Institute of Virology & Environmental Microbiology

Annual Report 1995 - 1996

Centre for Ecology and Hydrology
Natural Environment Research Council

Institute of Virology & Environmental Microbiology

Annual Report 1995 - 1996

Centre for Ecology and Hydrology
Natural Environment Research Council

Front cover

Main picture: Tick habitat

Inserts (top to bottom):
IVEM
Host range testing of a viral biopesticide
Lesions produced by a plant virus
Ixodes ricinus, the common sheep tick

The IVEM mission is to:-

- Advance the science of environmental microbiology (including virology) through high quality internationally recognised research leading to a better understanding of the functional roles and structure of micro-organisms in the environment and the interactions of micro-organisms with their natural hosts.

- Investigate, through experimentation, monitoring and modelling, natural changes in the ecological environments of micro-organisms and to assess past, present and future effects of man's impact on them.

- Secure, expand and provide data relevant to environmental microbiology (including virology) to further scientific research and provide the basis for advice on environmental conservation and sustainable development to governments and industry.

- Promote the use of the Institute's research facilities, expertise and data to provide research training and education of the highest quality, and to enhance the United Kingdom's industrial competitiveness, research basis and quality of life.

Report of the Institute of Virology and Environmental Microbiology (IVEM)

1995–1996

Centre for Ecology and Hydrology

Natural Environment Research Council

Contents

Contents

Foreword by CEH Director

Robust and timely responses to major environmental problems, such as the sustainability of natural resources, climate change, pollution etc, are only possible if based on sound interdisciplinary science.

Recognising the importance of this holistic approach, the Natural Environment Research Council restructured its activities during 1994. A major element of this reorganisation was a grouping of four NERC Institutes into the Centre for Ecology and Hydrology (CEH). Research within CEH is concerned with the land on which we live, its fresh waters and the living organisms which share the environment with us. Component Institutes of CEH are:

- Institute of Freshwater Ecology
- Institute of Hydrology
- Institute of Terrestrial Ecology
- **Institute of Virology and Environmental Microbiology**

CEH has some 625 staff (475 Scientists) and about 300 visiting scientists and students, well equipped laboratories located throughout the UK (Appendix 1), and a reputation for excellence in national and international research, monitoring and data collection. As such CEH must have one of the strongest capabilities in the world for undertaking holistic research in the terrestrial and freshwater sciences.

The CEH capability to address multidisciplinary issues has been strengthened during the past year following a full review of its research and a reshaping into ten new Research Programmes. These set the course for research over the next five years and beyond. The new Programmes have been formulated by the CEH Directors and scientists working closely with the external assessors who constitute four Programme Review Groups. In drawing together the new Research Programmes the scientists have also been cognisant of: the "wealth creation" and "quality of life" thrust in the 1993 White Paper, "Realising our Potential - a Strategy for Science and Technology"; the National Technology Foresight Programme; the UK Government's and European Union's legislation and policy; and the content of the major international science programmes.

The Institutes' activities have been further cemented by cross-Institute interdisciplinary research projects. These form part of the Integrating Science Fund which was established last year. Some seven projects were funded during 1994/95. All have made good progress. During the present year a further 6 have been approved (Appendix 2).

During 1994 the CEH Institutes were required to prepare papers and give evidence to the Office of Public Science and Services as part of the "Efficiency Survey of the Public Sector Research Establishments". The published report of the Survey left unresolved the role of many Research Council

Prof W B Wilkinson

Centre for Ecology & Hydrology

Institutes within Public Sector science. In September 1995 the Government announced that 42 Public Sector Research establishments would be subject to further review to assess whether the "functions" of the establishments were needed and whether the Public Sector should provide these. Scope for further rationalisation was also to be examined.

