TRENDS IN VETERINARY PARASITOLOGY

A TWO-DAYS COURSE

DEPARTMENT OF VETERINARY PATHOLOGY, MICROBIOLOGY & PARASITOLOGY

FACULTY OF VETERINARY MEDICINE

UNIVERSITY OF NAIROBI

10^{TH} & 11^{TH} AUGUST 2011

VECTOR CONTROL AND PESTICIDES RESISTANCE

By

Professor W.K. Munyua, B.V.Sc., M.Sc, Dip An. Husb., PhD

VECTOR CONTROL AND PESTICIDES RESISTANCE

Presentation by:
Professor W.K. Munyua, B.V.Sc., M.Sc, Dip An. Husb., PhD.

What is a vector?

- A vector can generally be defined as an arthropod ectoparasite that transmits disease causing pathogenic micro-organisms including
- Protozoa, rickettsiae, spirochaetes & viruses to Man & animals, i.e.,

✓ Domestic livestock

✓ Companion animals

Reasons for vector control

- They cause irritation to their hosts through their bites when taking blood meals
- The host spend most its time scratching & grooming in an effort to dislodge the irritant
- This leads to loss in production as the host does not feed properly

- The vectors transmit disease causing pathogenic microorganisms

Examples of important vectors & the major microorganisms they transmit

Ticks: Various genera of ticks are biological vectors, i.e.,

- ***Rhipicephalus appendiculatus*** transmit ***Theileria parva parva*** & ***T. parva lawrencei*** the causal agents of East Coast Fever (ECF) and Corridor disease in cattle, respectively
- ***Boophilus decoloratus*** transmit ***Babesia bigemina*** & the rickettiae ***Anaplasma marginale*** & ***A. centrale***
- These organisms cause babesiosis & anaplasmosis in cattle, respectively

Important vectors contd.

- ***Amblyomma variegatum*** is the major vector of heart water that affect cattle and small ruminants and caused by ***Ehrlichia*** formally (***Cowdria***) ***ruminantium***

- ***Rhipicephalus sanguineus*** *transmit* ***Ehrlichia canis*** *the aetiological agent of canine monocytic ehrlichiosis(CME)*
- *Also transmit* ***Babesia canis*** *in dogs*

Important vectors contd.

- ***Oxides* spp**. (not identified in Kenya) have been shown to transmit in humans several organisms including ***Borrelia***, ***Anaplasma***, ***Coxiella*** & ***Babesia*** species as well as viruses such as tick-borne encephalitis (TBE) virus
- ***Glossina* spp**. (Tsetse flies) are the biological vectors of trypanosomes that cause human sleeping sickness & nagana of livestock
- Both conditions are collectively known as African trypanosomisis

Mosquitoes

- *Anopheles* spp. transmit protozoan parasites, ***Plasmodium*** spp. that cause human malaria
- ***Aedes*** mosquitoes (especially **Aedes meintoshi** that lays virus-infected draught-resistant eggs) are implicated in the transmission of Rift Valley Fever(RVF) that affect domesticated ruminants, camels & man
- Mosquitoes act as intermediate hosts of ***Dirofilaria immitis*** (a dog heartworm)

Important vectors contd.

- ***Simulium* spp** are commonly referred to as Black flies because of their color or buffalo flies, because of their hump-shaped thorax
- They are notorious vectors of ***Onchocerca volvulus***, a filarial parasite that cause river blindness in humans
- They also transmit the non-pathogenic ***Onchocerca gutturosa*** *&* ***O. linealis*** to cattle
- The protozoan pathogen ***Leucocytozoon*** is transmitted by ***Simulium* spp**. to poultry

Important vectors contd.

- **Culicoides spp.** or the Biting midges also transmit ***Leucocytozoon*** to poultry, ***Onchcerca cervicalis*** to horses & ***Dipetalonema spp***, a filarial nematode to man & some livestock
- They also transmit viruses which cause African horse sickness, Rift Valley Fever, Bovine ephemeral fever & Bluetongue of sheep & goats
- ***Phlebotomus* spp** – are the Sand flies that transmit ***Leishmania* spp**. to dogs & man

Control of vectors

- This can conveniently be considered under two separate parts viz.

