Epidémiol. santé anim., 1997, 31-32

STUDIES ON COWPOX IN ITS NATURAL RODENT HOSTS IN GREAT BRITAIN

Feore S.[1], Chantrey J., Bennett M., Baxby D., Begon M.

Le virus de la variole bovine est endémique en Europe. On le trouve le plus souvent chez le chat, parfois chez l'Homme. Les rongeurs sauvages représentent probablement le réservoir. En Grande-Bretagne, il s'agit du mulot (Apodemus sylvaticus), du campagnol roussâtre (Clethrionomys glareolus) et du campanol agreste (Microtus agrestis). Nous avons étudié les liens entre l'infection et la dynamique de population de ces espèces au laboratoire et sur le terrain comme modèle de poxvirose et de relation virus-espèces sauvages. Le travail expérimental s'est concentré sur la réceptivité, la période infectieuse, l'impact sur la croissance, le développement, la moratlité, la fécondité et les modes de transmission. Sur le terrain, des piégeages mensuels ont eu lieu dans deux endroits. Chaque animal est identifié, sexé, examiné et du sang est prélevé. Cela permet un suivi longitudinal et horizontal, au niveau individuel et populationnel. Le campagnol roussâtre est plus sensible à une inoculation oronasale et cutanée. Il n'y a pas de mortalité mais la reproduction est touchée. Autant de mulots que de campagnols roussâtres sont touchés, mais la séroprévalence est supérieure chez les campagnols et ils présentent seuls un cycle saisonnier.

INTRODUCTION

Cowpox virus is an orthopoxvirus which, despite its name, rarely infects cattle (Baxby & Bennett, 1994). It is found in eastern and western Europe where it circulates primarily in wild rodents. It is seen most commonly in cats and occasionally humans (Bennett *et al.*, 1990) but wild rodents are believed to be the reservoir hosts. Antibody and virus have been detected in wild ground squirrels (yellow suslicks, *Citellus fulvus*) and gerbils (*Rhombomys opimus*, *Meriones libicus* and *Meriones meridianus*) in Turkmenistan and Georgia (Marrenikova *et al.*, 1984 ; Tsanava *et al.*, 1989), from root voles (*Microtus oeconomus*) on the Kolskiy Peninsula in northern Russia (Lvov *et al.*., 1988), and evidence of infection has been obtained by PCR from various rodents in Norway (Sandvik & Tryland, 1996). In Great Britain and parts of western continental Europe, a high prevalence of cowpox virus antibody has been detected in wild wood mice (*Apodemus sylvaticus*), bank voles (*Clethrionomys glareolus*) and field voles (*Microtus agrestis*) (Kaplan *et al.*, 1980 ; Crouch *et al.*, 1995 ; Boulanger *et al.*, 1996). We are studying the interaction between cowpox and the population dynamics of wood mice and bank voles in the laboratory and field to establish the epidemiology of the disease but more particularly to use this system as a model to study an endemic virus in a wildlife population.

The potential role of subclinical endemic disease in the population dynamics of a wild vertebrates has been largely overlooked in the past. The potential role of parasites and pathogens in the population dynamics of their hosts is now being recognised (Grenfell & Dobson, 1995) and theoretical studies (e.g. Anderson & May 1978, 1981) have been highly influential in bringing about this change of perception. However, empirical confirmation of theoretical possibilities has remained rare (Dobson & Hudson 1995 ; Gulland 1995). Studies have tended to be either opportunistic investigations of epidemics with measurable increases in mortality such as myxomatosis in rabbits (Flowerdew *et al.*, 1992) or seal morbillivirus (Osterhaus *et al.*, 1995) or evocations of endemic infections where other effects on demographic variables appear unable to explain observed population time series (Laine & Henttonen, 1983 ; Mihok *et al.*, 1985). That natural populations support infections of microparasites including bacteria, viruses and protozoa which are endemic but have no obvious or widespread effects on mortality is certain. The importance of such infections for the dynamics of their hosts, however, remains profoundly uncertain. Cowpox is such a disease.