CEH was subject to this so called "Prior Options" review. CEH provided extensive documentary evidence and made a verbal presentation to the Steering Committee undertaking the NERC Prior Options review. CEH took the opportunity to highlight the major benefits that have and will continue to flow to the ecological and hydrological sciences as a result of the strong interdisciplinary focus that is now possible through CEH. The NERC Steering Committee presented its report to Government in late July 1996. Neither the recommendations of the Steering Committee nor the Government response to these are known at the time of writing.

It is to the credit of all CEH staff that the research has remained buoyant and the science outputs have been maintained at a high level during the year against this background of uncertainty and potential change.

During the course of the year the **Institute of Virology and Environmental Microbiology** has made a major contribution in developing the new CEH research programmes. Professor Nuttall the Director of IVEM took the responsibility for steering two of the programme areas. It is also pleasing to note that IVEM are involved in a number of the CEH Integrating Fund Projects and that the Institutes links with other CEH Institutes have been enhanced during the past year. Under Professor Nuttall's leadership the science at IVEM is flourishing. Several of the more recent scientific achievements are described in some detail in this annual report which I commend to you. During the course of the year Professor Nuttall was confirmed in the post as the Institutes Director

I would like to take this opportunity to draw your attention to the complementary Annual Reports for 1995/96 from the other CEH Institutes and to the CEH Overview Report.

Centre for Ecology & Hydrology
Institute of Freshwater Ecology
Institute of Hydrology
Institute of Terrestrial Ecology
Institute of Virology & Environmental Microbiology

Natural Environment Research Council

Prof P A Nuttall

Introduction by IVEM Director

During 1995 to 1996, further progress has been made towards integrating IVEM's research within the science portfolio of the Centre for Ecology and Hydrology (CEH). This has been achieved on two fronts. First, in response to the New Funding Model of its parent Research Council, the NERC, CEH has mapped its science projects against the Environmental Issues flagged by the NERC. The result has been the creation of ten Core/Strategic CEH Science Programmes. These are:

- Programme 1: Soil and Soil/Vegetation Interactions.
- Programme 2: Land Use Science.
- Programme 3: The Urban Environment
- Programme 4: Freshwater Resources.
- Programme 5: Biodiversity and Population Processes
- Programme 6: Pest and Disease Control and Risk Assessment for GMO's
- Programme 7: Pollution.
- Programme 8: Environmental Risks and Extreme Events
- Programme 9: Global Change.
- Programme 10: Integrating Generic Science

These ten CEH Science Programmes are funded by the Science Budget allocation from NERC and by complementary Commissioned Research. They cut across all the science undertaken within the CEH Institutes (Appendix 1) and thereby bring together scientists from different backgrounds who nevertheless have a common goal. Thus, for example, IVEM has played a lead co-ordinating role in the Biodiversity Programme, and has worked together with scientists from all Institutes to define projects within the programme. The scientific achievements described within this Annual Report are presented in line with the contributions from IVEM to six of the Core/Strategic Programmes.

The second front on which IVEM has strengthened its links within CEH is through the Integrating Science Fund (Appendix 2).

The organisation of IVEM has also seen some changes. Following interviews, I was appointed Director on 1st October, 1996. My background is in virology and bacteriology. To take advantage of funding opportunities, a new team, the Microbial Biodiversity Group, was formed with Dr Ian Thompson as Project Leader, under the Group Leader of the Molecular Microbial Ecology Group, Dr Mark Bailey. Another new project, PRIVEM (Products of IVEM), was created to exploit commercial spin-offs from IVEM science, the aim being to generate profits that can be ploughed back into research. Personal accolades won during the year went to Pat Nuttall who was given the title, Professor of Virology, by the University of Oxford, to Bob Possee, visiting Professor of Oxford Brookes University, and to Rosie Hails who accepted two lectureships in Oxford colleges, and had to decline the offer of one more!

After a year of restructuring and excessive paperwork, we look forward to focusing on what we do best - science!

Our aim for the forthcoming year is to demonstrate how widely microbiology underpins the science portfolio of CEH and the NERC.