✓Control of ticks

✓Control of tsetse & other flies

Control of Ticks: Will be confined to the hard ticks (Ixodidae)

- The control of ticks is by integrated pest management, in which different control methods are adapted, the major of which is the use of acaricides

Acaricides

- An efficient acaricide must: Be able to kill ticks
- ✓ Be stable & retain its acaricidal properties for a long time after mixing with water
- ✓ Be safe in use with no serious danger to humans or livestock
- ✓ Not be too expensive
- ✓ Be effective when the wash in dipping tanks has been fouled by mud, dung, urine & hair from animals

Acaricides contd.

- **NB**: The acaricides most widely used are sold as liquid "**concentrates**" which are diluted with water to form an "**emulsion**" before use as dips or sprays. After dilution, the concentrate is known as the "**dip-wash**" or "**spray wash**"
- The dilution instructions given on label **should always be followed accurately** in order that the final dip-wash or spray-wash contains the correct concentration of the acaricide so as to give good tick control results

Chemical groups used as aaricides

- The type of acaricide group to be use will generally depend on:-
- the ticks to be controlled, i.e.,
  - ✓ One – host tick
  - ✓ Two – host tick
  - ✓ Three – host tick
- The possible presence of resistance in the ticks
- The cost for the control
- The method of acaricide application in use

Acaricide groups

Clorinated hydrocarbons - Examples

✓Toxaphene

✓Dieldrin

✓Aldrin

✓Chlordane

Organophosphates (OP) – or organic phosphoric acid esters (OPAE)

- These were introduced after ticks started to develop resistance to chlorinated hydrocarbons

Organophosphorus compounds

- They exert their neurotoxic effect by inhibiting the enzyme acetylcholinesterase at the endplate of the nerves
- Thereby prolonging the resting time of acetylcholine at cholinergic pathways

- Examples: **Coumaphos** – asuntol, **Quinthiophos** – Bacdip, **Chlorphenvinphos** – Supona, Steladone, **Dichlorvos** – Vapona, **Dioxthion** – Delnav

Carbamates

- They inhibit cholinesterase & block hydrolysis of acetylcholine, acting somehow like the Ops
- Example: **Carbaryl** – Sevin

Cyclic Amidines (Formamidines)

They belong to a heterogeneous group of compounds which must principally be distinguished from the classical acaricides/insecticides as far as the way they work is concerned

Formamidines

- **Cyclic amidines** are also known as "**detaching**" **agents**
- They interfere with the metabolism of the tick; reduce the glycogen & glucose level & block the development of the ova
- They also interfere with the respiratory enzyme system of the arthropods
- They also cause a neuromuscular blockage
- Example: **Amitraz** – Triatix, Triatox

Plant derivatives

- **Pyrethroids**; **Pyrethrins**: Are derived from the flowers of Pyrethrum, ***Chrysanthemum cinerariaefolium***

- Several products have been synthesized & are used as acaricides & insecticides

- Examples: - Flumethrin, Deltamethrin & Permethrin

Pyrethrins contd.

- Some of the pyrethrins have now been combined with organophosphates:-

  Flumethrin 1.2 % + coumaphos 16

  Cypermethrin 5.6% + Diazinon 56%

Avermectins (Macrocyclic lactones)

- These are derived from **Actinomyces** & the active principle are **mycotoxins** which are active against both ecto- & endo- parasites hence being referred to as **endocticides,** Example: **Ivermectin** (ivomec)

Acaricide application methods

Dip baths (Plunge dips, dip tank)

- A tank set in the ground 1.0 m wide, 2.0 m deep & 4.0-6.0 m long containing 12,000-15,000 litres of water plus appropriate acaricide (i.e. dip-wash)

Spray – races (Mechanical or hand sprays)

- The acaricide is formulated in the same way as dip-baths

Application methods contd.

