparative tabular data of reported occurrences of a variety of diseases subject to continuous surveillance;
- b) periodic, more detailed data on selected diseases;
- c) on-going reports of progress in specific control efforts;
- d) alerts warning of existing or potential disease problems;
- e) notices of relevant changes in disease legislation;
- f) informative synopses of specific follow-up investigations of cases or outbreaks;
- g) predictions of future disease patterns or events.

Uses of Surveillance Data

Some of the more important uses of surveillance data in veterinary medicine include:

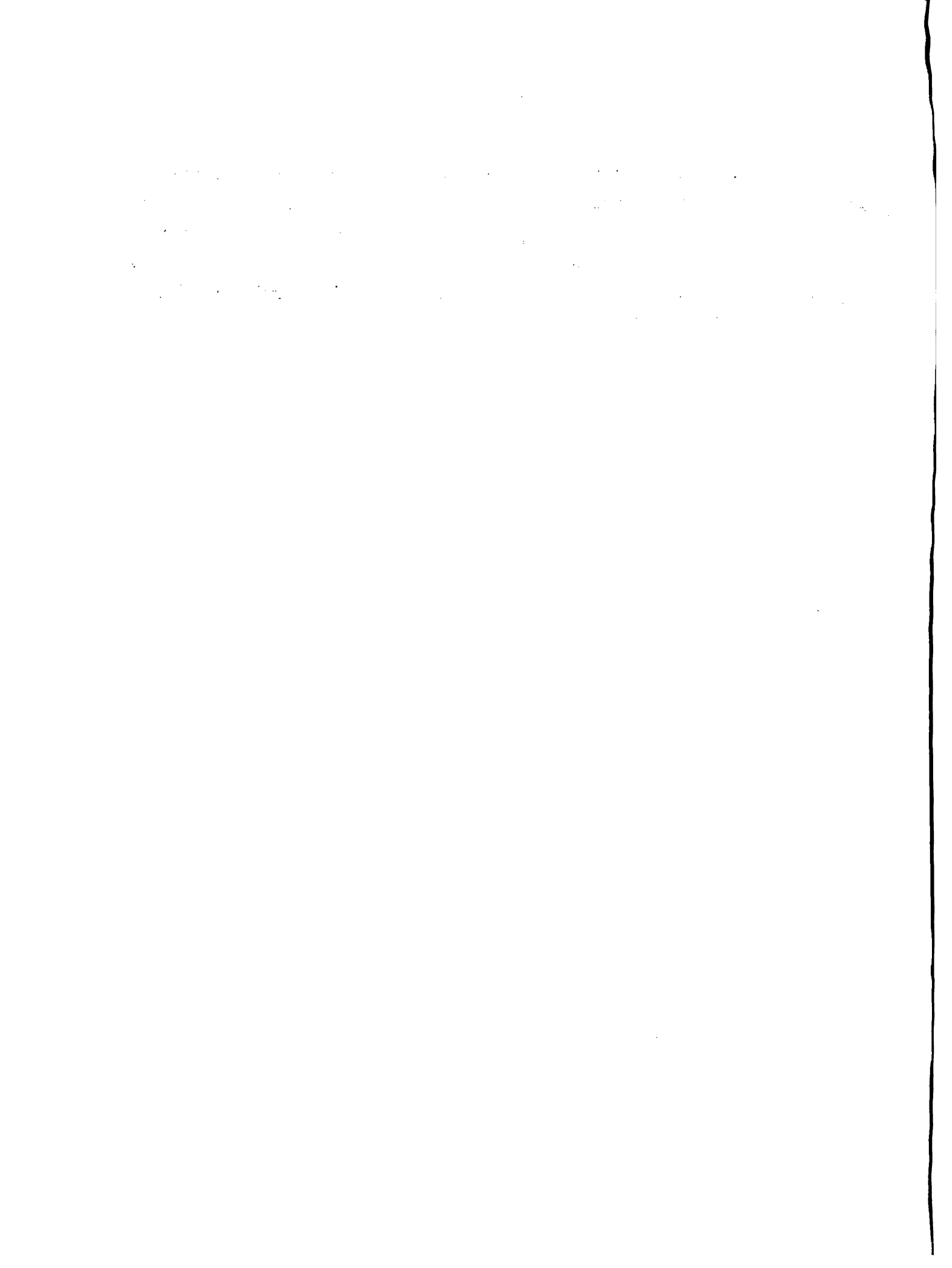
1. determination of endemic levels of disease;
2. characterization of disease behaviour;
3. early detection of epidemics and other changing patterns of disease;
4. prediction of future disease incidence;
5. establishment of priorities in animal health programmes;
6. evaluation of preventive medicine programmes;
7. monitoring of disease control and eradication programmes.

Development of a Disease Surveillance System for the Caribbean

It is important to develop a system that will rapidly identify the disease problems constraining improvements in livestock production in the Caribbean and that will determine practical strategies for their cost-effective control. Such a system would evolve in four stages:

1. The prevalence of diseases and infections would be determined by serological and parasitological surveys and by examination of animals passing through abattoirs. The serological surveys would utilize sera collected from scientific samples of each animal species. The sampling procedure chosen must ensure that the samples were representative of each animal population in the Caribbean nation or island with respect to variables such as geographic location, breed, production type, sex, and age. Parasitological surveys for ectoparasites (ticks, mites, lice, keds, etc.) and for endoparasites by fecal and blood examination would use the same animals as selected for the serological surveys. Samples of animals passing through abattoirs would be examined for parasites and for gross pathological lesions. Serum samples collected for the serological surveys would be stored to form a Caribbean serum bank.
2. Each disease or infection of high or moderate prevalence would be examined to determine its economic impact on animal productivity.
3. The prevalent diseases and infections which either had significant economic effects on animal productivity or posed threats to public health, would be divided into two groups: those with and those without established methods for their control. Control programmes would be instituted immediately for the former group of diseases and infections. For the latter group, research programmes would be established to develop cost-effective methods for the control of each disease or infection on a priority basis.
4. A system would be developed to regularly monitor changes in prevalence and incidence of those diseases and infections of economic importance to the animal industries and of public health significance. Such a system would be computerized so that data could be rapidly collated, analyzed, retrieved, and disseminated.

The major long-term benefit from such a surveillance system would be a marked increase in animal protein available for human consumption by the peoples of the Caribbean through development of a rational approach to animal disease control. An additional benefit should be an increase in international commerce in animals and animal products through improved information on the distribution and incidence of diseases and infections.



TICKS - DISTRIBUTION AND ECOLOGY BY SPECIES
OF IMPORTANCE IN THE CARIBBEAN

by
Glen I. Garris

Drummond (1980) estimates that there are 11 300 000 cattle in the Caribbean. Although this figure represents a large potential source of animal protein for human consumption, most Caribbean countries import over half of their red meat needs. There are many factors that affect the production of red meat; primary among these factors are ticks and the diseases they transmit.

Lombardo (1975) estimates that approximately 70% of the 175 million head of cattle in Central and South America and the Caribbean Area are moderately to heavily infested with ticks.

There are probably two genera of ticks that are primarily responsible for the limitations on the growth of the livestock industry in Latin America. Boophilus microplus (Canestrini) is the most important tick and probably accounts for the majority of the infestations.

Species in the genera Amblyomma comprise the second most important group of ticks that infest cattle in Latin America.

It is the purpose of this discussion to present the distribution and life history of those ticks that are of importance to the livestock industry in the Caribbean Area. Also included are some species of ticks that are not known to be parasitic on livestock except on incidental basis, but presence and wide distribution and host range warrant mention.

Tick Identification

Before the distribution of a tick species can be mapped, it is very important that accurate identification be made. For the purpose of this meeting, I will not discuss the techniques used to separate individual species of ticks. However, it is useful to understand that ticks are separated into 2 distinct families, the hard ticks (Ixodidae) and the soft ticks (Argasidae).

There are 2 features that are important in distinguishing between these two families. One is the presence of a scutum, a hard usually ornate covering that extends over the entire abdomen of the male and over about half of the

abdomen of the female. The second feature is the protrusion of the mouth parts from the front end of the body. This is clearly visible from above. In the soft ticks these two distinct features are different in that there is no scutum present and the mouth parts are usually not visible from above.

It is important to separate these two families of ticks because the hard ticks present a greater threat to the livestock industry in the Caribbean than do the soft ticks. There are a few soft ticks that may be of economic value to the livestock industry. However, there is little information on their life history and distribution. A few of these will be mentioned later in this presentation.

There are several genera of hard ticks that are of economic importance to the livestock industry in the Caribbean Area as well as other parts of the world. I will restrict my comments to the following four genera: Amblyomma, Boophilus, Anocentor (= Dermacentor like), and Rhipicephalus. The general characteristics used to separate these genera are as follows:

1. Shape of the basis capitule
2. Length of the hypostome and corresponding palps
3. Presence or absence of eye spots
4. Length and shape of the second segment of the palps
5. Shape and ornamentation on the scutum of primarily the female

General Life Cycles

The life cycles of ticks can be separated into essentially two phases, the parasitic and non-parasitic phases. The non-parasitic phase is spent off the host and is affected by the micro-environment in the various habitats encountered.

There has been some attempt to characterize the various habitats that are associated with an individual species. However, these characterizations have not included details about micro-climate and its effect on tick biology or behavior.

The parasitic phase can be conveniently classified based on the number of hosts utilized as sources of food during the development from egg to adult.

Ticks are blood feeders and thus attack animals. The range of hosts can be quite wide as is apparent with Amblyomma variegatum (Fab.) This species of tick may feed on lizards, snakes, birds and all types of mammals including man. Although the range of hosts that a particular tick species can be found to feed on may be wide, the hard ticks are separated into groups. The first group, the 1-host tick, spends its entire parasitic phase on one host. For example, Boophilus microplus is a 1-host tick. A typical life cycle of a 1-host tick is presented in Fig. 1. The second group, the 2-host ticks, use one host for 2 life stages such as larvae and nymphs may feed on a lizard while the third life stage will seek a second host such as a cow or other mammal. There are no 2-host ticks of importance to livestock in the Caribbean; thus I have not included a schematic diagram of a general life cycle. The last groups, the 3-host ticks, utilize a different host for each developmental stage, Fig. 2.

It is important to keep in mind the general life cycles as presented above since this greatly influences the management procedures necessary to control these ticks. The 1-host tick, for example, is generally more host specific and spends a longer period of time on a single host than the 3-host ticks, thus a control programme can be directed toward treating the most preferred host. Where a 3-host tick is involved, the parasitic phase of the life cycle is usually of short duration on any one given host and the wide range of hosts complicates the control programme that could be directed toward it.

Ixodidae of the Caribbean Area

Table 1 shows the 6 species of ticks that are of economic importance to the livestock industry in the Caribbean. The known distribution and ecology of each species as listed in this table will be discussed on an individual basis below.

Amblyomma cajennense: Table 2 shows what information is known concerning the life cycle of this tick species. This tick species is found from Southern Texas to Argentina and it occurs in Tobago, Trinidad and Jamaica.

It is generally found on the animal host year-round and usually all 3 life stages can be found infesting the animals at the same time. When found on cattle, this species will generally be found attached to any portion of the

animal surface that it is able to penetrate with the mouth parts. It does not have a preferred feeding site on the animal. There is little known about the habitat preference of the free-living forms of this tick. This is an area that deserves considerable research effort in the future.

The survival of the free-living forms is considerable. The free-living forms may survive for well over 1 year, especially the flat adults. This makes them a very difficult species to control.

Amblyomma maculatum: Although A. maculatum is not currently present in the Caribbean, it is a species which has a tremendous potential of being introduced in this area. Its potential for disease transmission and destruction to the livestock industry is considerable.

Table 3 gives what is known about the life cycle of this species. It is found in an area covering the entire south-eastern coast including Oklahoma and parts of Kansas of the U.S.A. and Mexico, Colombia, and Venezuela. It is usually found in small numbers on cattle throughout the year. It prefers to attach and feed on or in the ears of cattle. There is little known about the survival or habitat preference of the free-living forms in Latin America. However, the free-living forms generally will survive for long periods of time off the host.

Amblyomma variegatum: The life cycle is shown in Table 4. It is found in Antigua, French Guiana, Guadeloupe, Martinique, St. Kitts, Suriname, Puerto Rico, and probably St. Lucia. It was eradicated from St. Croix in the U.S. Virgin Islands in 1970. The seasonal variation, habitat preference and survival off the host is not known. It will attach to almost any part of the animal.

Boophilus microplus: This is a 1-host tick. Its life cycle is represented in Table 6. It is widely distributed and there appears to be about 5-6 generations per year on cattle in Puerto Rico. This probably is true for all of the Caribbean area. It will attach to any part of the animal but in light infestations prefers the thin-skinned area such as the escutcheon and axillary areas of the animal. This species has a close relation to rainfall.