Soil and soil-vegetation interactions

CEH Core Strategic programme 1

Fig. 1. Agar print of bacteria colonising the root, whilst degrading phthalate

Microscopic examination of samples by Victoria Goddard (Student)

Microbial degradation of phthalate in soil

Phthalate is produced in large quantities in the manufacture of plastics. It is toxic, commonly detected in water ways and implicated in lowered human sperm counts (it is believed to be an analogue of oestrogen). The detrimental impact phthalate may have on male fertility has stimulated interest in developing effective methods of removing and preventing the compound from entering the environment. The chemical degradation of phthalate (Fig. 1) by microorganisms was elucidated in culture over twenty years ago. However, until the present study, it had not been investigated *in situ*. Surprisingly, the degradative pathway of phthalate by soil microbes was found to differ from that observed in the laboratory. In culture, the side chains of the phthalate molecule are systematically removed to give monophthalate, phthalic acid and eventually protocatechuate. However, evidence obtained in collaboration with the University of Kent suggests that in soil, phthalate is converted to phthalic acid by demethylation of the side chain groups, leaving the ester bond intact. The implications of this novel pathway on exploiting microbial degradation of phthalate are currently being investigated.

Biodiversity and population processes

CEH Core Strategic programme 5

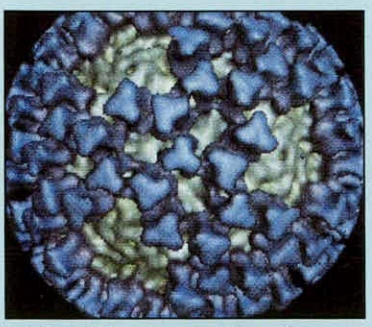


Fig.2 A 3D reconstruction of a baculovirus expressed BTV core-like particle. VP7 trimers = blue VP3 = Green

Claire Hill, Student

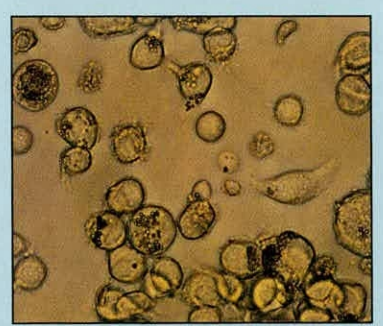


Fig. 3 Disper insect cell line infected with a cypovirus

Structural biodiversity in Reoviruses

Cryogenic-transmission electron microscopy (cryo-TEM) is a powerful technique for structural investigations of aqueous systems. It is unique in allowing direct observation of molecular structures and interactions, and of suspensions and solutions, in a thin layer of vitreous ice. Which is formed when cooling takes place at a high rate, and is essentially an amorphous form of water which preserves the specimen cryogenically in "suspended animation", remaining stable when observed in the microscope at temperatures between -140°C and -185°C. The technique is ideal for looking at microbes e.g. viruses, bacteria, fungi, protozoa. There are no artefacts (such as those that occur with conventional preservation techniques) which damage or alter the structure of the specimen (Fig. 2).

IVEM is using cryo-TEM to investigate the structure and function of a diverse range of viruses and microbes as well as comparing and characterising different isolates of an organism. Cryo-TEM is a rapidly developing technique which is gaining new applications in analytical biochemistry and biosensors as well as in other biophysical investigations.