- **<u>Hand-dressing</u>:** This is manual application of an acaricide especially a grease containing acaricide on to specific areas of an animal like the ears,peri-anal region
- **<u>Pour – on</u>:** The method uses acaricides at a high concentration in oil, & a set volume per weight of the animal is poured along its back
- The acaricide (especially a pyrethroid) will spread superficially to give a fairly good coverage of the whole animal

Application methods contd.

Ear – tags: Some pesticides especially synthetic pyrethroids have been incorporated into plastic, from which they slowly diffuse to have their effects

- Also organophosphorus compounds are also used
- Obviously ear tags are effective against ear ticks
- **Injectables & Ruminal boluses**: The avermectins can be injected & a ruminal bolus formulation is available although their economic application to control ticks need evaluation

Control of tsetse & other flies

- The control of flies that attack livestock can be a difficult task but it generally requires the use of insecticides.
- To control ***Simulium,*** the insecticides are applied to rivers & with mosquitoes, drainage may also be necessary
- Aerial spraying can particularly be used in the control of tsetse flies
- **Endosulphan** (Thiodan) has been used against tsetse

Control of Tsetse flies

- **Traps** for the control of tsetse flies are now being produced
- They consist of a framed cloth screen of about 2.0 metres square, set up close to ground level so that it can rotate and act as a visual attractant – black & blue have been found to be the most attractive colors
- At the base of the screen, bottles slowly release the attractants **acetone** & **octenol** (the latter is a synthetic food flavour)

Tsetse control contd.

- The screen is treated regularly with a residual deposit of a special formulation of the synthetic pyrethroid deltamethrin insecticide (Glossinex)

- These traps can be effective at densities as low as four per square kilometer(km^2) & they can be used together with aerial spraying

Tsetse control contd.

Sterile male release

- Males are treated with gamma radiation to sterilize them but leave them mobile & sexually active
- They are released to unmated females that were fed as larvae artificially
- When mating occurs between the sterile males & the females, sterile eggs are produced
- (NB, a female tsetse is mated once)

Problems encountered in using chemical pesticides

Development of acaricide / pesticide resistance

- Often, there is some degrees of cross resistance within the same chemical class of pesticide/acaricide
- so that pests/ticks with resistance to one pesticide / acaricide are resistance to a related pesticide / acaricide even without prior exposure
- In ticks it has been shown that resistance can be passed to the next progeny indicating that it is heritable
- No resistance has so far been reported in tsetse flies

Problems encountered in using chemical pesticides contd.

- **Other problems include**:

✓Environmental contamination

✓Contamination of milk & meat products with chemical residues

✓The cost of the existing pesticides, or their unavailability especially in developing countries including Kenya

✓The high cost of developing new pesticides

New strategies in the control of vector arthropod ectoparasites

- Considering the problems that are likely to be encountered in the use of chemicals (acaricides/ insecticides) for the control of vector arthropod ectoparasites, it is clear that alternative effective control strategies are necessary

- Such methods for the control are:-

✓ Biological control

✓ Vaccines

Biological control

- Numerous potential pest biocontrol agents have been documented including

✓Pathogens

✓Parasitoids

✓Predators

- Among these agents, pathogens have widely been applied in the biocontrol of various vectors
- These pathogens include **bacteria**, **fungi** & **entomopathogenic nematodes**

Biological control contd.

- Of the bacteria, the bacterium ***Bacillus thuringiensis*** which attacks the midgut of insects has been found to be a useful biopesticide & indeed, its serotype H-14 has been used against larvae of mosquitoes & black flies
- Also, this bacterium cause mortality to ticks
- It has been established that crystalline delta-endotoxin of **B. thuringiensis** is produced during sporulation & this disrupts the insect's midgut walls

Biological control contd.

- Among the **entomophagous fungi** at least two species ***Beauveria bassiana*** & ***Metarhizium* anisopliae** have been shown to cause pathogenicity to ticks & are being used as biopesticides

- The entomopathogenic nematodes (EPN^{S}) of the families **Heterorhabditidae** & ***Steinermatidae*** are known to be obligatory parasites of insects

Biological control contd.