The present study concentrates on the effect of cowpox virus infection in bank voles and wood mice. Field data are presented which reveal the dynamics of the disease in wild populations as well as experimental data which indicate the susceptibility of bank voles and wood mice to cowpox virus and its effect on their mortality, morbidity and fecundity.

METHODS

1. FIELD WORK

Two field study sites have been established in 1 hectare plots of mixed woodland habitat on a private estate in South Wirral in north west England. Both sites support large populations of bank voles and wood mice. Each population has been sampled intensively every four weeks from March 1995 to the present. Longworth traps are set in pairs at 10m intervals on a 10 x 10 grid over three days and three nights. All animals are identified using electronic transponder tags (AVID identity tags, Labtrac Ltd.). This allows them to be recognised on subsequent recapture for capture-mark-recapture analysis. Information on sex, reproductive state, weight, site of capture and presence of ectoparasites (ticks and fleas) is collected from each animal as well as a blood sample which is taken from the tip of the animals tail. Traps are routinely sterilised between sites and cleaned with ethanol swabs between captures at each site to avoid possible cross-infection. Serum samples are tested for antibody to

[1] Centre for Comparative Infectious Diseases, University of Liverpool, P.O. Box 147, Liverpool, L69 3BX, U.K.

cowpox in an immunofluorescence (IF) assay using a mixture of FITC-conjugated polyclonal anti-mouse and anti-rat IgG (Sigma) (Crouch *et al.*, 1995).

2. EXPERIMENTAL WORK

Captive-bred colonies of bank voles and wood mice originally obtained from Dr. J. Clarke, Department of Zoology, Oxford University, have been established (Baker & Clark, 1987). Previous studies (Bennett *et al.*, 1997) have demonstrated that bank voles and wood mice are susceptible to 0.1-20 plaque-forming units (p.f.u.) of cowpox virus depending on species, age and route of inoculation although it causes no obvious disease.
In this study,the effect of infection on reproductive rate was investigated. Bank voles and wood mice weaned at approximately 18 days old and distantly-related were placed in mixed sex pairs. Within a few days, each pair of animals was inoculated oronasally with 40µl of either cell medium alone or medium containing 800 p.f.u. cowpox virus. Time to first litter and litter size were recorded for all those that produced young within a 120 day period. All animals were killed and bled at the end of the experimental period.

RESULTS

1. FIELD WORK

Field data has revealed seasonal patterns of abundance of bank voles and wood mice at both sites with bank voles peaking in August/September and wood mice peaking in December/January. Numbers of each species decline to their lowest numbers in February/March in bank voles and April/May in wood mice. The number of seropositive bank voles and wood mice are roughly similiar at the two sites, but the seroprevalence is higher in bank voles. There is also a seasonal trend in the bank vole seroprevalence which increases from a lowest level in April (5%) to a peak in September (74%). No obvious seasonal trend has been seen in wood mice in which seroprevalence varies between 4% and 24%. Capture-mark-recapture allows the serological status of the animals in the populations to be monitored over time and so determine when an individual animal becomes seropositive. The seasonal trend observed in seroprevalence among bank voles is reflected in a similiar seasonal trend in the number of animals seroconverting. No such trend is observed in wood mice.

2. EXPERIMENTAL WORK

While cowpox does not cause increased mortality, a marked reduction in reproductive rate was observed in both species. The time to first litter was significantly longer (20-30 days) in infected compared to uninfected pairs of bank voles (Mean time to first litter: uninfected = 43 days, infected = 66 days, $t=2.85$, $d.f.=26$, $p<0.05$) and wood mice (Mean time to first litter: uninfected = 56 days infected = 75 days, $t=2.15$, $d.f.=31$,$p<0.05$) but no difference in litter size was seen.