Virus biodiversity at the structural level

Elucidation of viral structure, function and evolution at the genetic, molecular and atomic level underpins identification of the higher and lower order relationships of virus taxa. At IVEM, we are focusing on two different groups of RNA viruses:
(1) the gnat-transmitted complex, non-enveloped orbiviruses, for which transmission to particular vertebrate hosts is determined by the distribution and feeding habits of the insect vectors. Cypoviruses are single shelled icosahedral virions which characteristically become occluded within polyhedra in the cytoplasm of infected cells (Fig. 3) and (2) lipid enveloped, animal retroviruses whose transmission is determined by direct contact between hosts. By studying the genes and gene products of closely and more distantly related viruses, we have identified the extent of virus genetic relationships. For orbiviruses such as bluetongue virus (BTV),

extent of genetic relationships that exist between them. For orbiviruses such as bluetongue virus (BTV), and African horsesickness virus (AHSV), close relationships were shown by simple sequence comparisons (alignments of genes and their products). These observations were recently confirmed by comparisons of protein structures at the 3D level for the major capsid protein, VP7, of BTV and AHSV (Fig. 4). However, for more distantly related viruses, such as simian immunodeficiency and bovine leukaemia retroviruses (SIV and BLV, respectively), a genetic relationship was only demonstrated by protein 3D structural studies (Fig. 5), i.e., for situations where the constraints against evolution due to structural requirements meant that the 3D structures were comparable although the actual amino acid sequences involved were totally different (e.g., for proteins associated with virus assembly).

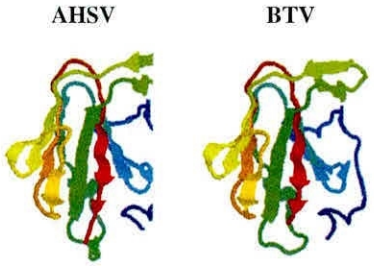


Fig. 4. The close relationships of orbiviruses is demonstrated by identical 3D crystallographic folding of major capsid protein VP7.

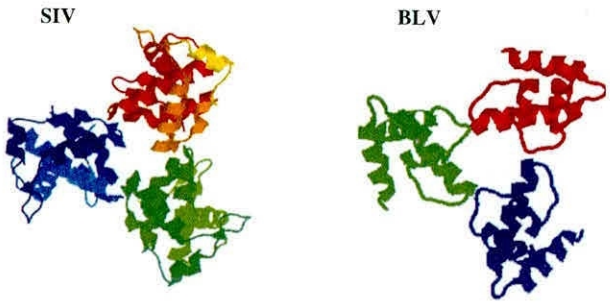
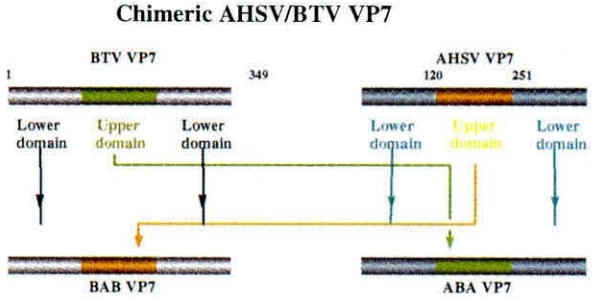


Fig. 5. The trimeric structures of the SIV and BLV matrix antigen, a major structural protein. Note their similar pattern of interaction despite their distant sequence relatedness.



The close relationship between orbiviruses is demonstrated by synthesis of domain-switched chimeric molecules for the major capsid protein VP7.

Soil nitrogen determines microbial population dynamics in the Ecotron

Microbial diversity in Ecotron soil

A new study has been initiated to investigate the microbial diversity in soil contained within the Ecotron. The Ecotron is a unique NERC sponsored controlled environment facility consisting of small replicated terrestrial ecosystems (mesocosms), sustaining up to 40 species of plants and animals in four trophic levels, interacting over several plant generations (Lawton JH *et al.*, 1993). It also represents a rare opportunity for environmental scientists working on organisms from different trophic levels (plants, insects and microbes) to work together in the same model habitat.

The initial part of the study has been to investigate the base line microbiology of the Ecotron soil. When the Ecotron is first prepared for experimentation, the soil is 'sterilized' to prevent germination of unwanted plant species. The indigenous microbial community is then introduced back into the sterilized soil. Reinoculation with a suspension of the original soil, resulted in the establishment of a soil microbial community that was fairly representative of the natural environment. Furthermore, replication between the replicate chambers was found to be excellent.