- The free-living stage of the nematode, the **third infective juveniles** (IJ^s) actively locates & enters the insect via natural openings & then releases symbiotic bacteria that kill the host, insect within 1-3 days
- The nematode then multiplies within the host cadaver & 6-18 days post infection thousands of (IJ^S) are released into the environment
- Most common natural habitat of these nematodes is moist ground

Biological control contd.

- The IJ^{S} are well adapted to the changing conditions of moisture, temperature, texture and chemical composition associated with different soil types

Parasitoids (wasps)

- The only species shown to control ticks is ***Ixodiphagus hookeri*** which is widespread naturally

Vaccines

- The only vaccines available or being developed against vector ectoparasites are vaccines against ticks

Vaccines

- These offer an attractive alternative to the use of acaricides for tick control

<u>Some advantages of anti-tick vaccines based on recombinant antigen</u>

- ✓ They do not pose a health risk
- ✓ They are environmentally safe & friendly
- ✓ Tick resistance through selective adaptation is less likely to occur

Vaccines contd.

- ✓ Anti tick vaccines allows for inclusion of multiple antigens in order to target a broad range of tick species as well as pathogen-specific antigens

<u>Tick antigens for anti tick vaccine development</u>

- Two distinct types of antigenic targets have been explored for tick vaccine development
- These are:
- ✓ Conventional antigens
- ✓ Concealed or ‘Hidden’ antigens

Conventional antigens

- These are secreted in saliva during tick attachment & feeding on the host
- They are also referred to as **Exposed antigens**
- These antigens are usually proteins or peptides synthesized in the salivary glands
- Exposed antigens are taken up at the tick feeding site by host **dendritic cells**, which process & present them to T-lymphocytes, priming a cell-or antibody-mediated immune response

Concealed antigens

- These are normally hidden from host immune mechanisms
- Typical ‘concealed antigens’ are those found on the tick gut wall & interact with specific immunoglobulin's taken up in the blood meal

- Anti tick effects include:

✓ Death of ticks

✓ Death of eggs

Concealed antigens contd.

- ✓ Decreased weights of egg mass laid Prolonged tick feeding time before engorgement
- ✓ Inhibition of moulting

- Vaccination with a ‘concealed’ antigen induces specific immunoglobulin's that are taken up with the blood meal as the tick feeds

Concealed antigens contd.

- If directed against certain gut-derived antigens antibodies interact with the concealed antigen on the surface of the gut, causing rupture of gut wall, leakage of blood into the body cavity, (the so-called "**red-legged ticks**" and death

- Concealed antigens do not induce natural immunity; they rely on adequate levels of vaccine-induced antibodies to be effective

Concealed antigens contd.

- Hence repeated vaccination may be necessary to maintain sufficient levels of protection that targets concealed antigens

<u>Ideal characteristics of an anti tick vaccine</u>

- ✓ Should be active against all tick species
- ✓ Should be active against all tick stages
- ✓ Should give a long-lasting immunity
- ✓ Should deter ticks from attaching

Concealed antigens contd.

- ✓ Should reduce disease incidence
- ✓ Should have No vaccine resistance
- ✓ Should be cost-effective
- Vaccination using ‘concealed’ antigen derived vaccine is recommended in conjunction with strategic use of acaricides in integrated tick management system
- Two commercial vaccines have been developed so far for the control of tick infestations on cattle. These are:-

Commercial vaccines

- Tick GARD Plus® in Australia & GAVAC® in Cuba
- Both vaccines are base on the same recombinant antigen - **Bm 86**,
- **Bm 86** is a glycoprotein & is located predominantly on the surface of midgut digest cells

Pesticides resistance

- **<u>Tick resistance to acaricides</u>**
  When a group of acaricides or one type of an acaricide within a group is used very frequently to dip or spray animals, there is often a chance for ticks to develop resistance to such an acaricide

- Ticks, mites (or other insects) when they acquire resistance to a chemical, this ability to survive the acaricide treatment can be passed to the forthcoming progeny of the arthropod

Tick resistance to acaricides

- When this happens on a farm, the only possible course of action is to change to another acaricide which will deal with the resistant ticks since an increase in concentration of the dip in use is rarely effective & may even lead to toxicity in the treated animals

- Often, a one-host tick lick ***Boophilus*** is more likely to become resistant to acaricides compared to a two - or three-host tick