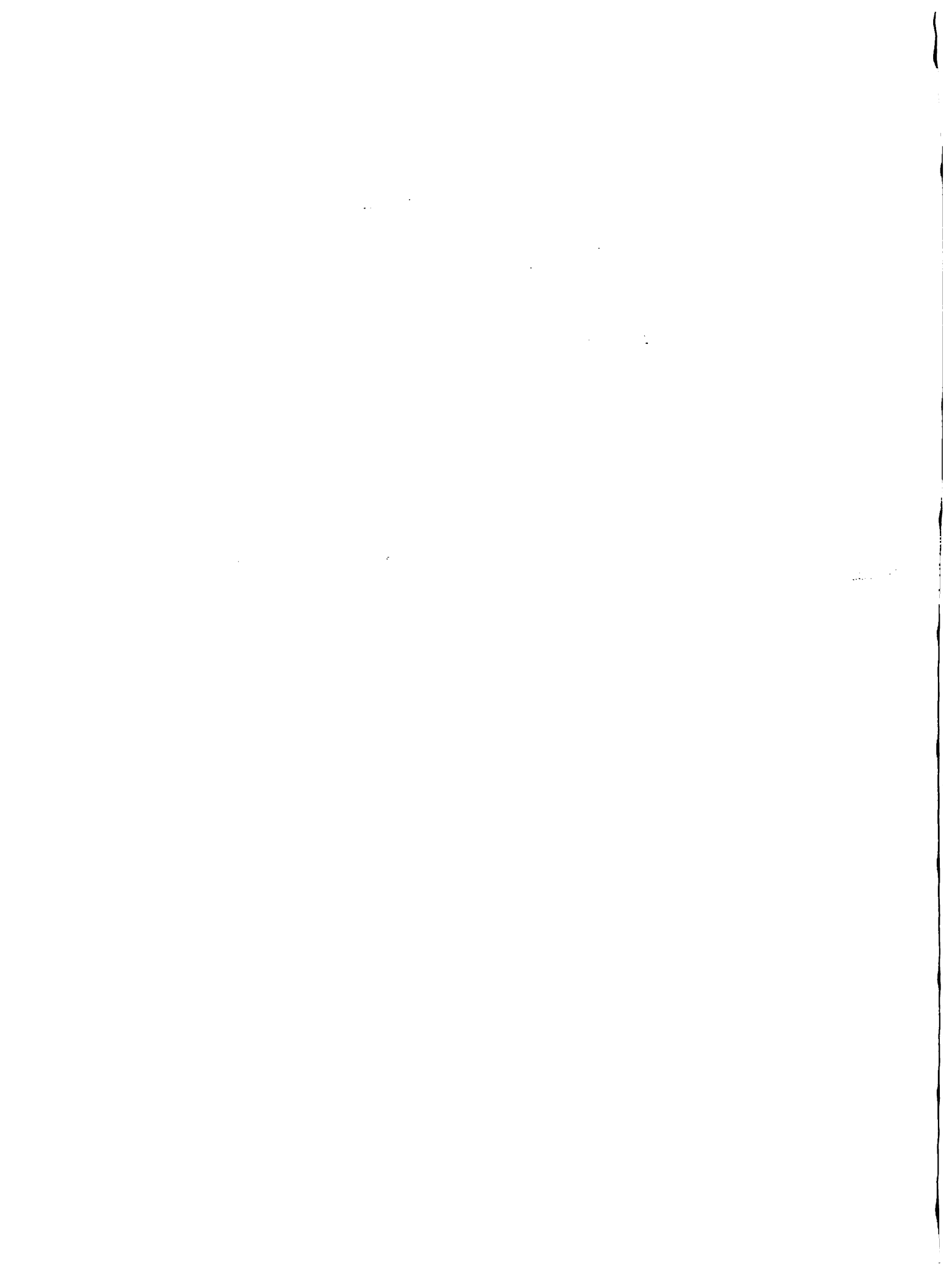
Anocentor nitens: Widely distributed throughout the Caribbean and world. It feeds primarily on horses and probably has a similar survival and habitat preference as does B. microplus. Table 5 shows what is known about its life cycle.

Rhipicephalus sanguinius: Its life cycle is presented in Table 7. It is widely distributed and feeds primarily on dogs. However, in the tropics its host range is quite wide.

Soft Ticks

I will only mention that soft ticks are present in the Caribbean which may have some relation to the livestock industry. However, there is very little information available on these ticks. There are two groups of soft ticks and one species is of interest, these are as follows:

1. Ornithodoros puertorecensis
2. Ornithodoros talaje group
3. Argas persicus group



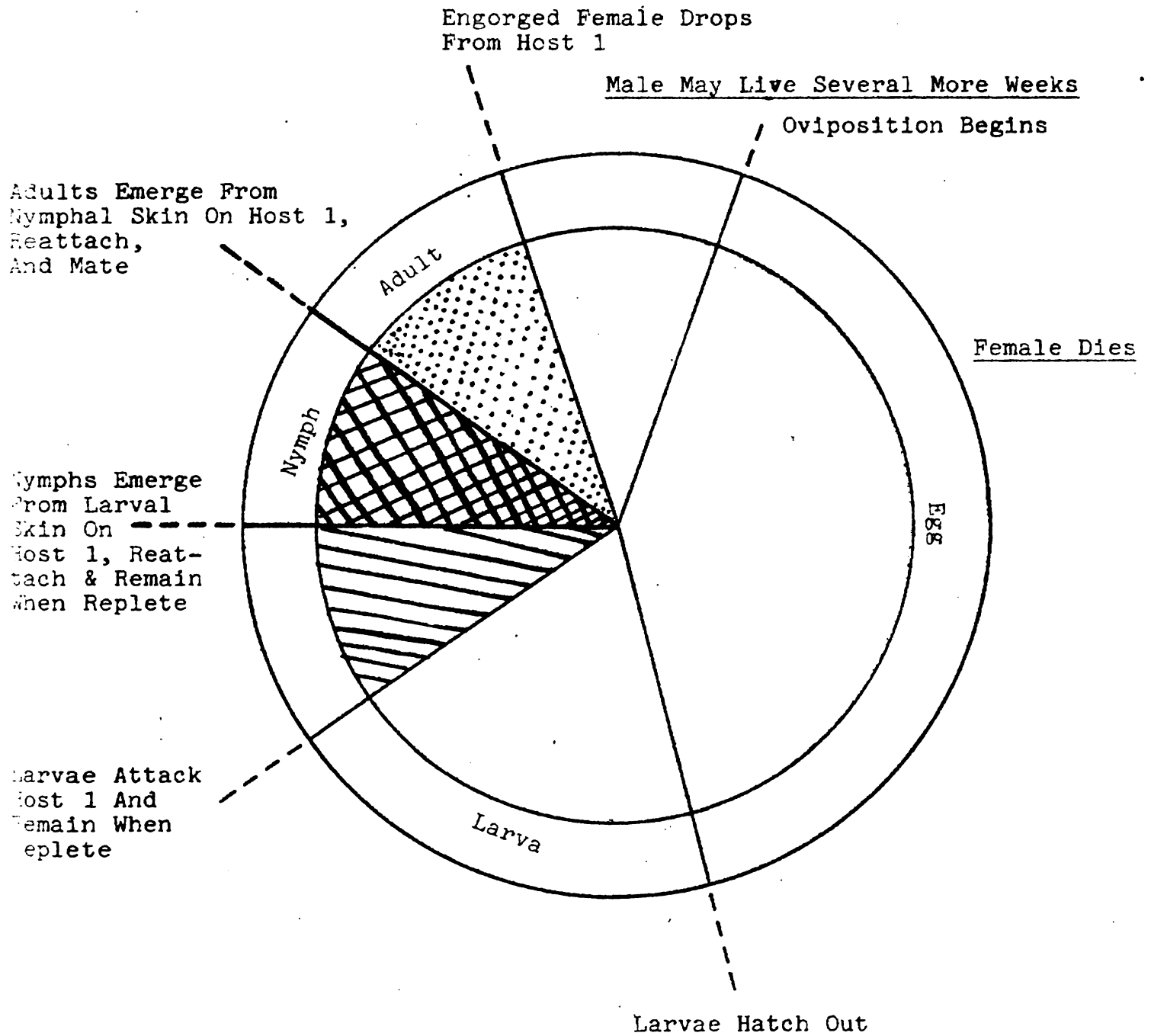


FIGURE 1. SCHEMATIC LIFE CYCLE OF A 1-HOST TICK.

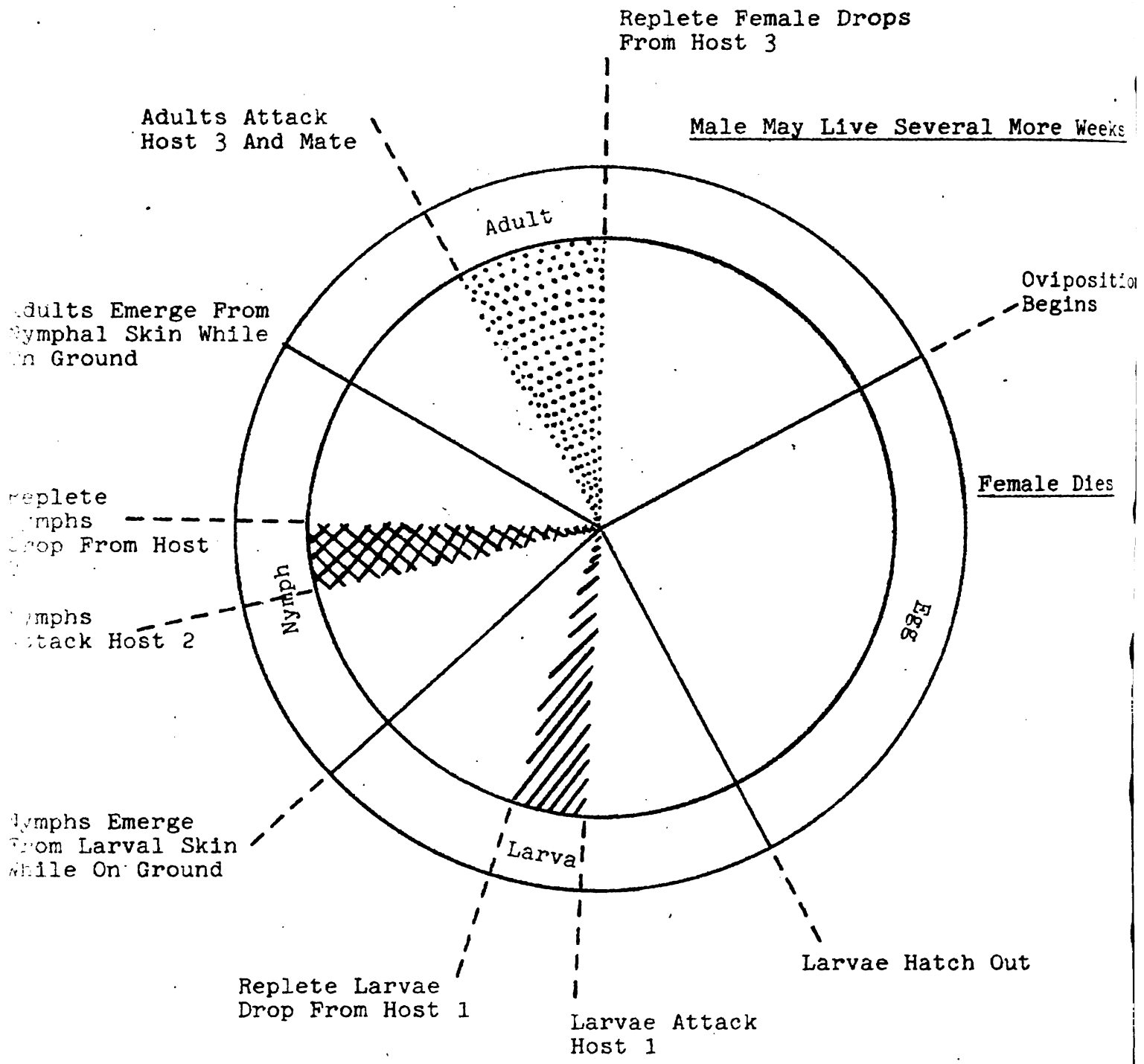


FIGURE 2. SCHEMATIC LIFE CYCLE OF A 3-HOST TICK.

TABLE 1.--TICKS OF IMPORTANCE TO THE LIVESTOCK INDUSTRY IN THE
 CARIBBEAN AREA.

TICKS	HOSTS
<u>AMBLYOMMA</u> <u>C</u> <u>JENNENSE</u> (F.)	WIDE RANGE
<u>AMBLYOMMA</u> <u>M</u> <u>ACULATUM</u> KOCH	WIDE RANGE
<u>AMBLYOMMA</u> <u>V</u> <u>ARIEGATUM</u> (F.)	WIDE RANGE
<u>ANOCENTOR</u> <u>N</u> <u>TENS</u> (NEUMANN)	HORSES, CATTLE, GOATS, SHEEP
<u>BOOPHILUS</u> <u>M</u> <u>ICROPLUS</u> (CANESTRINI)	CATTLE, HORSES, GOATS, SHEEP
<u>RHIPICEPHALUS</u> <u>S</u> <u>ANGUINIUS</u> (LATREILLE)	DOGS, CATTLE, SHEEP, GOATS

TABLE 2.--LIFE CYCLE OF Amblyomma cajennense (F.)

STAGE	DEVELOPMENT PERIOD	IN DAYS	
		AVERAGE	RANGE
EGG	INCUBATION	--	37-154
LARVA	PREFEEDING	--	-
	FEEDING	--	3-7
	PREMOLTING	--	10
NYMPH	PREFEEDING	--	-
	FEEDING	--	3-13
	PREMOLTING	--	12-105
MALE	PREFEEDING	--	-
	FEEDING	--	-
FEMALE	PREFEEDING	--	-
	FEEDING	--	7-12
	PREOVIPOSITION	6.23	3-20.
	OVIPOSITION	28.47	17-36

FROM: DRUMMOND AND WHETSTONE (1975); STRICKLAND ET AL. (1976).

TABLE 3.--LIFE CYCLE OF Amblyomma maculatum Koch.

STAGE	DEVELOPMENT		IN DAYS	
	PERIOD	AVERAGE	RANGE	
EGG	INCUBATION	--	21-142	
LARVA	PREFEEDING	--	-	
	FEEDING	--	3-7	
	PREMOLTING	--	7-121	
NYMPH	PREFEEDING	--	-	
	FEEDING	--	5-11	
	PREMOLTING	--	17-71	
MALE	PREFEEDING	--	-	
	FEEDING	--	-	
FEMALE	PREFEEDING	--	-	
	FEEDING	--	14-18	
	PREOVIPOSITION	3.7	1-6	
	OVIPOSITION	18	0-26	

FROM: DRUMMOND AND WHETSTONE (1970); STRICKLAND ET AL. (1976).