The population dynamics of the culturable bacterial community was then monitored closely over several plant generations, and revealed that the number of bacteria was strongly influenced by the availability of nitrogen in the soil. Using a combination of culture and molecular methods, the characteristics of the soil microbial community in the Ecotron (Fig. 6) will be compared to the microbial community in the field site from which the soil originated.

Fig. 6. Inside the Ecotron Reproduced by kind permission of J H Lawton.

Lawton J.H *et al.*(1993). The Ecotron: a controlled environment facility for the investigation of population and ecosystem processes. *Philosophical Transactions of the Royal Society of London (Series B)*, 341, 181-194.

Pest and disease control and risk assessment for GMO's

CEH Core Strategic programme 6

Fig. 7 Field site for release of recombinant baculoviruses

Fig. 8 Healthy caterpillars being placed on a plant

Fig. 9 Mamestra brassicae larvae infected with wild type Autographa californica nucleopolyhedrovirus

Risk assessment of genetically modified biopesticides

Fundamental studies of the baculovirus genome and genetic modification go hand-in-hand with laboratory and field assessment of genetically engineered products (Fig.7). Before genetically engineered baculoviruses can be developed as bioinsecticides, it is crucial to determine how they interact with their potential hosts at an organismal, population and community level.

The main risk to be assessed following release of modified baculoviruses into the environment is whether or not they (or the transgene) will become established in non-target hosts. Experiments designed to assess the range of hosts that are susceptible to baculovirus have shown that Lepidoptera differ in their response to the baculovirus virus *Autographa californica* NPV (AcNPV). We have therefore compared the behaviour of wild type baculovirus and AcNPV expressing an insect-selective scorpion toxin insect hosts of varying susceptibility. These studies have measured a range of parameters, such as virus productivity, pathogenicity and virulence each of, which is are crucial to the fitness and survival of the virus in insect populations. 7

Using a host which is highly susceptible (permissive) to, the cabbage looper *Trichoplusia ni* (Fig. 8) and a much less sensitive host (semi-permissive), the cabbage moth *Mamestra brassicae*, we have demonstrated the complex relationship that exists between the key biological parameters defined above and their dependence on both host species and virus type. For example, the recombinant virus that expresses scorpion toxin does not kill the semi-permissive host (Fig. 9) any faster than the wild-type virus, contrasting markedly with the response found in the permissive host *T. ni*. This has important consequences for virus productivity, a measure of virus fitness. Whether or not these relationships hold true for other less susceptible hosts is currently being investigated.

In a recent field trial, the relative susceptibility of two semi-permissive insect hosts with varying life history strategies were compared. One host was a

foliar feeder (and the cabbage moth), and the other a species which spends much of its larval stage in the soil (the common cutworm, *Agrotis segetum*).

Carefully controlled field experiments are essential for evaluating genetically modified biopesticides.

One consequence of infection with the recombinant virus that expresses the scorpion toxin is paralysis of the insects, which subsequently fall off the plant, thereby removing the inoculum from the vicinity of other leaf-feeding caterpillars. However, this may make the recombinant virus more available to insects which are soil dwelling.

Preliminary analysis showed that the foliar feeding *M. brassicae* only acquire infection following treatment with the wild type virus, as expected. However, the soil-dwelling cutworm becomes fatally infected following infection by either virus, indicating that these mobile larvae acquire virus from the plants (wild type virus) and the soil (recombinant virus). The difference in virus-host response has important implications for the spread of genetically modified viruses from target hosts, such as *T. ni*, to non-target hosts such as *A. segetum*.

Both these laboratory and field experiments demonstrate that the effects of genetically modified bioinsecticides, throughout their host range, are unlikely to be easy to predict. Our results underline the difficulties of extrapolating from laboratory data to the natural environment.