Host acquired resistance to ticks

- With regard to hosts, it has been shown that an acquired resistance to ticks can occur with repeated tick infestation & this can be expressed by the following:-

✓ Reduced engorgement weight
✓ Longer feeding period before full engorgement
✓ Decreased ova production by the females
✓ Reduced egg viability (or increased egg mortality)
✓ Increased adult tick mortality

Tick resistance

- To test for acaricide resistance use:-
  - ✓ Engorged female tick method
  - ✓ Larval packet method

<u>Engorged female tick method</u>

- An acaricide when received initially, it is formulated as an **Emulsifiable concentrate** containing:-
  - ✓ 25 % active ingredient (AI)
  - ✓ 65 % xylene (solvent)
  - ✓ 10 % Triton X-100 (emulsifier)

Engorged female tick method

- When available, commercially supplied emulsifiable concetrates & wettable powders (WP) are used
- The formulations are diluted with water to 1.0-,0.1-, and 0.01- per cent concentrations immediately before the ticks are treated
- Usually 50 ml of the 1.0% concentration is prepared
- 5.0 ml of this concentration is diluted with 45 ml of water to 0.1%
- In turn, 5 ml to 0.01%

Testing procedure

- Engorged female ticks are collected
- They are washed in tap water to remove any debris on them
- They are dried & placed in groups of 10-ticks
- They are then weighed
- The weighed group of 10 ticks is placed in each dilution of the acaricide under test
- The liquid & the ticks are vigorously stirred for about 30 secs

Testing procedure contd.

- The liquid and ticks are then poured out on wire screen to recover the ticks
- Ticks are allowed to dry for few minutes and then they are placed on paper towels to dry
- After ticks are dry, they are placed in cotton stopper glass vials
- They are then placed in an incubator and held at 27º$\pm$1ºc and above 80% relative humidity for oviposition

Testing procedure contd.

- After 2-3 weeks in the incubator the oviposition should be over & the female ticks are discarded
- The eggs are weighed
- The eggs are allowed to remain in the incubator

- After about a month the eggs will have hatched & the hatch is estimated visually by comparing the number of larvae or empty egg shells with the number of unhatched eggs

Testing procedure contd.

- For **controls**, several groups of 10 ticks collected at the same time from the same animals as above group are dipped in 50 ml of emulsion
- The emulsion contains 2.6% xylene & 0.4% Triton X-100 (the amount in a 1.0% active ingredient (AI) concentration or the combination with N-methyl-2-pyrrolidone
- Thereafter, the **controls** are handled in the same way as the toxicant-treated ticks

Testing procedure contd.

- The effectiveness of a candidate acaricide is determined by the formulars

✓ Drummond, R.O; Ernst, S.D; Trevino, JL,Gladney, W.J. and Graham,O.H. (1973) ***Boophilus annulatus*** and ***Boophilus microplus*** laboratory tests of insecticides, *J. Econ. Entomol*., **66**: 130-133]

- **First**: Estimated reproduction(**ER**) is calculated for each group of treated ticks & its control groups as follows:-

Testing procedure contd.

- **ER** = wt of eggs laid (gm) X Estimate hatch % X 20,000 wt of females (gm)
- In the formular, 20,000 is a constant for the larvae in 1.0 gm of eggs

- **Second**: The **ER** of each group of treated ticks is compared with the **ER** of its control groups, to find the percentage of control afforded by each acaricide concentration

Testing procedure contd.

- Control % $= \frac{\text{ER control ticks} - \text{ER treated ticks}}{\text{ER control ticks}} \times 100$

- In these tests, a concentration of a candidate acaricide affording more the 90% control of ER is considered effective

Larval packet test

- Already the FAO World Acaricide Resistance Reference Centre (WARRC) in Berlin, Germany has developed an acaricide resistance Test Kit
- The test entails the exposure of tick larvae for 24 hours in the incubator to acaricide impregnated filter papers at different concentrations
- Subsequently, per cent mortality is determined for every acaricide concentration
- The acaricide concentration dose & per cent mortality graph is plotted