TABLE 4.--LIFE CYCLE OF Amblyomma variegatum (F.)

STAGE	DEVELOPMENT PERIOD	IN DAYS	
		AVERAGE	RANGE
EGG	INCUBATION	66.9	30-70
LARVA	PREFEEDING	2.4	1-7
	FEEDING	--	5-11
	PREMOLTING	--	15-34
NYMPH	PREFEEDING	--	7
	FEEDING	--	5-9
	PREMOLTING	--	23-31
MALE	PREFEEDING	--	4-16
	FEEDING	--	-
FEMALE	PREFEEDING	--	4-16
	FEEDING	--	9-25
	PREOVIPOSITION	12.17	10-15
	OVIPOSITION	33.9	17-40

FROM: GARRIS (UNPUBLISHED DATA) AND HOOGSTRAAL (1956).

TABLE 5.--LIFE CYCLE OF Anocentor (=Dermacentor) nitens
(Neumann).

STAGE	DEVELOPMENT PERIOD	IN DAYS	
		AVERAGE	RANGE
EGG	INCUBATION	--	21-28
LARVA	ENGORGE AND MOLT	--	8-16
NYPH	ENGORGE AND MOLT	--	7-14
FEMALE	FEEDING	--	9-23
	PREOVIPOSITION	2.8	2-4
	OVIPOSITION	14.5	9-17

FROM: DRUMMOND ET AL. (1969); STRICKLAND ET AL. (1976).

TABLE 6.--LIFE CYCLE OF Boophilus microplus (Canestrini).

STAGE	DEVELOPMENT PERIOD	IN DAYS	
		AVERAGE	RANGE
EGG	INCUBATION	--	14-146
LARVA	ENGORGE AND MOLT	--	7-12
NYMPH	ENGORGE AND MOLT	--	5-17
FEMALE	FEEDING	--	5-23
	PREOVIPOSITION	3	2-4
	OVIPOSITION	17	12-21

FROM: DAVEY ET AL. (1980); STRICKLAND ET AL. (1976).

ANAPLASMOSIS

by
Dr. Robert E. Ormiston

I would like to thank all the involved individuals and organizations who have been responsible for this seminar. It is certainly an outstanding example of cooperation in response to a long existing need for an exchange of ideas and technical information. These presentations which I have the honor to give owe their content almost entirely to observations of many scientists who have published extensively concerning the diseases and parasites with which we are concerned. I would especially like to acknowledge Dr. Ralph Bram, the distinguished research entomologist, whose work I have gratefully plagiarized.

Bovine Anaplasmosis

Bovine anaplasmosis is caused by an arthropod transmitted rickettsia, Anaplasma marginale. It is an acute or sub-acute, infectious non-contagious disease of cattle characterized by anemia, high fever, and icterus. The disease has a wide distribution in the tropics and sub-tropics and extends into some temperate zones. It has been recorded from Africa, the Middle East, the Far East, India, Australia, Southern Europe, Russia, South and Central America, Mexico, and the United States.

In the continental United States, anaplasmosis is found in virtually every state; however, the incidence of infection varies considerably in different regions of the country. Areas of highest incidence include the Southwest (particularly the Mississippi Delta), the Intermountain West, the West Coast, and Puerto Rico. Estimates of the economic impact of anaplasmosis vary, but by any standard the cost is considerable. Oglesby (1962) estimated that costs to the cattle owners of the United States, because of anaplasmosis, were at least \$34 to \$35 million each year; McCallon (1976) estimated an annual loss in the United States of over \$100 million; whereas Goodger (1978) estimated financial losses in California, alone, to be somewhere between \$5 and 11 million per year. (Prevalence - 30%).

Anaplasma marginale is an intraerythrocytic parasite appearing as rounded inclusion bodies measuring 0.3 - 0.8 microns in diameter, the majority being at or near the margins of the red blood cells (with A. centrale, a related

species which does not occur in the United States, the inclusions are more frequently seen in the center of the red blood cell).

When mature, the marginal bodies divide by binary fission and the resulting 2-8 initial bodies penetrate the envelope of other red blood cells. As a result, marked changes are seen in the blood of infected bovines, associated with extreme anemia and in many cases, jaundice. Hemoglobinuria is not a part of the pathology of anaplasmosis.

At least 19 tick species of the genera Boophilus, Dermacentor, Rhipicephalus, and Ixodes have been incriminated as biological vectors of anaplasmosis. Although both trans-stadial and transovarial methods of transmission have been reported, transovarial transmission remains subject to question. Biological transmission through intransdial feeding by male ticks may play a very important role in the transmission cycle.

Trans-stadial Transmission

The arthropod ingests the pathogen when feeding during one of the pre-adult stages; the pathogen undergoes development and/or multiplication, and after molting to the next stage, the arthropod introduces the pathogen into a susceptible host when feeding. Trans-stadial transmission is encountered most frequently with tick-borne diseases where the larval or nymphal stages feed on an infected host, molt to the respective nymphal or adult stages, and transmit the pathogen to a susceptible host during feeding.

Transovarial Transmission

Also known as hereditary transmission or transgenerational passage, it commences when an adult female vector feeds on an infected host. The pathogenic agent then undergoes development and/or replication within the arthropod, and the pathogen is transmitted through the egg to the next generation. Feeding by individuals of the first generation can introduce the pathogenic agent into susceptible hosts and, in some instances, the pathogen is maintained in the vector, amplifying host, and pathogen reservoir. Transovarial transmission was long considered exclusively in the realm of tick-borne diseases; however, in the past decade, evidence

has accumulated that even aquatic Diptera (e.g., sand flies and mosquitoes) are capable of transovarially transmitting certain arboviruses.

The fate of the marginal body after tick ingestion remains clouded. Using fluorescent antibody techniques, Anthony (1964) detected the organism within intact erythrocytes in gut contents, but lost track of the pathogen after 24 hours. Although Friedhoff and Ristic (1966) suggested that anaplasmata multiply in the Malpighian tubules of nymphal ticks by binary fission, Bram and Romanowski (1970) could not confirm these results. In addition to ticks, Anaplasma marginale is also transmitted mechanically by a number of biting fly species, particularly horse and deer flies.

The role of different arthropod groups as vectors of anaplasmosis varies in different geographical regions of the United States. In the South-eastern United States, blood-sucking Diptera, particularly horse flies, are the major anaplasmosis vectors. In the Intermountain West, where anaplasmosis is transmitted in spring and early summer, the tick Dermacentor andersoni is thought to be the principal biological vector. On the West Coast, the tick, Dermacentor occidentalis is probably the principal biological vector. Both are 3 host ticks. Unique to this area is the presence of the Columbian black-tailed deer as an important wild-life reservoir of anaplasmosis which greatly complicates the epizootiology. Mule deer may assume the same role in the Intermountain West, but white-tailed deer have not been found to be naturally infected in the Eastern United States.

Studies of the natural transmission of bovine anaplasmosis have been badly neglected over the years. As a result, it is often difficult or impossible to determine precisely which species, or even arthropod group, is responsible for anaplasmosis transmission in different regions. Such basic questions as the relative importance of different tick species and tabanids or other mechanical vectors in different areas, and the significance of interhost movement of infected ticks remain to be answered. Furthermore, the epizootiology of anaplasmosis may be complicated by unsanitary management practices, such as dehorning, castration, or multiple use of hypodermic syringes and needles, which mechanically transfer the infectious agent from infected to susceptible cattle.

Pathogenesis

Electron Microscopy has revealed that the anaplasma inclusion body or marginal body is composed of 1-8 initial bodies or sub-units. Initial bodies are also dispersed extracellularly within the plasma. Infection of a susceptible host follows introduction of infective initial bodies from either an acutely infected or carrier animal, including previously mentioned deer species. Blood from acute cases diluted as high as 10^6 will infect healthy animals.

Mechanical transmission by insects depends on 3 principal factors:

1. Number of organisms in the donor's blood
2. Density of insect population
3. Lapse of time between feeding on infected and recipient animal

Research suggests that the incubation period is decreased and the degree of anemia increased in direct proportion to the size of the infecting dose.

1-2 marginal bodies are observed per erythrocyte in mild infections and up to 7-8 in animals with acute infections. The normal r.b.c. count of 7 million per mm^3 may be reduced to 1 1/2 - 2. Infected animals remain life-long carriers with $\leq 1\%$ of the erythrocytes parasitized unless they are treated successfully by chemotherapy.

Signs

Normal urine.

Severe respiratory distress, abortions, depressions, weakness, dehydration and constipation. Mortality may reach 80% in animals over 1 year. The temperature may go as high as 105-107°F but is normal in the carrier.

Calves under 6 months old do not develop signs of illness unless splenectomized.

Lesions

Pale, watery blood and pale mucous membranes, splenic enlargement petechiation of the heart and abdominal viscera.

Diagnosis

In addition to history, signs, and lesions, various laboratory procedures are of considerable value.

1. The Complement-Fixation (C.F) Test is the current definitive test and is considered to be 97% accurate.
2. The card test is widely used as a screening test.
3. Blood Smears with Wrights or Giemsa stain are time consuming and difficult because of confusion caused by artifacts. Chronic infections (carriers) may be missed by this procedure.
4. Acridine Orange Vital Stain - a screening test which utilizes fresh whole blood. Fast and inexpensive.

Treatment

Before the introduction of the tetracyclines, treatment was limited to supportive or non-specific measures including good nursing and care, blood transfusions, and hematinics. There is always risk associated with the treatment of acutely affected, weakened, and anemic animals, particularly range cattle. The stress resulting from treatment may do more harm than the benefits resulting from the treatment.

The tetracyclines, oxytetracycline (terramycin) and chlortetracycline (allreomycin), are the only effective specific compounds approved for anaplasmosis therapy in the United States. The treatment is practical and effective when priority is given to severe Anemia cases. The tetracyclines destroy parasites through alteration of the membranes of the structures surrounding the initial bodies.

Chlortetracycline - Oral administration. 5 mg/lb at 45-60 days will destroy carriers. 0.1 - 0.5 ml/lb have been used when vectors appeared in order to avoid transmission.

Injectable Oxytetracycline (LA-200) seems effective in protecting carrier animals, as well as seriously infected cattle, and the recommended dose is

20 mg/kg twice with an interval of 7 days. It is possible for some isolated anaplasmas to develop a tolerance to the drug.

Two additional compounds, Gloxazone and Imizol have been shown to be effective therapeutic agents but their use in animal food has not been permitted in the United States. Both are produced by the Burroughs Wellcome Co.

Vaccines - Various methods have been used in different parts of the world to establish resistance to anaplasmosis with varied degrees of success. These include:

1. Pre-immunization of calves less than 6 months old by injecting them with the blood of carrier animals, with and without simultaneous chemotherapy.
2. A. centrale - infection with this parasite seems to reduce the severity of A. marginale infections.
3. Attenuated vaccines - A. marginale has been attenuated by ovine passage and a vaccine developed by this process.
4. Killed adjunct vaccine - The Ft. Dodge Company produces a vaccine called Anaplaz which is licenced and in use in the U.S.A. A problem with Neonatal Isoerythrolysis (NI) in nursing calves, has reduced its popularity, although the manufacturer claims that these problems have been largely eliminated.

The recommended vaccination schedule is as follows:

Bulls - Can be vaccinated any time.

Females - Vaccinate with two doses - when open boost - 1 year later - when open subsequent boosters - every two years - when open.

THANK YOU.

BABESIOSIS

by
Dr. Robert E. Ormiston

I hope those of you who have considerable personal experience with Babesiosis will tolerate and forgive my lack of first-hand knowledge. Once again I must rely on the wisdom of others in making this presentation. If my comments stray too far from the truth, I trust someone will kindly set me straight at an appropriate time prior to the termination of this seminar.

Bovine Babesiosis

It is only natural that bovine babesiosis be selected as one example of tick-borne animal disease transmission. The classic work of Smith and Kilbourne (1893) demonstrated that Texas fever caused by Babesia bigemina, was transmitted by the tick, Boophilus annulatus. There are at least 15 distinct species of Babesia, from various vertebrate hosts, a number of which are of considerable economic importance. Currently, it is felt that there are four valid species of Babesia in cattle: B. bigemina, B. major, B. bovis, and B. divergens.

Bovine babesiosis is probably one of the most important diseases of cattle in the tropics and subtropics, but there is wide variation in clinical signs. Members of the genus do not show uniform pathogenicity and, even within a species, strains may differ in this characteristic. For example, the Australian strain of Babesia bigemina rarely causes disease, but the African strain is highly pathogenic. Furthermore, the susceptibility of the host may be altered by factors such as age, breed, environmental stress, and, in the young, passive immunity conferred by colostrum from immune dams.

Affected animals are dull and listless; they fail to eat and stop ruminating. Animals become thin, emaciated, and icteric. Severe anemia is common and usually accompanied by hemoglobinuria (not present in anaplasmosis). Death may occur in 4 to 8 days in acute cases, and mortality may be as high as 50-90 percent in previously unexposed herds. In chronic cases, animals lose condition quite rapidly and remain thin, weak, and emaciated for weeks before finally recovering. Growth rates and milk production are reduced in chronic carriers even when the animals do not demonstrate clinical signs of babesiosis. Calves less than nine months to a year of age, are seldom seriously affected due to resistance conferred by the colostrum of the immune dam as well as

physiological resistance; however, these animals may suffer relapses as adult cattle, especially when not regularly exposed to reinfection by infestation with Babesia-infected ticks.