Evolution of baculovirus genomes

The isolation of over 400 baculovirus species from all over the world supports the notion that these viruses are very successful pathogens of insects. Analysis of their DNA genomes reveals wide variation in total size (90,0000 - 160,000 base pairs), indicating

Fig. 10. Computer predicted 3D structure of the baculovirus chitinase

considerable genetic heterogeneity. Baculoviruses probably acquire and lose genes according to selective pressures in the insect host, which would account for this variability. Although baculoviruses are generally regarded as very stable genetic entities, this perception does not take into account the enormous periods of time (millions of years) available for mutations to occur. Furthermore, a single virus "life cycle" is completed in less than 24 hours, which contrasts sharply with our own, "three score years and ten". We are particularly interested in how baculoviruses acquire and lose genes in order to estimate the stability of genetically modified virus insecticides. A chitinase gene (Fig. 10), apparently acquired from a bacterial species (*Serratia marcescens*) has been studied to determine if it was acquired in distant evolutionary history. We argued that if this hypothesis is correct, it should be present in most baculovirus genomes. However, if it is found in only a few, closely related viruses, this would suggest that it was acquired more recently.

Our studies have shown that many baculoviruses have chitinase genes, supporting the idea of an ancestral virus integrating chitinase into its genome. We are in the process of sequencing a number of these genes to

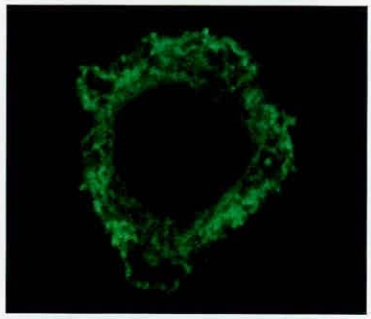


Fig. 11. Chitinase staining in AcMNPV infected cell

determine phylogenetic relationships and to improve our understanding of their evolutionary characteristics.

In related studies, we have also investigated the localisation of the chitinase protein within virus-infected cells. Surprisingly, chitinase was found associated with the endoplasmic reticulum (ER), the major highway within the cells for proteins destined for secretion or incorporation within the plasma membrane (Fig. 11). Chitinase appears to form aggregates within the ER.

Further examination of the predicted protein sequence of chitinase revealed results consistent with the presence of a signal peptide secretory signal at the amino terminal of the protein and an ER localisation motif (KDEL) at the carboxl end of the sequence. The signal peptide is required for entry to the ER and the KDEL for retention in this organelle. We believe that this unusual distribution of protein within the insect cell is a consequence of the chitinase gene being incorporated into an ancestral virus sequence bearing these important signals; the bacterial gene lacks these sequences. The chitinase protein is also of particular significance in the insect world as the liquefaction process observed in virus-infected insects depends on the synthesis of this protein.

Fig. 12 A site for the study of cabbage virus interactions

Integration of IVEM and ITE science has produced a model system for the assessment of genetically-modified crop plants.

Interactions of virus, aphids and wild brassica - a GMO impact study

This project is funded under the CEH integrating scheme, involving collaboration between IVEM and ITE (Furzebrook).

The distribution and impact of viruses in natural populations of plants is largely unknown, thus many of the early results of this study are providing a genuinely novel view of the interactions between plant hosts and viral pathogens.

The aim of this project is to investigate the ecology of virus-host interactions in natural populations (Fig. 12) of the crop relative of *Brassica oleracea* (wild cabbage). This work encompasses studies of the insect vectors of plant viruses and the interaction between these insects and the plants on which they feed. Variation in the plants affects their attractiveness and utilisation by the insects.