DISCUSSION

It has long been recognised that infectious agents which cause obvious morbidity and mortality, and which are often characterised by epidemic spread, affect wild mammal populations: for example myxomatosis in European rabbits (Flowerdew *et al.* 1992), phocine morbillivirus in seals (Osterhaus *et al.* 1995), canine distemper in lions (Roelke-Parker *et al.* 1996) or black-footed ferrets (Appel & Summers 1995). However, subclinical endemic infections, probably because they often cause minimal or no obvious disease and mortality, have generally been regarded as relatively unimportant as determinants of host population dynamics. This has been especially true of microparasites, although a small number of studies of nematode infection in lagomorphs and game birds, for example, have demonstrated parasite-infection causing increased vulnerability to predation (Hudson *et al.* 1992 ; Murray *et al.* 1997) or decreases in natality (Yuill 1964 ; Dunsmore 1981 ; Hudson 1986).
Cowpox represents such an endemic infection. The prevalence observed in the two field sites in this study supports the role of small wild rodents as a reservoir for the disease. The greater seroprevalence and seasonal variation observed in bank voles suggests that they are more susceptible to cowpox and this is supported by experimental work which indicates that bank voles are susceptible to a dose ten times lower than that required to infect wood mice (Bennett *et al.*, 1997). However reproductive rate in both bank voles and wood mice is significantly affected by cowpox infection. Reduction in fecundity has profound implications for the population dynamics of field populations in which the disease is endemic. A delay in reproductive rate of the order of 20-30 days, as observed here, is tantamount to the death of a whole litter. The higher prevalence among bank voles in the field may mean that the implications of cowpox for their population dynamics will be more marked but both species will be affected. Future work will continue to address the implications of these findings for the epidemiology of endemic diseases in wild populations.

ACKNOWLEDGEMENTS

We are grateful for funding from the Natural Environment Research Council (GR3/9304). J.C. is supported by a BBSRC Veterinary Fellowship.

BIBLIOGRAPHY

Anderson R. M. & May R. M.,1978. Regulation and stability of host-parasite population interactions. I. Regulatory processes. Journal of Animal Ecology 47, 219-249.

Anderson R. M. & May R. M., 1981. The population dynamics of microparasites and their invertebrate hosts. Philosophical Transactions of the Royal Society London, Series B 291,451-524.

Appel M. J. G. & Summers, B. A., 1995. Pathogenicity of morbilliviruses for terrestrial carnivores. Veterinary Microbiology 44, 187-191.

Baker J. P. & Clarke J. R., 1987. Voles . In The UFAW Handbook of Laboratory Animals. (ed. T.B. Poole). pp. 331-345. London: Longman Scientific and Technical.

Baxby D. & Bennett M., 1994. Cowpox virus. In Encyclopedia of Virology. (ed. R. G. Webster & A. Granoff). pp. 261-267. London: Academic Press.

Bennett M., Crouch A. J., Begon M. E., Duffy B., Feore S. M., Gaskell R. M., Kelly D. F., McCracken C. M., Vicary L. and Baxby D., 1997. Cowpox in British voles and mice. Journal of Comparative Pathology 116, 35-44.

Bennett M., Gaskell C. J., Baxby D., Gaskell R. M., Kelly D. F. and Naidoo J., 1990. Feline cowpox virus infection. Journal of small Animal Practice 31, 167-173.

Boulanger D., Crouch A., Brochier B., Bennett M., Clement M., Gaskell R. M., Baxby D. & Pastoret P. -P., 1996. Serological survey of orthopoxvirus infection of wild mammals in areas where a recombinant rabies virus is used to vaccinate foxes. Veterinary Record 138, 247-249.

Crouch A. C., Baxby D., McCracken C. M., Gaskell R. M. & Bennett M., 1995. Serological evidence for the reservoir hosts of cowpox virus in British wildlife. Epidemiology and Infection 115, 185-191.