Babesia bigemina is found in Mexico, Central and South America, the Caribbean, Europe, the Middle East, North Central, and South Africa, and Australia. The disease, called Texas tick fever in the United States, was eradicated from the continental United States by means of a Federal/State cooperative programme to eradicate the vector ticks, Boophilus microplus and B. annulatus. Through a centrally planned and coordinated programme of quarantine, compulsory dipping of cattle, and/or pasture vacation, the campaign, which was initiated in 1907, gradually progressed to a successful conclusion by 1943. B. Microplus was eliminated from Puerto Rico in 1954, following an 18 year eradication programme.

Babesia bigemina is transmitted not only by Boophilus microplus and B. annulatus, but also by B. decoloratus (in South Africa), Haemaphysalis punctata (in Europe), Rhipicephalus appendiculatus and R. evertsi (in East and South Africa), and R. bursa (in North-West Africa).

Riek (1968) has provided a comprehensive review of bovine babesiosis caused by B. bigemina. In the bovine, Trophozoites of B. bigemina occur in the erythrocytes where they multiply by binary fission, budding, or schizogony. They break out of the erythrocytes and enter new red cells in a cycle which continues indefinitely, sometimes for the life of the animal host. When a female boophilus ingests infected bovine blood, the parasites invade the cells of the gut epithelium where multiplication by multiple fission occurs. Only a comparatively small number of ingested parasites survive in the tick to invade and develop in the gut epithelial cells. At 48 to 60 hours, the epithelial cell ruptures and liberates club-shaped bodies into the lumen of the gut. These migrate through the gut wall into the tick's hemolymph. After about 96 hours, a secondary cycle becomes evident when the parasites enter cells of the hemolymph and Malpighian tubules where they undergo multiple fission. Upon entering the ovary, the parasites penetrate the eggs where they round up and divide a few times, forming very small, round or vermicular forms. Their numbers increase as the egg develops, and they are distributed throughout the organs of the embryonic larva. The parasites do not develop further in the larval tick which hatches from the egg, but when the tick molts to the nymphal stage, the parasites enter

the salivary glands and continue their development. Here they undergo a series of binary fissions and enter the cells of the glandular acini where they multiply further until the host cell contains thousands of minute parasites. When the infected nymph or subsequent adult feeds on a bovine, the parasites infect the new host through the salivary secretions. In the case of Babesia bovis, the infected larval tick is capable of pathogen transmission. It is reported that as many as five successive generations of female ticks may transmit Babesia without feeding on an infected host through this process of transovarian transmission.

The epizootiology of babesiosis is quite complex. Young cattle up to 9-12 months of age are not only resistant to the clinical effects of Babesia spp. but when infected, develop protective antibodies which persist to adulthood. This immunity is reinforced by the frequent natural challenge of infected ticks. Although infected, the animal never exhibits clinical disease unless placed under severe stress or challenged by antigenically different strains. Thus, in enzootic areas where all cattle are infected at an early age and periodically challenged by infected ticks, there is little apparent babesiosis. In areas where the vector tick populations are very low and the incidence of babesiosis is correspondingly low, many cattle reach maturity without being exposed to infested tick vectors. When these adult cattle eventually become exposed to infested ticks, they respond with acute clinical disease and an outbreak or epizootic may occur. Similarly, when mature cattle from babesiosis-free areas are introduced into enzootic or epizootic areas, they inevitably succumb to babesiosis, frequently with high mortality rates. Problems of babesiosis in epizootic areas and in cattle moved from free areas to enzootic or epizootic areas, have been moderated by the use of live, attenuated vaccines and by premunition of immature cattle in certain areas.

Equine Piroplasmosis has a worldwide distribution and occurs in Asia, the U.S.S.R., India, the Mid-East, Europe, Africa, Mexico, Central and South America and in parts of the U.S. including Florida and Puerto Rico. The incidence of equine babesiosis in Puerto Rico is 89%, U.S.V.I. 50% and very slight in Florida partly due to a vector control programme.

Each animal species has its own protozoan species and babesia are not capable of producing infections in animals outside of their normal host species e.g. equine piroplasmosis cannot be transferred to cattle and Texas fever is not

transmissible to horses. Equine babesiosis is caused by two species: B. caballi and B. equi. Both species affect horses, mules, and donkeys. B. equi also infects zebras.

B. caballi appears in parasitized erythrocytes as paired pyriform bodies with their pointed ends meeting at an acute angle. B. equi exists in a 4 part maltese cross shape form. B. equi has a wider distribution, is smaller, more pathogenic and more resistant to chemotherapy than B. caballi.

In enzootic areas equines usually become infected during their first year of life while they are relatively resistant. Losses in young horses may be high as 5-10% depending upon the strain of the parasite, general health of the host, availability of treatment and general environmental stress factors. Serious losses sometimes exceeding 50% have occurred when susceptible and infected adult horses are exposed to each other, through movement.

Numerous ticks serve as biological vectors including Dermacentor nitens, Rhipicephalus sanguineus, and Rhipicephalus evertsi. R. sanguineus is capable of transovarian transmission for up to 4 generations.

Signs and lesions: The natural incubation period is 1-3 weeks. Commonly observed signs are: fever, anemia, pale-icteric mucous membranes which are sometimes hemorrhagic, depression, weakness, and edema. Hemoglobinuria is not common with B. caballi. In severe cases, respiratory distress is noted. Digestive tract involvement results from inflammation of the mucous membranes and is reflected by: Anorexia, emaciation, colic, constipation, and later diarrhea. The depression and dullness observed in severe cases is associated with encephalitis.

At necropsy pathology may include enlargement of the spleen and liver, and excessive pleural, pericardial, and peritoneal fluids.

DIAGNOSTIC TEST

- (1) CF Test - Definitive
- (2) Capillary - Tube Agglutination Test (C-A Test) - This test has not been widely accepted.
- (3) AGAR-GEL immuno-diffusion test (AGID) - very accurate test run on micro slides or small plates.

- (4) Acridine orange vital staining - best as a screening test. Whole blood is stained and examined with a darkfield microscope and special filters.
- (5) Blood Smears - Wrights stain - Tedious and requires considerable skill.

When examining blood for evidence of the parasite, one should give consideration to the increased numbers of parasites present during the early febrile period. It is very difficult to locate the protozoa in the blood of carrier horses by direct visual means.

Clinically, babesiosis can be confused with equine infectious anemia (E.I.A.) and african horse sickness.

IMMUNITY

Clinical recovery confers a strong resistance to reinfection with the same strain only. This resistance or premunity may be lost by lowering of condition resulting from some other concurrent disease condition. FOALS UNDER ONE YEAR OF AGE ARE QUITE RESISTANT.

TREATMENT OF BABESIOSIS IN CATTLE AND HORSES

At least twelve proprietary drugs are available on the world market for the treatment of babesiosis. They are generally not available in the U.S. and are not cleared for food animal use in the U.S. Some of these drugs are reported to be so specific and effective that a single injection will clear an infected animal. A sterilizing treatment i.e. (one which entirely eliminates the Babesia) may be undesirable when animals are going to be re-exposed to infection in an enzootic environment. Reinfection may produce severe clinical illness depending on the degree of persisting sterile immunity.

Drug resistant Babesia can be produced experimentally and are felt to occur naturally; however, this problem, as yet, has not placed a major constraint on chemotherapy. As a general rule the larger Babesia are more responsive to therapy than the smaller ones such as B. equi.

TRYPAN BLUE was probably the first specific treatment for B. bigemina. Better products are available and used today.

QUINOLINE DERIVATIVES are effective against B. bigemina and to a lesser extent B. bovis. For many years they were the drug of choice for use in cattle.

DIAMIDINE DERIVATIVES - most are effective against B. bigemina and B. bovis.

ACRIDINE DERIVATIVES are reportedly effective against both B. bigemina and B. bovis but are no longer widely used.

IMIDOCARB is of recent introduction and is highly effective as a therapeutic and prophylactic agent. Imidocarb is slowly metabolized and eliminated. This characteristic probably enhances its effectiveness, but also is responsible for the persistence of tissue residues. Various authors have described the prophylactic effect of Imidocarb which is estimated to last an average of 4-6 weeks following treatment. Some reports suggest a longer duration of protection.

Equine Babesiosis is more refractory to Imidocarb treatment than are bovine infections; however, B. caballi can be successfully eliminated from carrier equines by two 2.2 mg/kg doses given at 24 hours intervals. B. equi sometimes can be eliminated by the indicated treatment but not always. These above compounds are also effective against Babesiosis in dogs, swine, and sheep.

THANK YOU

ARTHROPOD - BORNE DISEASES: AFRICAN SWINE FEVER

by
Dr. Gary Colgrove

In 1921, Montgomery described an acute, febrile, and usually fatal disease of domestic (European) pigs in Kenya. He noted that outbreaks of the disease occurred in areas inhabited by warthogs, and concluded that wild swine were reservoirs of infection for domestic pigs. It was subsequently demonstrated that three species of wild swine - the warthog, bush pig and giant forest hog - can be inapparently infected with the virus of African Swine Fever (ASF), but the mechanism of transmission from wild to domestic pigs was not immediately apparent. Pigs placed in contact with infected warthogs did not become infected, and the disease proved difficult to transmit by feeding warthog meat or offal to domestic swine.

An arthropod vector of ASF virus was first demonstrated in Spain in 1963. Ticks of the species Ornithodoros erraticus, collected from piggeries where outbreaks of ASF had occurred, transmitted the disease to susceptible swine. Ticks were able to transmit the disease for up to one year after feeding on infected swine. Later, ticks of the species Ornithodoros moubata, collected from warthogs and warthog burrows, were shown to be vectors of ASF virus in Africa. It was assumed that these ticks became infected by feeding on infected warthogs, but viremia has rarely been demonstrated in wild African swine. In addition, when viremia does occur, the titers of virus found in the blood are apparently beneath the level required to infect a tick. The finding that ASF virus is transmitted transtadially (stage to stage), transovarially (generation to generation) and sexually in the tick indicated that long term maintenance of ASF virus in enzootic areas does not depend on the continuing presence of a primary vertebrate host. This has led to the speculation that ASF virus may be a virus of ticks, with wild and domestic swine becoming accidental hosts.

Arthropod reservoirs of ASF virus are an important consideration in any eradication programme. In the Dominican Republic, a study was conducted to determine if potential vectors/reservoirs exist, but ticks were not found on any swine examined.

Ornithodoros erraticus is undoubtedly a factor in the maintenance of ASF virus in the Iberian peninsula, but fortunately the tick has a rather limited geographic distribution. In the United States, ticks of the species Ornithodoros coriaceus have been shown to be capable of transmitting ASF virus.

HEARTWATER

by
Dr. M.J. Burridge

Etiology

Heartwater (cowdriosis) is an acute febrile disease of ruminants caused by the rickettsia Cowdria ruminantium. The rickettsial organisms occur in closely packed colonies in the endothelial cells of blood vessels, especially in the capillaries of the cerebral cortex. The number of granules in any one colony varies from less than 10 to several hundreds.

Geographical Distribution

Heartwater has been reported from many countries in Africa south of the Sahara, but in most areas little is known about the prevalence of infection or disease due to the difficulty of diagnosis and the lack of any serodiagnostic test. Heartwater has recently been diagnosed in Guadeloupe in the Caribbean (Perreau et al., 1980). C. ruminantium presumably was introduced to the French island of Guadeloupe by ticks infesting cattle imported from Senegal, an ex-French colony on the West African coast.

Susceptibility

Cattle, sheep, goats, and water buffalo are susceptible to C. ruminantium infection. Some species of African antelope, as well as European fallow deer, have been shown to be susceptible to experimental infection; natural fatal cases have been seen in springbok and eland.

Transmission

C. ruminantium is transmitted by 5 species of Amblyomma ticks: A. gemma, A. hebraeum, A. lepidum, A. pomposum, and A. variegatum. The tropical bont tick, A. variegatum, was introduced into the Caribbean in the late 1800s on cattle from Senegal, and is now established on Antigua, Guadeloupe, Martinique, Puerto Rico, and St. Kitts (Morel, 1966; USDA, 1976). Two other species have recently been shown to be experimental vectors of C. ruminantium: they are the elephant tick, A. tholloni and the Gulf Coast tick A. maculatum. The latter tick is found in the United States, primarily in the south-eastern states bordering the Gulf of Mexico and the Atlantic Ocean; it has also been reported from Colombia, Ecuador, Jamaica, Mexico and Venezuela.

Amblyomma species are three-host ticks. Transmission is trans-stadial only so that unfed larvae are always free of infection. Nymphs and adults may start to transmit C. ruminantium within 24 hours of attachment, with the number of infective rickettsiae in the tick increasing during the first three days of engorgement.

A. variegatum ticks are found on a great variety of mammals, with cattle a preferred host. Adults are commonly found on the lower part of the dewlap, brisket, axillae, abdomen, groin, udder, and external genitalia of the male, whereas immature stages have no preferred site of attachment. Adults of A. maculatum are found on sheep, cattle, equines and deer, with nymphs and larvae primarily on birds and small mammals; however, the immature stages will feed freely on domestic ruminants. Common sites of attachment for the latter tick are the head, neck, and ears.

Clinical Symptoms

Heartwater is commonly mild or sub-clinical in local indigenous stock in endemic areas. However, in exotic breeds disease is typically acute, especially when susceptible foreign breeds of ruminants are introduced into endemic areas. A high fever is followed by development of nervous symptoms such as staggering drunken gait, circling movements, twitching of eyelids, and muscle tremors. Finally, the animal may collapse in convulsions. Profuse fetid diarrhea is common. Bronchial rales may be heard. The mortality rate is usually over 50% in exotic breeds, while it may be less than 5% in local breeds.