The work so far has revealed widespread infection by virus species in natural populations of the wild cabbage with several plants simultaeously infected by at least 4 viruses. Significant between-population variation in virus distribution and distinct patterns of virus species co-infection have been revealed. Analyses of vector distribution, and of population genetic structure using isozymes and microsatellite DNA loci, are providing a spatial framework for the study of virus distribution. By analysis of natural progeny differences in individual tolerance to viruses have been shown to have a heritable basis. The effects of virus infection on plant mortality and fecundity are being measured both in the natural populations and experimental trials. Preliminary analyses indicate that a relationship between plant age and viral infections is detectable at the scale of the survey. Effects of viruses on host performace, obscured by high variation in other environmental factors, may only be measurable over long periods.

Understanding and Controlling Arthropod-Transmitted Diseases

Many disease-causing agents (pathogens) are transmitted to animals (hosts) by the bite of blood-sucking arthropods

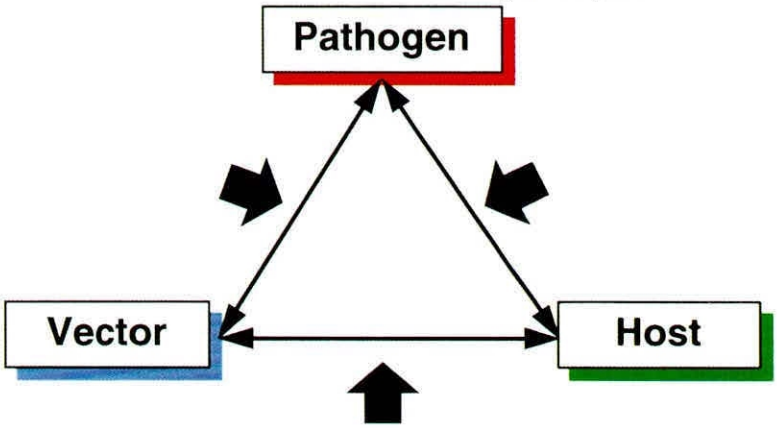


Fig. 13 The Parasite Triangle

(vectors) such as mosquitoes and ticks. Most of these arthropod-borne pathogens depend for their survival on wildlife species on which the vectors feed. The consequent interactions between pathogen, vector and vertebrate host can be viewed as a vector-borne Parasite Triangle (Fig. 13).

In the UK, the most important diseases caused by arthropod-borne pathogens are louping ill which affects sheep and grouse and is caused by louping ill virus, and Lyme disease, which affects humans and dogs and is caused by *Borrelia burgdorferi*, a bacterium.

Measures to control arthropod-transmitted diseases rely heavily on chemicals that kill the arthropod vectors.

Unfortunately, such chemicals can pollute the environment and may affect human health. By contrast, control methods focused on the Parasite Triangle are environmentally sustainable. However, despite the desirability of biocontrol compared with chemical control methods, there are commercial conflicts of interest. Hence the need for impartial research to demonstrate the feasibility and potential economic viability of biocontrol for arthropod-transmitted diseases.

Environmentally sustainable methods of control are offered by intervention at the level of the vector-host, pathogen-host, or pathogen-vector interaction (indicated by ➨ in the Parasite Triangle Fig. 13).

Intervention at the level of the vector-host interface requires an understanding of what happens when an infected blood-sucking arthropod attaches to the

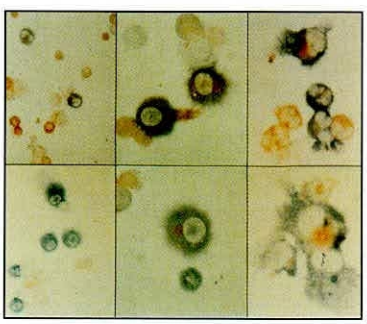


Fig. 14. Skin cells infected with tick-borne encephalitis virus (stained red). Langerhans cells are stained blue.

skin of its host and feeds. Vectors such as ticks inject their host with saliva as they feed. Tick saliva is a

Fig. 15. Cartoon of an infected tick (red highlight) and uninfected tick feeding together; arrows show the transmission of virus through the host's skin.