Dobson A. P. & Hudson P. J., 1995. Microparasites: observed patterns. In Ecology of Infectious Diseases in Natural Populations (ed. B. T. Grenfell & A. P. Dobson). pp. 52-89. Cambridge: Cambridge University Press.

Dunsmore J. D., 1981. The role of parasites in population regulation of the European rabbit (*Orictolagus cuniculus*) in Australia. In Proceedings of the Worldwide Furbearer conference, Frostburg, Md., 1980 (ed. J. A. Chapman & D. Pursley). pp. 654-669. Worldwide Furbearer Conference, Inc.

Flowerdew J. R., Trout R. C. & Ross J., 1992. Myxomatosis and ecological effects in the United Kingdom. Revue Scientifique et Technique Office International des Epizooties 11, 1109-1113.

Grenfell B. T. & Dobson A. P., 1995. Ecology of Infectious Diseases in Natural Populations. Cambridge: Cambridge University Press.

Gulland F. M. D., 1995. The impact of infectious diseases on wild animal population-a review. In Ecology of Infectious Diseases in Natural Populations (ed. B.T. Grenfell & A.P.Dobson). pp. 20-51. Cambridge: Cambridge University Press.

Hudson P.J., 1986. The effect of a parasitic nematode on the breeding of red grouse. Journal of Animal Ecology 55, 85-92.

Hudson, P. J., Dobson, A. P. & Newborn, D. 1992 Do parasites make prey vulnerable to predation? Red grouse and parasites. Journal of Animal Ecology 61, 681-692.

Kaplan C., Healing T. D., Evans N., Healing L. and Prior A., 1980. Evidence of infection by viruses in British field rodents. Journal of Hygiene, Cambridge 84, 285-294.

Laine K. & Henttonen H., 1983. The role of plant production in microtine cycles in northern Fennoscandia. Oikos 40, 407-418.

Lvov S. D., Gromashevskyi V. L., Marennikova S. S., Bogoyavlenskyi G. V., Bajluk F. N., Butenko A. M., Gushchina E., Shelukhina E. M., Thukova O. A. and Morozova T. N., 1988. Poxvirus isolation from *Microtus oeconomus* Pal. 1776 in Colsky peninsula. Vop Virus 1, 92-94.

Marennikova S. S., Shelukhina E. A. and Efremova E. V., 1984. New outlook on the biology of cowpox virus. Acta Virologica 57, 461-464.

Mihok S., Turner B. N. & Iversen S. L., 1985. The characterization of vole population dynamics. Ecological Monographs 55, 399-420.

Murray D. L., Cary R. & Keith L. B., 1997. Interactive effects of sublethal nematodes and nutritional status on snowshoe hare vulnerability to predation. Journal of Animal Ecology 66, 250-264.

Osterhaus A. D. M. E., De Swart H. W. Vos, Ross P. S., Kenter M. J. H. & Barrett T., 1995. Morbillivirus infections of aquatic mammals: newly identified members of the genus. Veterinary Microbiology 44, 219-229.

Roelke-Parker M. E., Munson L., Packer C., Kock R., Cleaveland S., Carpenter M., O'Brien S. J., Pospischil A., Hofmann-Lehmann R., Lutz H., Mwamengele G. L. M., Mgasa M. N. Machange G. A., Summers B. A. & Appel M. J. G., 1996. A canine distemper virus epidemic in Serengeti lions (*Panthero leo*). Nature, London 379, 441-445.

Sandvik T. & Tryland M., 1996. Orthopoxviruses in Norway: Distribution, reservoirs and characteristics., Thesis, University of TromsÆ.

Tsanava S. A., Sakvarelidze L. A. and Shelukhina E. M., 1989. Serologic survey of wild rodents in Georgia for antibodies to orthopoxviruses. Acta Virologica 33, 91.

Yuill T. M., 1964. Effects of gastrotestinal parasites on cottontails. Journal of Wildlife Management 28, 20-26.