Post Mortem Findings

In acute cases, the most common gross lesions at necropsy are pulmonary edema with froth in the trachea and bronchi, hydropericardium, hydrothorax, ascites, and hemorrhagic enteritis. Histopathological changes include leucocytosis and peri-vascular cellular infiltration in the brain and other organs.

Pathogenesis

The pathogenesis of heartwater is poorly understood. Marked pulmonary edema may in some cases be directly responsible for death by asphyxiation. The

histopathological changes in the brain do not appear to be constant or severe enough to explain all nervous symptoms associated with the disease. A terminal decrease in arterial blood pressure may be involved in the pathogenesis, and it is associated with a terminal fall in plasma volume. The latter is thought to be due to an increase in capillary permeability, producing the transudates and edema so often seen at necropsy. These accumulations of liquid frequently coagulate, demonstrating that molecules as large as fibrinogen are able to pass through the capillary wall.

Diagnosis

A definitive diagnosis of C. ruminantium infection can only be made by demonstration of the organism by brain biopsy or sub-inoculation of susceptible ruminants. When handling brain material from an animal which has exhibited nervous symptoms, the possibility of rabies should always be considered and due precautions should be taken. After opening the skull, a small fragment of gray matter the size of a match head is snipped off the surface of the brain with curved scissors, placed on a microscope slide, crushed with another slide and, while pressure is maintained, the two slides are drawn over one another lengthwise, producing two separate smears. These smears are fixed in methanol and stained with Giemsa. Areas containing capillaries are located under low magnification, and individual capillaries are then searched with the oil-immersion lens. A positive diagnosis depends upon demonstration of colonies of C. ruminantium which stain from a reddish purple to blue with Giemsa. These colonies may be extremely scanty, especially in peracute cases.

Sampling the cerebral cortex necessitates opening the skull, often a tedious exercise. Schreuder (1980) has developed a method whereby samples of the cerebellum can be taken through the foramen magnum after severing the head from the neck through the occipital articulation. The cerebellum is seen by lifting the dorsal meninges from the medulla oblongata with forceps, and superficial cerebellar tissue is collected with a curette (or even an ordinary teaspoon) inserted between the medulla and the meninges. Smears are made as described above.

There is no serological test presently available for the diagnosis of C. ruminantium infection.

Treatment

The tetracyclines are most active against C. ruminantium. Two treatments of oxytetracycline ("Terramycin") or rolitetracycline ("Reverin") 1-2 days apart are usually effective when used at a dosage of 5-10 mg/kg. One treatment with long-acting oxytetracycline ("Terramycin L.A.") at 20 mg/kg may also be sufficient. However, in the field treatment is often too late, especially when nervous symptoms or hemorrhagic diarrhea are evident.

Control and Prevention

1. Tick control: Amblyomma ticks are difficult to control since they are three-host ticks which can survive on many wild and domestic animal species. While intensive dipping with acaricides will greatly reduce the number of cases of heartwater, bi-weekly dipping is necessary to prevent all transmission.
2. Immunization: Attempts have been made to immunize ruminants by intravenous injection of blood from a reacting donor animal, followed by tetracycline treatment at the beginning of the febrile response. Results have been inconsistent with some mortality and some breakdowns of immunity. Acceptable methods for immunization against heartwater on a herd basis are not expected until the organism can be grown in vitro or in laboratory animals. So far, the organism has survived in mammalian cell cultures for only 2 weeks.

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TICK CONTROL: ACARICIDES

by
Dr. Glen I. Garris

Summary

For the next few years and probably much longer, any tick control or eradication programme will have to be based on the use of conventional chemicals, the acaricides that are presently available or very similar ones. In such programmes we usually are most concerned with the selection of the efficient acaricide and the proper dosage-safety, cost stability, availability, etc., are secondary considerations that are evaluated only after we know that the chemical is an efficient tick killer. As a result, arthropod toxicology is usually studied in three phases. With Boophilus species, for example, candidate acaricides are first tested in vitro against a non-parasitic stage of the tick, either the engorged female or the unfed larva. In the second phase, the most promising candidates are applied to a small number of infested cattle to determine their toxicity to datanymphs, young adults, and engorged or partially engorged females. The third phase of testing conducted in the field in a variety of practical situations that provide information on the protection of cattle from reinfestation by the residual action of the chemical.

In regions in which insecticide resistance is a major consideration, studies in arthropod toxicology should be intensified in order to minimize the impact of resistance on the control programme. In every programme, means of monitoring resistance should be provided for in the early stages as prompt recognition might make it possible to alter programme strategy and avoid failure. While experience in Australia and Brazil indicates that resistance to other chemicals usually rapidly follow the first appearance of resistance, there is a possibility of developing treatment regimes that will delay the appearance of resistance or soften its impact on the programme. The greatest need seems to be for the discovery and development of entirely new chemicals unrelated to those presently in use which kill ticks by an entirely new mode of action.

Acaricide resistance is merely the phenotypic (outward) expression of an evolutionary process accelerated by chemical selection, and any compound (acaricide) is likely to fail eventually against certain individuals. This is a genetically based response by the organism for survival of its species.

Pesticide Use

As with the information on ticks affecting livestock, there is little published information available on the use of pesticides on livestock in the Caribbean. Many of the pesticides used on livestock throughout the world have been or are being used in the Caribbean. Pesticides being used in Jamaica and Barbados illustrate this:

<u>PESTICIDE</u>	<u>JAMAICA</u>	<u>BARBADOS</u>
Carbaryl (Sevin)		X
Chlorfenvinphos	X	X
Coumaphos	X	X
Diazinon		X
Dioxathion	X	X
Lindane	X	
Malathion		X

Table I shows the known acaricide resistance in ticks that has been discovered to date. This table represents a summary and is adapted from work by Wharton 1976, and Laurens 1979.

Conclusions

The technical aspects of an organized tick control programme that is national in scope can be properly planned and controlled only if current, reliable technical information is readily available. As a general rule, research carried out in other countries, even though some of it may be pertinent, will not be adequate or fully applicable to the country with the programme and some research will be needed that is local and is designed to solve local problems. Also, acaricide resistance should be anticipated and research to predict and avoid this possibility must be conducted. The magnitude of this research will depend on the size and probable duration of the programme but even a limited research effort can substantially improve the efficiency of a tick control programme and contribute to the ultimate success of an eradication campaign.

WHY IS TICK CONTROL OR ERADICATION CONSIDERED?

by
Dr. Robert Ormiston

Ticks are well known ecto-parasites found throughout the world and are considered the most detrimental and costly ecto-parasites to large livestock animals, particularly in tropical and sub-tropical regions. They belong to the class Arachnida, order Acarina and family Ixodidae. They are all parasites but some live in a free state during the ovulation phase of adult females and, generally, in most species, when moulting.

During the parasitic phase of the cycle in which they remain on domestic animals they affect their hosts in different and significant ways.

1. The average female tick needs from 0.5 to 1.0 ml of blood to complete its development. Larger species like the Amblyomma variegatum need much more. The loss of blood, without counting infectious anaemias, leads to weakness, greater susceptibility to diseases, drastic growth reduction and losses in meat and milk production.
2. Some species (Gegen A. variegatum), cause injuries which are not noticed due to the lesions formed during the feeding process, especially in females. These lesions, which can be severe in themselves, can become secondarily infected by Dermatophilus congolensis which causes the bovine condition streptothricosis or dermatofilosis. This is considered one of the main diseases among cattle in parts of Africa, and is generally associated with Amblyomma spp. infestations. Where there are fly larvae in the primary stage, wounds due to tick bites become responsible, in many cases, for myiasis. During the time when Callitroga hominivorax was a serious problem in Texas, it is estimated that some 90% of the infections were associated with wounds caused by the feeding of ticks. The success of the Cooperative Eradication Programme between Mexico and the U.S.A., has largely eliminated the problem of the disease in Texas.
3. In man and animals they can produce paralysis and poisoning due to the injection of saliva secretions from certain ticks as they feed.
4. Ticks are responsible for the biological transmission of pathogens which cause many mortal diseases in animals, some of which have

been discussed in this seminar. When the different dangers represented by the numerous species of ticks are considered, an obvious conclusion is that it would be wise to avoid the introduction of exotic ticks into countries free of them. Control and/or eradication plans should be designed for the most destructive species of native or imported ticks whenever these procedures are practical and possible.

Prevention of the Introduction of Exotic Ticks

Undoubtedly this needs an efficient expenditure account before any subsequent control or eradication scheme. However, certain considerations must be made in this respect since they could be overlooked or given merely casual consideration due to inexperience. I am going to give a list of some of those which come to mind:

1. Can the tick in question be introduced to free zones by migratory birds or animals?
2. Is there sufficient legislation to effectively control ports of entry whether they be by air, sea or land?
3. Is there an organization or unit within the government to manage the necessary regulatory functions? If this is not so, can such a group be created in good time before the invasion of ticks?
4. Is there certainty that the funds necessary to operate the regulatory government agencies will be available?
5. Once a government is involved in a specific tick control or eradication programme it will have to maintain vigilance with respect to the possible introduction of other species. For example, A. variegatum was found in Puerto Rico in 1974. B. microplus was found again in 1978 for the first time since its previous eradication. Field investigations suggest that Boophilus was present and spread for many months before its eventual detection and identification.

The Programme in progress, led by the veterinary services of the United States Department of Agriculture and the Animal Health

Commission of Texas along the frontier of Mexico and the United States, has dramatically demonstrated the value of this effort which is an example of a well managed Prevention Programme.

6. In 1907 an area of 1 813 000 km² became infected with Boophilus spp. in the southern part of the United States. An Eradication Programme was initiated during that year using quarantines and vaccination of infected pastures as well as compulsive immersion of cattle in baths containing arsenical substances. Various types of spraying equipment was used in a limited manner especially during the last years of this programme. In 1943 the Programme reached a successful conclusion as Boophilus spp. was completely eliminated from the continental territory of the United States. By 1954 this type had also been eradicated from Puerto Rico after 18 years of effort which began in 1936 but was briefly interrupted during the Second World War.

After 1943, all cattle entering the United States from Mexico, coming from infested areas, have had to presented with certificates that they are free from ticks and taken straight to the border where each animal is carefully inspected and bathed under the official supervision of the United States Department of Agriculture.

In addition to this, the border is vigorously patrolled by inspectors on horse-back, in pick-up trucks and occasionally in aeroplanes Well trained and highly motivated specialists are constantly looking to capture horses and cattle in order to avoid the entry of these animals. This type of programme is supported by ranchers on both sides of the border who recognize the danger which this entry represents. Nevertheless, infected Mexican cattle have been found beyond the free zone for cattle in the United States and the zone is placed under strict quarantine and any infected animal is quickly eliminated. On rare occasions when ticks are found infecting animals in the U.S., the North American cattle are moved carefully under regulated conditions to pastures which are known to be free of ticks. No type of cattle, horses, sheep and goats are kept in infested pastures until all the larvae are dead. This procedure, generally

called vacant pastures, has proven to be somewhat controversial in the areas along the border where there are high livestock populations. Some officials think that in pastures infested with this tick, the livestock should be left with the deer if the population is significant. A systematic bathing programme, twice a week, is then established which will eventually be a complete eradication in the infested areas. If the deer alone are present in pastures infested by larvae, only these will be attacked and, due to the migratory habits of the deer, the infestations can be spread to other free pastures. It is not practical to try pesticide treatments against wild deer populations. The value of the programmes to maintain the United States free from Boophilus spp. and bovine babesiosis was estimated at \$500,000,000 per year in 1977.

Important Points for Control Programmes

When the decision is taken to control or reduce the population of one or more species of ticks, some questions and alternatives must be considered in the management of the programme. Getting sincere support for the programme from the industry is of great importance as well as basing the activities on real necessities and a deep economic meaning.

1. At present, is it desirable to reduce the general population within an area starting from the premise of infected individuals? Would using this approach increase the prevalence risk of the tick transmitted diseases?

Some countries with infestations of this tick and bovine babesiosis are not making every effort to eliminate the ticks in their infected herds. They prefer to maintain the tick populations at a low level in order to pre-immunize their calves. The result of control and eradication of the Dermacentor nitens with respect to the incidence of equine babesiosis is presently being disputed in Puerto Rico. We are aware that severe babesiosis in horses could temporarily increase if its main vector is eliminated and more horses reach maturity without having been exposed to the disease when young.

2. What degree of control could the industry tolerate on the movement of animals? What are the present restrictions on the movement of livestock? What is the best way to gain the confidence of the cattle dealers? If a decision had to be made for complete elimination or eradication, would the need for more controls on the movement of cattle be intensified?
3. If a decision was taken to reduce the tick population and eliminate the abolition of infections, how would this be achieved? Many countries have unfortunate experiences with the use of pesticides in very diluted concentrations. Weak concentrations are cheaper and leave some ticks around to guard against the risk of severe babesiosis, but they generally create a resistance to pesticides in tick families. The few ticks surviving each application of pesticide will be more tolerant each time to the chemical action. Consequently, each new generation will be more and more resistant to pesticides. A more in-depth approach to reduce but not eliminate ticks can be to reduce the interval between treatments or to limit the application of pesticides to a specific season (seasonal treatment) and the interval between immersions must be calculated according to the life cycle of the specific species of tick involved. These approaches are based on the use of pesticide concentrations which will kill 100% of the ticks exposed and will not leave any to reproduce. Control over the concentration of pesticides used will be difficult if the producers (e.g. landholders and ranchers) are applying it.
4. Who is going to do the work? The answer will depend on factors like:
 - (a) Method of application - spraying equipment - ear branding.
 - (b) Educational level of the producers.
 - (c) Tradition.
 - (d) Available funds, equipment and people.