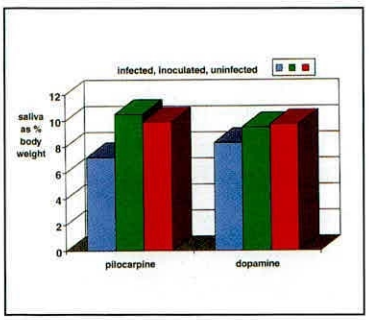


Fig. 16. Secretory activity of infected and uninfected ticks.

cocktail of pharmacologically active proteins and other chemicals that aid blood-feeding. New collaborative studies with the Institute of Zoology in Bratislava and of cells, thus carrying the virus through the body of the host from infeted ticks to uninfected co-feeding ticks. (Fig. 15). As a result, the virus was transmitted very efficiently to new ticks with very little adverse effect on the animal on which the ticks were feeding. Experiments are now underway to identify the components of tick saliva that effect host cell migration.

Another important component of the Parasite Triangle is at the level of the pathogen-vector interaction. Arthropod-borne pathogens apparently have little, if any, detrimental effect on their natural arthropod vector. This is presumably because the pathogen relies on the mobility and feeding success of its vector for survival; consequently, any adverse effect on the vector would reduce the pathogen's survival rate. However, there may be subtle effects of pathogens on their vectors that have been overlooked. Recent work has shown that this is the case.

When investigating the mechanism and control of arbovirus transmission by ticks, scientists at IVEM and their Canadian collaborators discovered that the secretory activity of ticks infected with Thogoto virus was impaired if compared with uninfected ticks (Fig. 16). There are two possible interpretations of these results: (1) the virus has a deleterious effect on the secretory fluid response, or (2) the virus stimulates a more vigorous secretion by infected ticks early during blood-feeding, a situation which would obviously benefit the virus. Understanding the nature of this virus-tick interaction will provide insights into how viruses may be used to manipulate their tick vectors.

Fig. 17. Blue mountain hare in Scotland

Louping ill virus causes high rates of grouse mortality

Louping ill persistence on the British moorlands

Louping ill is a disease of sheep and red grouse caused by a virus transmitted between vertebrates and the sheep tick, *Ixodes ricinus.* The disease occurs on the moorlands of Scotland, northern England, Wales and Devon. Mortality amongst grouse is high, reaching 80%, and attempts to reduce mortality rate have not been very successful. For a long time it was presumed that intensive sheep farming on the moorlands was the primary cause of virus persistence because they sustain the tick population and amplify the virus.

Disease control measures have focused on immunisation and acaricide treatment of sheep. In theory, this should reduce tick numbers, and also residual infectious virus, to levels resulting in eradication of the disease. In practice, this has not happened, even when the control measures have been carefully and painstakingly implemented.

Research on the epidemiology of louping ill involved the collection of ticks from a wide variety of vertebrate hosts. In the course of studying these ticks, it was noticed that mountain hares (Fig. 17) consistently yielded ticks containing louping ill virus, implying that the virus was transmitted between ticks by the hares. This surprising finding was investigated in the laboratory and it has now been demonstrated that the mountain hare is an efficient host for virus transmission between ticks.

Previously it was believed that, in common with many other wild species on the moorlands, the mountain hare was not significantly involved in maintaining louping ill virus because the quantity of virus is produced in the blood of a hare following infection is very low and below the threshold for efficient transmission to ticks when they take a bloodmeal. It has now been demonstrated that when ticks feed on hares, they aggregate together (co-feed); during this process, non-infected ticks may acquire the infection from infected ticks without developing a significant viraemia. This co-feeding mechanism of virus transmission from tick to tick is efficient and can occur on hares which have previously developed immunity to virus.

During the winter, when ticks are not active, hares are not exposed to virus and their immunity drops to a low level. In the spring, when the ticks start to feed, hares with low level immunity transmit the virus from between ticks during co-feeding. Thus the mountain hare has a major role to play in louping ill persistence in the environment.