The Puerto Rican Department of Agriculture is planning a control programme which is expected to operate independently and separately

from the present Eradication Programme for Boophilus, but only in the areas outside of the two quarantine treatment areas we have. It is proposed that producers be trained by extension personnel and Puerto Rican teams employed by the Department of Agriculture who would assist dairy farmers with the application of pesticides to dairy cattle with deep spraying machines. The Government of the United Kingdom plan to subsidize up to 50% of the construction cost of baths for operation with bovine livestock and to assist producers in doing away with the waste in the said baths.

5. How will the persons applying pesticides be protected from the pesticides? If spraying equipment is going to be used, security equipment has to be available, and used and maintained in an appropriate manner. If this is not done, there will be serious problems with respect to the health and longevity of their employees.
6. How will the tick samples be identified and collected? Once again, a manageable system has to be established and the personnel must be trained.
7. What type of regulations exist for the availability of pesticides? In what way will the pesticide residues in the baths be disposed of? What pesticides must be used? This is a crucial point. The reputation and credibility of the programme will be on trial. If the pesticides selected have been widely used by producers in a controlled manner, then you can be almost certain that it will have resistance or tolerance problems. For example, we have a clinical report on tolerant ticks in two areas of Puerto Rico. Co-ral has been used extensively in both areas by landowners. The specimens have been laboratory tested to measure the degree of tolerance present. We are using a concentration of 0.25% in the Eradication Programme of B. microplus and A. variegatum. Up to now no tolerance has been observed, reported or found in our laboratories with respect to any of the two.

I have surely only mentioned a few of what would hopefully be the most important areas for consideration. Whatever it has been, just

start working on the daily operation of an Eradication or Control Programme and you will be able to better appreciate the challenge of this adventure.

Eradication

The main difference between control and eradication is probably in aptitude and a bit of confidence with regard to the protection of herds and areas once they have been liberated.

The world record for eradication of ticks suggests that precaution, planning and decision are necessary in order to be able to achieve eradication and maintain the area free after eradication, as well as having an available system which responds to any outbreak which may occur.

I would like to read a letter which was sent to an eradication official in the States from Puerto Rico by a competent Entomologist. It illustrates the confusion resulting when an exotic tick is introduced and regulations officials are not completely prepared before the tick becomes widespread.

Now, I would like to discuss in detail various aspects of our Eradication Programme in Puerto Rico.

Thank you.

OUTBREAKS OF EQUINE ENCEPHALITIS IN THE DOMINICAN REPUBLIC

by
Dra. F. Rosario Cabrera T.

Introduction

Since 1948, outbreaks of Equine Encephalomyelitis disease have been taking place, diagnosed for the first time in that same year.

After this first outbreak, others followed in 1955, 1960, and the last one in 1978. With the exception of the year 1960, the outbreaks took place in the same months of the year, approximately from February to April.

In places where epidemics of encephalitis appear similar ecological conditions are observed such as the presence of lagoons, swamps, an abundant population of mosquitoes and birds, dense vegetation and a considerable equine population.

With regard to control measures taken with these epidemics, several activities take place from the time the active virus is identified:- the positioning of sanitary cordons to avoid its spread to other areas, control and investigation of the vector population, vaccination of susceptible animals and slaughter of the diseased ones.

The type of virus isolated as causing the disease in all the outbreaks is the Eastern Equine Encephalomyelitis (EEE) type.

The summary given below was taken from the written account of authorities and persons who took part in the control of this disease in each one of the outbreaks.

First Outbreak

The knowledge of the presence of this disease in 1948, in the month of November, has been considered as the first outbreak of Equine Encephalomyelitis in the Dominican Republic, and it lasted until April, 1949.

The first cases analysed were in the Monte Cristy area (next to the sea) in the north-east of the country, and slowly the outbreak continued towards the east covering an area of 28 km in length by 6 km in breadth, between Carretera Duarte and the northern margin of the Yaque River.

The human population was affected with 9 deaths taking place out of a total of 13 patients, 12 children and 1 adult; equine deaths reached 600 and 27 138 horses were vaccinated.

The diagnosis was done by Complement Fixation (CF), the serum samples being processed in the National Public Health Laboratory.

The result was a high incidence of Eastern Equine Encephalomyelitis (EEE) and a low one for St. Louis Encephalomyelitis (SLE) and Eastern Encephalomyelitis (WEE), taking the two latter ones as non-specific reactions.

The Eastern virus type was isolated by brain sample in the U.S.A. by Dr. Randall of the North American Army Veterinary School.

Other cases occurred in March, 1949 in the area of Sánchez to the north-east of the country, some 200 km from the first cases of the previous year.

A report of 136 horse deaths was recorded in 1949 with no reports of human cases. The presence of the Eastern type virus was confirmed again.

A control campaign of the epidemic encephalitis was implemented in which 19 547 animals were vaccinated and the zone isolated by a sanitary cordon which prevented any horse or bird from leaving.

Second Outbreak

In February 1955 the presence of the Equine Encephalitis disease was reported in the south-eastern provinces of the country, especially between the provinces of Independencia and Bahoruco (Neyba).

The same action was taken as in the 1948-49 outbreak.

The disease analysed, some 22 750 horses were vaccinated with a vaccine effective for both types (EEE and WEE).

There was no report of human cases. 320 dead horses were found; with the objective of detaining any spread of the disease 210 animals were sacrificed.

One of the first measures taken by the State Secretariat for Agriculture was the installation of a sanitary cordon to isolate the affected zone prohibiting all kinds of animals from leaving.

The Maraliological service of the State Secretariat for Public Health disinfected dwellings and vehicles passing through the cordon.

The last case was observed in March, the quarantine being lifted in April of the same year.

Third Outbreak

In June, 1960 a new outbreak was detected on which there is not much information. It took place in the province of Monte Cristy in the Castanuelas area, township of Villa Vásquez in the same section as the 1948 outbreak.

This epizootic lasted 5 months. Other new cases appeared in the city of Mao, located 30 kms to the east of Mao, with the upsurge of isolated cases in the provinces of Duarte and Samaná more than 100 kms to the east of Santiago.

Vaccinations were given as in previous outbreaks with more than 40 000 horses being vaccinated from the beginning to the end of 1960, with 400 animals dying from the disease. No human case was recorded.

The epidemic was controlled by vaccinations and the sanitary cordon which isolated the affected zones.

Fourth Outbreak

On February 17, 1978 the Ministerial Department for Livestock received notification of the possibility of Equine Encephalitis cases from the Sánchez in the north-western part of the Dominican Republic.

After being clinically analysed by pathologists of the Central Veterinary Laboratory, brain and blood serum samples were sent to the U.S.A. Laboratory in Ames, Iowa, who confirmed by serology and isolated the Eastern Equine Encephalomyelitis (EEE) virus. Serology tests through Serum Neutralization (SN) and Haemagglutination Inhibition (HI) were carried out.

Control measures were discussed by a special multi-disciplinary commission coming to the following agreements:

- (a) Dilimitation of the problem zone.
- (b) Establishment of a sanitary cordon.
- (c) Control of mosquito-vectors through the disinfection of vehicles, houses and swampy areas.
- (d) Dissemination of sanitary education on the risks of the disease.
- (e) Vaccination of animals with vaccine effective for both diseases (EEE and WEE).
- (f) Slaughter of diseased animals.

A large quantity of possible vectors of the disease, from which the virus was not isolated, were captured and different species of birds were also examined as carriers. A serological investigation was also carried out in the affected area with human and horse serum.

The presence of anti-bodies in horses was analysed owing to a recent infection, and in persons it was found that their infection with the said virus had possibly been produced long before. Of those persons three had been born between 1957 and 1967, four between 1947 and 1957, three between 1937 and 1947 and one before 1926.

Discussion

The Equine Encephalomyelitis outbreaks which occurred in the Dominican Republic have certain similarities like the ecological medium, duration of 3-5 months, the presence of the same type of virus (EEE), an approximate mortality of 34 out of 1 000. In spite of that, sufficient evidence does not exist to precisely determine the indices of EEE cases among horses and humans.

According to Dr. Eklund (1949), it is probable that the virus existed in natural reservoirs (birds) for some indefinite time and that special meteorological conditions encouraged the activation of the virus. This justified the simultaneous outbreak in different areas far away from each other.

This concept of Dr. Eklund's is valid even in following years since the migration of birds from the southern zone of the U.S.A. occurs year after year to the country in previously affected zones. However, the disease is not observed with a cyclical period but between the last and second to last outbreaks there was a difference of 18 years, unlike the previous years which had 5 year intervals.

According to Dr. Ulises Cruz Ayala, at the symposium which took place in 1949 revaccination for Equine Encephalitis was discussed which should be done within a three year period after the last outbreak with a single skin injection. However, in countries like ours, it should be done every six months due to the large vector population, but he concluded that massive vaccination of all horses was not necessary, because it turned out costly as well as useless.

A new insight could be made regarding the difference of years between each outbreak, other than climatic and meteorological disorders perhaps it could be the presence of a susceptible young animal population which, on contact with a large vector population, provoke the outbreak.

In recent investigations carried out in the Central Veterinary Laboratory we have been able to analyse the latent presence of the virus in reservoirs in some sampled zones where cases have been previously observed. Anti-bodies of the disease have been found in a hen, which reveals an important point since this bird lives closely with man.

In spite of the presence of the disease in the country for years and no other outbreak being observed after 1978, investigations, other than those done during the outbreaks, are beginning this year (1981) with the implementation of the diagnostic test in the Central Laboratory.

Sampling has still not been terminated in all risk areas since other conclusions can arise after these tests.

We will have to continue with a vigilance plan of this zoonosis which involves the threatened areas with knowledge of the situation of our neighbour country Haiti, with investigation of the reservoirs and vectors, serological follow-up of susceptible animals, investigation of the migratory course of birds

which act as reservoirs, and in addition, the joint determination of climatic changes.

The study of each one of these variables will be able to give us a greater vision to effectively prevent Equine Encephalomyelitis in the Dominican Republic.

LABORATORY DIAGNOSIS FOR EQUINE ENCEPHALOMYELITIS
(EEE, WEE, VEE)

by
Dra. Lucía Duval de Pou

For Equine Encephalomyelitis diagnosis of all types we can follow two courses: virus isolation and identification studies, and serological studies.

Virus Isolation

From consistent tests on brain tissue, blood of sick animals and mosquitoes, 10% purified suspensions are prepared (undiluted serum can also be used) which are inoculated through the brains of lactating rats 2-4 days before giving birth. The lactating rats are very sensitive to the virus and it is a sure, quick way of carrying out the virus isolation. Nervous symptoms or death of the young rats are seen within the next 48 hours after the inoculation. New passages of the virus in rats increases its virulence killing the young rats within an 18-24 hour period. The viruses isolated from this inoculation can be identified through neutralization tests.

The virus also multiplies in embryonic chicken eggs and in chicken fibroblast cell cultures, rat embryo, BHK (Baby Hamster Kidney) or it may be Rimón Hamster baby cells and in duck embryo cells.

Serological Diagnosis

Neutralising haemagglutination inhibitors and anti-bodies are present in the serum a few days after the symptoms start in the animal.

Through Serum Neutralization tests or Haemagglutination Inhibition tests we can detect these anti-bodies very early in sick animals. A second analysis, 2-3 weeks after having observed the first symptoms, will show an increase in the number of neutralizing anti-bodies and haemagglutination inhibitors. These two stereotyped signs at 2-3 week intervals confirm the diagnosis. The stereotyped signs must be observed by applying the same serological test.

The Complement Fixation anti-bodies appear later and also disappear first; they disappear within 2-5 years.

The neutralizing anti-bodies and haemagglutination inhibitors remain a few years.

The Haemagglutination Inhibition test is the simplest serological method of diagnosis, based on the ability of the virus to agglutinate the geese and chicken red blood cells. The virus agglutinating antigens are found in the infecting portion of the viral particle. The union between the antigens and the red blood cells is irreversible but it continues being infectious and can be made inactive by adding anti-bodies.

This Haemagglutination Inhibition test is the one used in the Central Veterinary Laboratory (Ministerial Department for Livestock, Dominican Republic) since it used inactive viral antigen, and at the moment, for reasons of security, we cannot work with the live virus.

The neutralization test, through plaque reduction using tissue culture, is the most specific for the serological diagnosis followed, in order of specificity, by the Complement Fixation test and then the Haemagglutination Inhibition (HI).

DIFFERENT STAGES OF ANAPLASMOSIS AND BABESIOSIS IN THE COUNTRY

by
Dr. Victor Mena Sánchez, Dr. Orlando Méndez
and Dra. Jocelyn Quirico de Pérez

Since the initiation of activities at the Central Veterinary Laboratory of the State Secretariat of Agriculture, the diagnosis of these diseases (anaplasmosis and babesiosis) is being made using the direct method (blood sampling and staining with Giemsa).

But in 1973 alone, we carried out 508 tests of which we have no record of procedures or animal species.

Then in 1974 we find the same situation, and in that year 724 tests were carried out.

In 1975, diagnosis through Complement Fixation of anaplasmosis and babesiosis began; the rapid method of Anaplasma Card test too.

During that time all tests conducted, especially with bovine and equine sera, were done through those two techniques (see Table 1).

DIAGNOSIS OF BOVINE ANAPLASMOSIS THROUGH CF (1975)

Disease	Positive	%	Negative	%	Total
Anaplasmosis	204	49.8	206	50.2	410
Babesiosis bigemina	57	12.2	408	87.8	465
TOTAL	261		614		875

The number of tests has no statistical value at a national or zonal level since this included two ranches in two different zones of the country.

These Complement Fixation tests were suspended due to the exhaustion of antigen supplies.

In that same year (1975), on an experimental ranch in Santo Domingo, 200 animals were examined, five of them proved positive for anaplasmosis and

this was equal to 2.5% positive animals. This figure was relatively low in relation to previous years when it varied between 10-15%.

In 1977, from two hundred and twenty-five (225) brains examined Babesiosis spp. was found in 33 of them, which is equivalent to 14.6% positive brains, from different zones of the country.

From 389 cows examined in that same year in the eastern zone of the country, 130 were proved positive for Anaplasma spp. and 7 positive Babesiosis spp. which is equivalent to 33.5% and 2.07% respectively. This low percentage of Babesiosis spp. in this zone is due to the moderate infestation of B. microplus in livestock. We are presently carrying out diagnosis by direct method although we will soon reintroduce diagnosis through Complement Fixation and Indirect Immuno-Flourescence (IFI).

Boophilus microplus in the Dominican Republic

The common tick in cattle in the Dominican Republic is the Boophilus microplus. This tick from the Ixodidae family causes great losses, not only through their spoliating action but also as transmitter of babesiosis and anaplasmosis diseases which cause at least 1% of the deaths among our bovine livestock.

This ecto-parasite has a world distribution which covers the entire area between the 32° north and south parallels. We can say that our Island is located in the centre of its highest activity since it is found in all zones of the Republic where there is bovine livestock.

The northern, north-western, north-eastern and central zones are the most affected since the majority of the cattle in those zones are dairy (Holstein, Brown Swiss, Charolais) breeds which are most sensitive to this tick. In the eastern zone the degree of infestation is moderate since the cattle population originates from Zebu crosses (Bos indicus) a breed which has shown its resistance to these ecto-parasites.

Several years ago the Boophilus annulatus was identified in the country but in our identification studies of the Boophilus we have not found this species again.

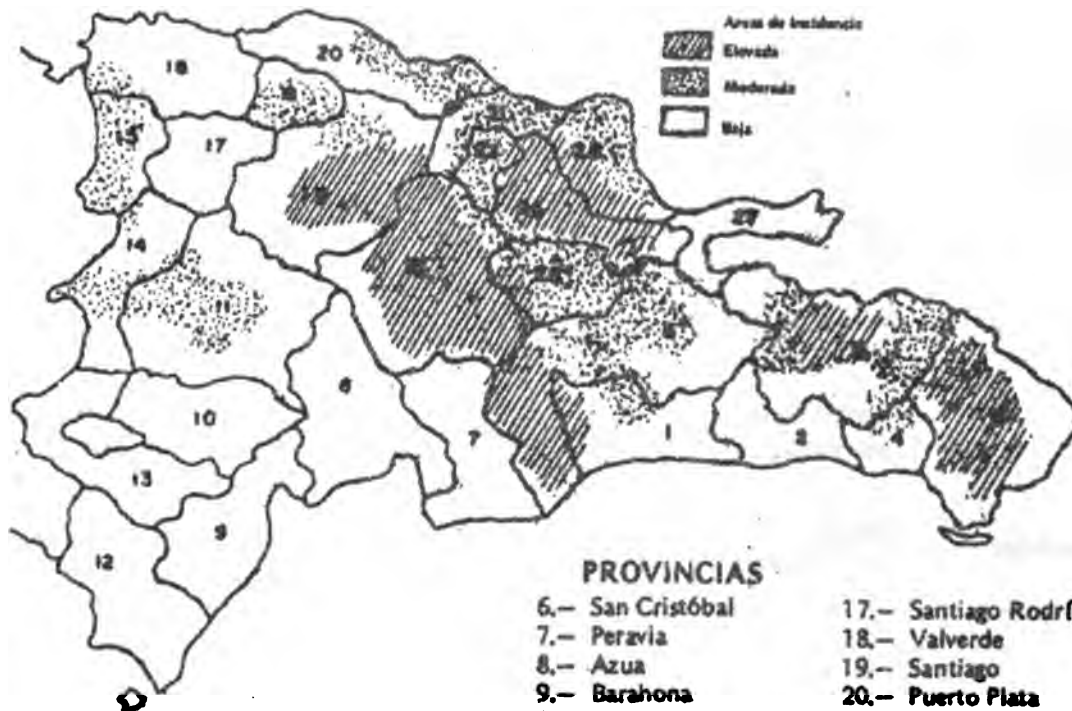
Other Investigated Ticks

In the distribution map for *Amblyomma* we see that the ruled areas are the places where tick-infested iguanas have been captured with *Amblyomma* *americanus*.

DISTRIBUTION OF TICK SPECIES IN THE D.R.

FAMILY	SPECIES	ANIMALS AFFECTED	GEOGRAPHICAL DISTRIBUTION
Ixodidae	Boophilus microplus	Bovine	The entire country
	Dermacentor nitens	Equine	The entire country
	Rhiphicephalus sanguineus	Canine	The entire country
	Amblyomma americanus	Iguana	South-eastern zone, Azua, Barahona, Estrelleta
Argasidae	Argas persicurs	Birds	The entire country

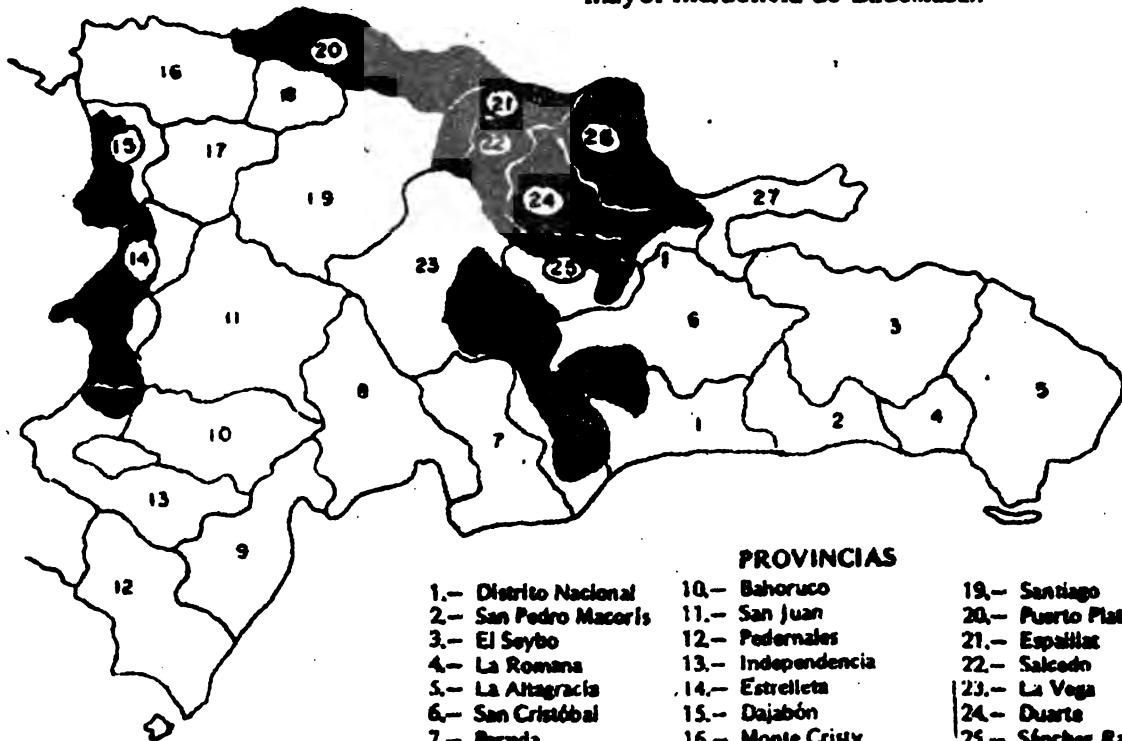
Fig. No.44: Anaplasmosis. Areas de mayor incidencia.



PROVINCIAS

- | | | |
|-----------------------|--------------------|-----------------------------|
| 1.- Distrito Nacional | 6.- San Cristóbal | 17.- Santiago Rodríguez |
| 2.- San Pedro Macorís | 7.- Peravia | 18.- Valverde |
| 3.- El Seybo | 8.- Azua | 19.- Santiago |
| 4.- La Romana | 9.- Barahona | 20.- Puerto Plata |
| 5.- La Altagracia | 10.- Bahoruco | 21.- Españat |
| | 11.- San Juan | 22.- Salcedo |
| | 12.- Pedernales | 23.- La Vega |
| | 13.- Independencia | 24.- Duarte |
| | 14.- Estrelleta | 25.- Sánchez Ramírez |
| | 15.- Dajabón | 26.- María Trinidad Sánchez |
| | 16.- Monte Cristi | 27.- Samaná |

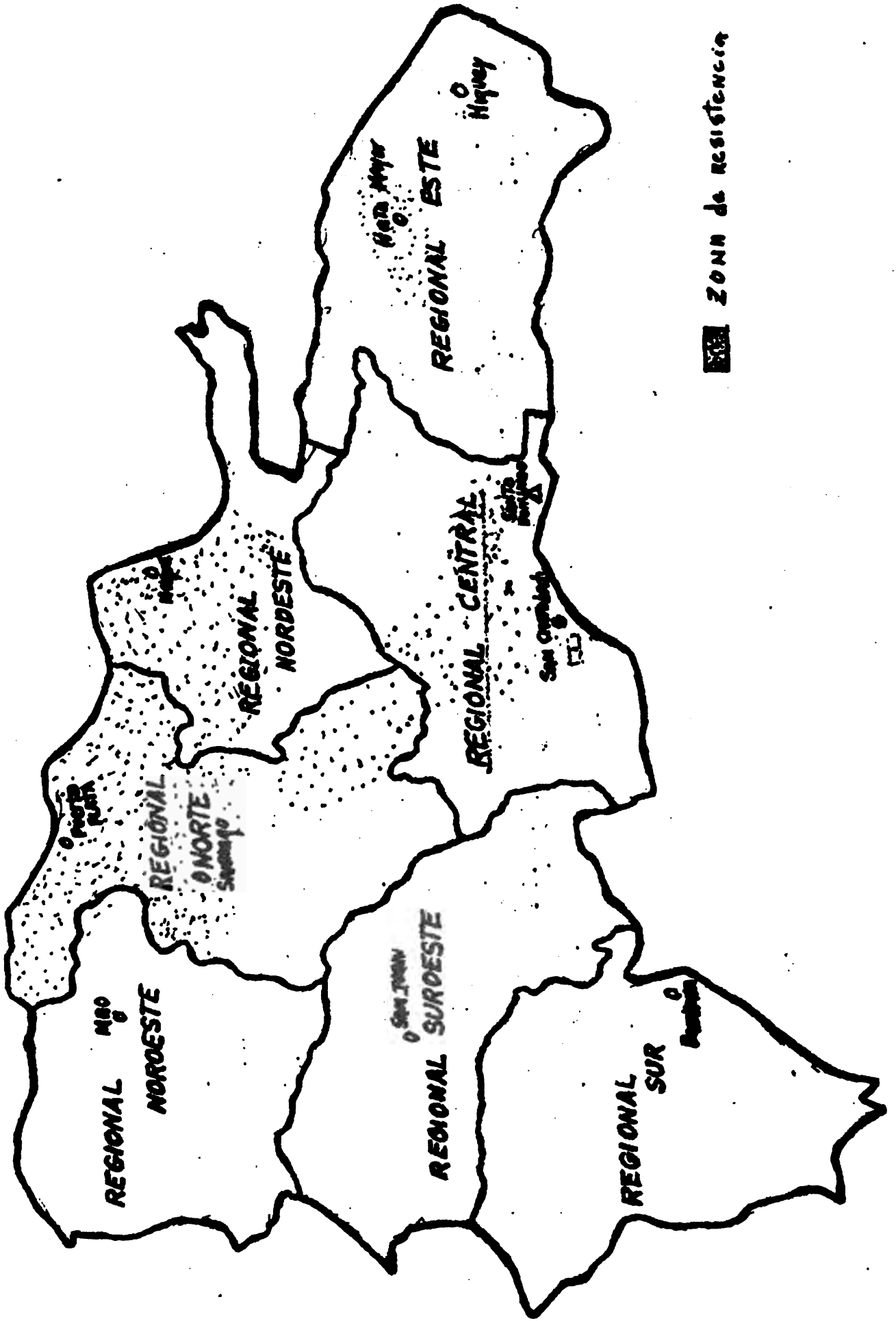
Fig. No.50: Babesiasis. Areas de garrapatois mayor incidencia de Babesiasis.



PROVINCIAS

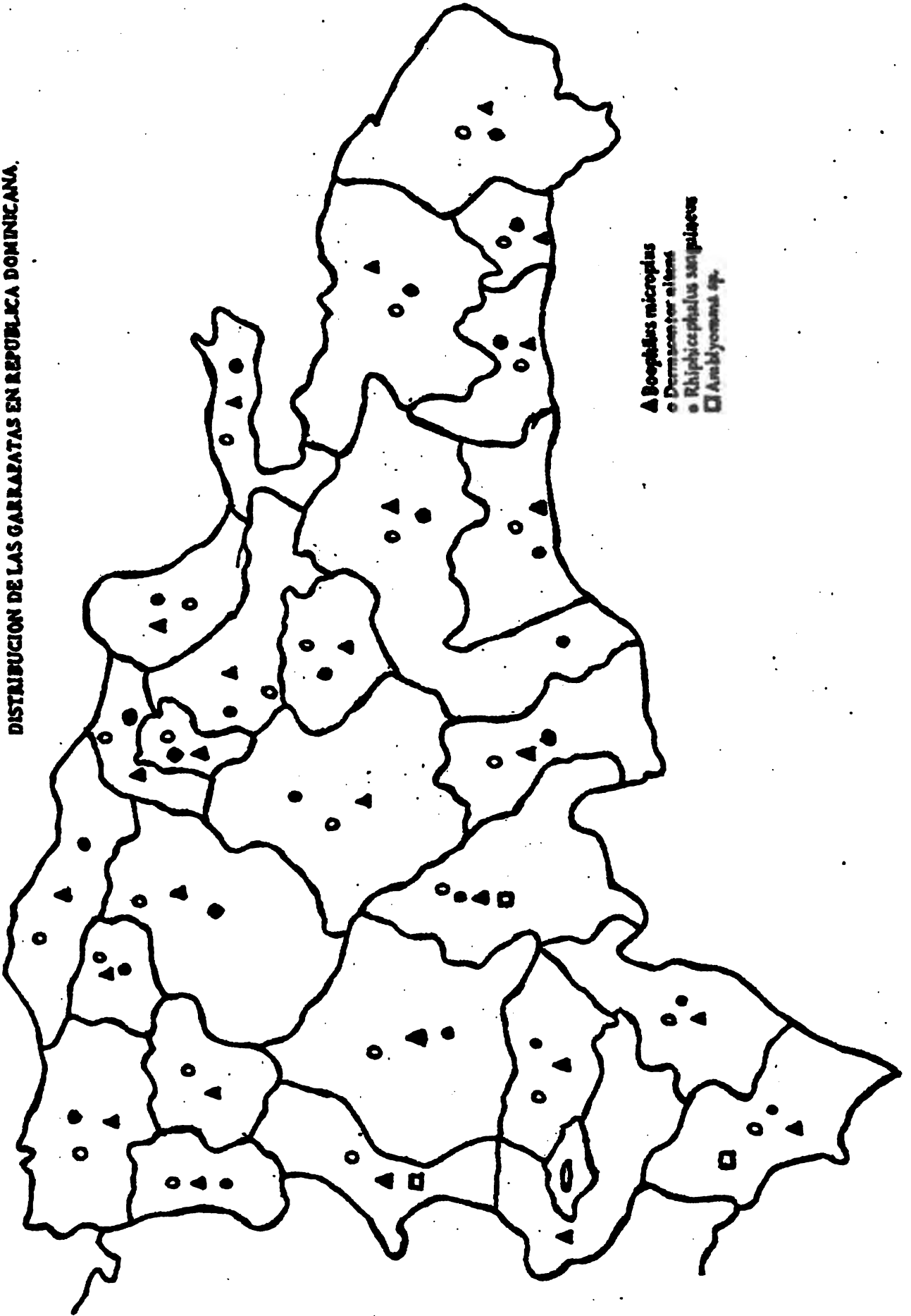
- | | | |
|-----------------------|-------------------------|-----------------------------|
| 1.- Distrito Nacional | 10.- Bahoruco | 19.- Santiago |
| 2.- San Pedro Macoris | 11.- San Juan | 20.- Puerto Plata |
| 3.- El Seybo | 12.- Pedernales | 21.- Española |
| 4.- La Romana | 13.- Independencia | 22.- Salcedo |
| 5.- La Altagracia | 14.- Estrelleta | 23.- La Vega |
| 6.- San Cristóbal | 15.- Dajabón | 24.- Duarte |
| 7.- Paravía | 16.- Monte Cristi | 25.- Sánchez Ramírez |
| 8.- Azua | 17.- Santiago Rodríguez | 26.- María Trinidad Sánchez |
| 9.- Barahona | 18.- Valverde | 27.- Samaná |

ZONAS DE RESISTENCIA A FOSFORADOS



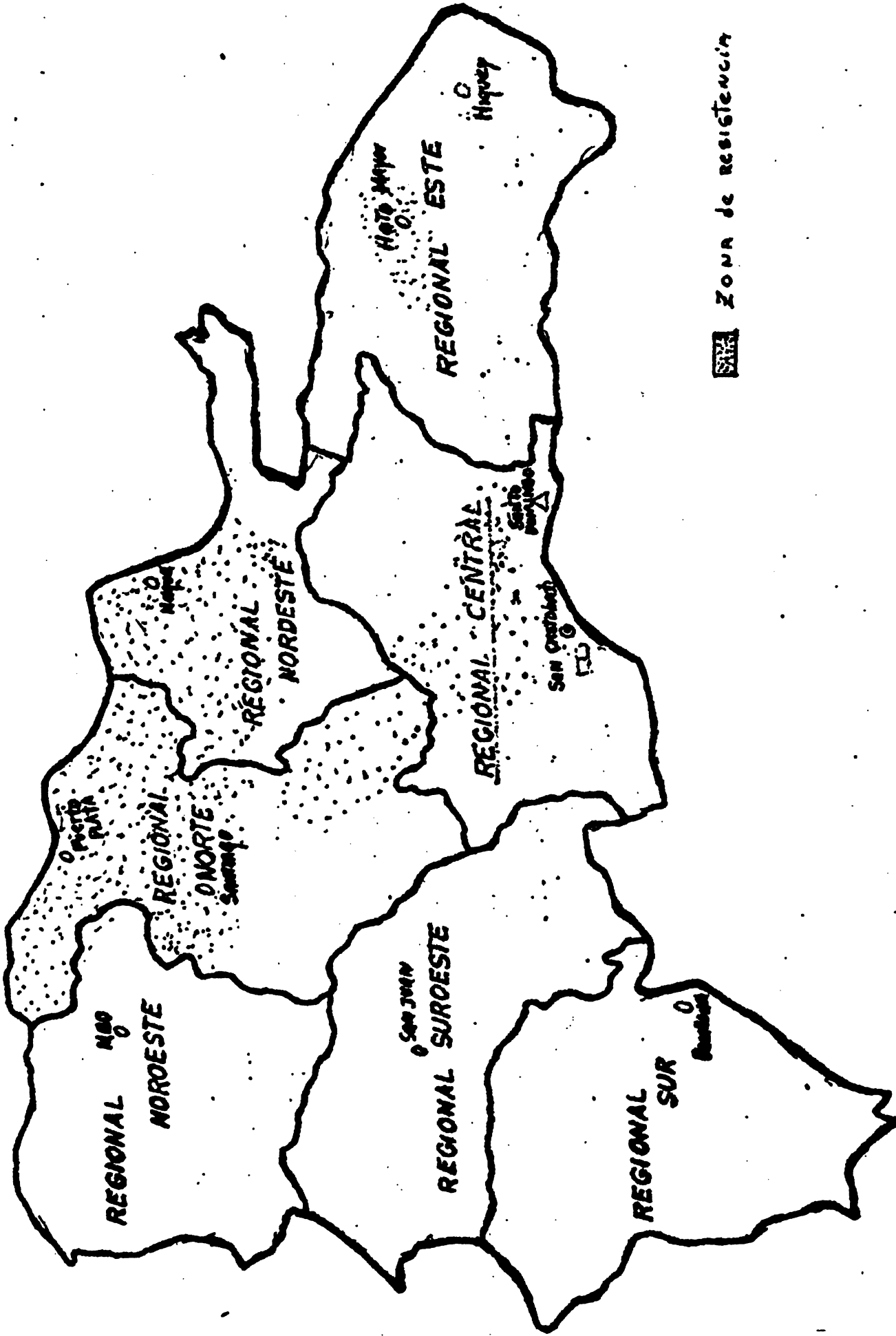
RES ZONA de resistencia

DISTRIBUCION DE LAS GARRAPATAS EN REPUBLICA DOMINICANA.



- ▲ *Boophilus microplus*
- *Dermacentor nitens*
- ◻ *Rhipicephalus sanguineus*
- ◻• *Amblyomma* sp.

"ZONAS DE RESISTENCIA A FOSFORADOS"



SARS ZONA de RESISTENCIA

REGIONAL SEMINAR ON VETERINARY EPIDEMIOLOGY

RECOMMENDATIONS ON EQUINE ENCEPHALITIS

Equine Encephalitis is an endemic disease for some areas of the Caribbean since the virus remains active in natural reservoirs (mosquitoes and wild birds) and it seems that when climate, susceptible animal population, and vector concentration conditions are all present, outbreaks occur.

The ideal situation of vaccinating horses annually would require the implementation of an entire vaccination campaign causing a considerable diminution of economic resources which would not be very justified due to the fact that the disease has only appeared sporadically.

- I. It is therefore recommended for the entire Caribbean and Antillean Area:
 - 1) To maintain a system of vigilance for the disease which covers the entire Caribbean area and includes, in addition, those countries outside of the area which also have this disease.
 - 2) To determine the way in which this vigilance will be implemented according to the resources available in the different countries, as well as the types of diagnostic tests to be adopted for the sample studies.
 - 3) To recommend the application of two different diagnostic tests which permit the comparison of results and, in this way, make available a much wider range of sensibility.

- II. For the Dominican Republic, where annual vaccination is presently almost impossible and considering that outbreaks have occurred at irregular intervals, the recommendations of the Seminar are:
 - 1) To maintain a system of vigilance with the realization of continuous samples and investigation of vectors and reservoirs of the virus which indicate the dynamics of the disease in the country.
 - 2) To vaccinate only in the case of an outbreak once it is known when an outbreak is appearing, together with other sanitary measures such as the control of the mosquito vector, control of the movement of susceptible animals, health education campaigns and other specific activities.

- 3) To maintain the production of a vaccine against EEE as a future goal of the Central Veterinary Laboratory as this is the only virus isolated in the country so far. This should be done when the laboratory equipment has the indispensable security requirements for working with the live virus.

REGIONAL SEMINAR ON VETERINARY EPIDEMIOLOGY

RECOMMENDATIONS ON TICK CONTROL

In view of the fact that cattle ticks are a constant threat to live-stock production, due to the injuries which they cause and the diseases which they transmit to animals, reflected in a diminution of food available to the human population, it is recommended that Governments of the area promote and support programmes for investigation, technical assistance and field control of ticks and the diseases they transmit, especially with regard to:

1. Proceeding with the installation of adequate laboratories for entomological and haemoparasitological studies.
2. Carrying out entomological studies which provide qualitative and quantitative information on the incidence and distribution of ticks in the respective countries.
3. Carrying out biological and population dynamics studies of ticks in the different ecological zones.
4. Carrying out studies on the susceptibility of cattle ticks to acaricides in use in the different zones.
5. Evaluating the most common tick control practices in each area and their incidence on the control of cattle haemoparasitosis.
6. Establishing control regulations on the importation, distribution and sale of acaricide products according to the strict requirements for each area.
7. Establishing norms and procedures for reducing the potential toxic danger of acaricides for persons exposed to them during their application and management.
8. Maintaining a health education programme which instructs on all aspects related to tick fevers and tends to reduce their effects on livestock production in general.
9. Encouraging the acquisition of equipment and inputs useful for the control of tick fevers of cattle.
10. Studying the prevalence and distribution of tick-transmitted diseases.

11. Carrying out tests on the control of tick-transmitted diseases.
12. Establishing norms and controls to reduce the spread of ticks in the different ecological zones of each country.

REGIONAL SEMINAR ON VETERINARY EPIDEMIOLOGY

GENERAL RECOMMENDATIONS

Epidemiology is an instrument of great value and efficacy for the study of the behaviour of diseases in animal populations since it permits a knowledge of the weak points where they can be attacked in the struggle to substantially diminish the losses of food for humans. In the light of experiences discussed in this Regional Seminar it is considered suitable to recommend:

- 1) That this science be incorporated into the studies of different faculties of Veterinary Medicine of the Antillean and Caribbean area.
- 2) That the number of seminars, courses and other activities which train and qualify technicians and professionals in the field of Epidemiology be increased.
- 3) To congratulate authorities of the Ministry of Agriculture in the Dominican Republic and the IICA Representation for sponsoring this interesting Regional Epidemiology Seminar which has given Animal Health field officials the tools necessary for combating diseases such as Tick Fevers and Equine Encephalomyelitis.
- 4) To thank governments of the area who allowed their livestock professionals to participate, as well as participants and observers in this Regional Epidemiology Seminar.

Santo Domingo, D.N.
July 10, 1981

SUMMARY
EVALUATION SHEET

REGIONAL SEMINAR ON VETERINARY EPIDEMIOLOGY

<u>OBJECTIVES</u>	<u>YES</u>	<u>PARTIALLY</u>	<u>NO</u>	<u>NO COMMENT</u>
Were these achieved?				
Epidemiological Principles.....	30	10	-	1
Collection and Analysis of Information.....	17	16	3	5
Tick Epidemiology.....	35	4	-	2
Tick Transmitted Fevers.....	38	2		1
Equine Encephalitis.....	36	4		1

Should more time be dedicated to some aspects? If the answer is YES, indicate below what these aspects would be.

1. 38 evaluations had affirmative answers.
2. 3 evaluations had negative answers.

What did you consider most valuable in this Seminar?

1. The majority said tick control and the exchange of ideas.
2. The lack of data or information on the participating countries was pointed out as a negative aspect.

Would you be capable of planning and implementing a programme related to the diseases mentioned below?

Vigilance 24 answered positively and 17 negatively.
Control 36 answered positively and 5 negatively.
Eradication 19 answered positively and 22 negatively.

What recommendations would you make for future seminars or activities?

1. That they are limited to diseases in the area.
2. That the epidemiological aspect is emphasized.
3. That the speakers are not of such a high academic level and, if possible, that they are not from developed countries.

Other comments: (write on the other side of the page)

10/10/10

10/10/10

SEA / IICA
VETERINARY EPIDEMIOLOGY SEMINAR

CLOSING SESSION

1. Address by Ing. Agrón José Daniel del Rosario, Director of the Office of International Cooperation of the State Secretariat for Agriculture.
2. Address by Dr. F.C.M. Alexander, Animal Health Specialist for the Antilles Zone Animal Health Programme under the auspices of IICA.
3. Address by Dr. Marcelino Vargas y Vargas, Director General for Livestock in the Dominican Republic.
4. Presentation of Certificates of Participation.
5. Toasts by the Secretary of State for Agriculture, R. Hipólito Mejía D.

SANTO DOMINGO, D.N.
July 10, 1981.-



