Deer Parapoxvirus: A new virus.

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The sudden appearance of a previously undescribed viral disease is an uncommon event but an event that we have seen at least three times in the last ten years. In 1978 canine parvovirus appeared on the scene (1), in 1981 we saw the emergence of AIDS and its associated viruses in man (reviewed in 2 and 3), and, although not in the same league as the other two, in 1985 we saw the appearance of a new parapoxvirus infection in red deer (Cervus elaphus)(4).

The reasons for these events are unknown but probably involve changes in husbandry or behaviour of the host as well as genetic changes in the virus. Sequencing studies have shown that it is likely that the canine parvovirus arose from a mutation in the virus normally infectious for cats (5) and there has been speculation that the mutant was introduced into the dog population via a contaminated vaccine to explain its rapid worldwide distribution. However, for such a hardy virus, it could have been just as easily spread by international air travellers. Serological studies have shown that the virus was not circulating in dogs as an inapparent infection prior to 1977 (6) which means that the switch from one host to another occurred over a very short space of time.

The appearance of AIDS has been just as rapid although it is now considered possible that HIV-1 had been circulating at a low level in a Central African population for many years and, due to a combination of the population drift to cities such as Kinshasa and the activities of jet-setting thrill-seekers from the western world, the conditions for selection and spread of the virus were created. HIV-2, however, is very closely related to a virus that has been isolated from healthy vervet monkeys and it is likely that this virus has somehow switched from monkeys to man (7).

The newly described parapoxvirus infection of red deer (4) has also appeared suddenly. Has it arisen from the virus causing scabby mouth of sheep or from the cattle parapoxviruses that cause papular stomatitis and pseudocowpox, or is it due to a virus that has existed in the red deer population for years and has only become prominent because of the intensification of deer farming? The evidence to date favours the latter conclusion and it is that evidence that I will present here.

The parapoxviruses constitute a rather unique genus of the poxvirus family. The appearance of the virus in the electron microscope is distinctive in that one sees a particle that has an oval outline and a surface that is covered with a regular array of criss-crossing bands (8)(9). It has been shown that this "ball of wool" appearance is due to a surface tubule

protein that is wound in a spiral fashion around the particle (10). All other poxviruses have the morphology of vaccinia virus (8) in that they are rectangular rather than oval in outline and their surface tubules are arranged on the particle surface in a relatively disorganised manner.

Another major difference between the parapoxviruses and other poxviruses is the make up of the DNA. The poxvirus genome is a linear double stranded DNA molecule contained within the particle (11). All DNA molecules are made up of the four bases, guanine, cytosine, adenine and thymine which are usually abbreviated to G,C,A and T. The ratio of G+C to A+T is commonly used to compare genomes of different viruses and is an important criterion in a number of biochemical procedures. The G+C to A+T ratio in parapoxviruses is 2:1 (12) whereas in other poxviruses the ratio is 1:2 (13). However, other features of the genome such as cross-linked ends, and inverted terminal repeats (14)(15) as well as the basic structure of the particle and the fact that other poxviruses share a common core antigen with the parapoxvirus, orf virus, (16) is convincing evidence that the parapoxviruses are members of the poxvirus family. It is probable that they have diverged from a common ancestor virus fairly early on in the evolution of poxviruses. On the basis of these criteria, there is no doubt that the virus isolated from red deer is a member of the parapoxvirus genus.

The International Committee on Taxonomy of Viruses (ICTV) currently recognise four members of the genus parapoxvirus (17). These are orf virus, bovine pustular (sic) stomatitis virus, milker's node virus, and chamois contagious ecthyma virus. This classification is currently under review and it has been recommended that the names of the members become orf virus, bovine papular stomatitis virus, and pseudocowpox virus (18). It has also been recommended that the parapoxvirus of red deer, as well as a parapoxvirus of seals, and a parapoxvirus of camels be considered as probable members of the genus. There is no good reason at present why the parapoxvirus that has been isolated from contagious ecthyma in chamois should be considered as a separate member because it is probably orf virus. However, as will be appreciated when the current methods of classification are discussed below, this is not certain.

How then do we decide whether or not a parapoxvirus isolate should be considered as a separate member of the genus? Before the advent of molecular biological techniques the parapoxviruses were classified on the basis of the species of animal affected and a pathological description of the disease. For instance, if an isolate came from scabby lesions in sheep it was an orf virus. If the disease was characterised by pocks on the teats of cows it was pseudocowpox virus and if it was isolated from papular lesions on the muzzle and in the mouth of calves it was papular stomatitis virus. Humans are affected by these viruses and isolates can be identified by noting a history of contact with sheep or cattle and by appropriate animal inoculations. Orf virus does not produce lesions in cattle, and neither pseudowcowpox virus nor papular stomatitis virus produce lesions

in sheep, or, if they do, the lesions produced are weak. Serological differences have been noted between these viruses but serological tests have not commonly been applied in distinguishing between isolates.

Orf or contagious pustular dermatitis has been reported as occurring naturally in domestic sheep (Ovis aries)(19), dall sheep (Ovis dalli dalli)(20), Rocky mountain big horn sheep (Ovis canadensis canadensis)(21), domestic goats (Capra hircus)(22), mountain goats (Oreamnos americanus)(21), chamois (Rupicapra rupicapra)(23), thar (Hemitragus emlaicus)(24), steinbok (Raphicerus campestris)(25), reindeer (Rangifer tarandus tarandus)(26), and musk oxen (Ovibos moschatus)(27). The viruses isolated from most of these species could reproduce typical orf lesions in sheep. The disease in musk oxen and reindeer in Norway differed from the others in that they presented as papillomatous lesions. It would be worthwhile considering the possibility that the musk ox disease seen in Norway was caused by dual infection with a papilloma virus and orf virus as protection against the disease could be achieved with ground-up glutaraldehyde-treated papillomatous lesions but not with orf virus alone (28). However the parapoxviruses do tend to cause dermal proliferation in other species and possibly the musk oxen show an exaggerated response to infection. The reindeer papillomas contained parapoxviruses as determined by electron microscopy but no attempts at protection were reported with either orf virus or ground up lesions.

Successful experimental infections of a moose (Alces alces) calf and a caribou (Rangifer tarandus) calf with orf virus derived from a dall sheep have been reported although the lesions were mild and orf virus was not able to be re-isolated (29). Similar mild lesions were reported in mule deer (Odocoileus hemionus), white tailed deer (Odocoileus virginianus), and pronghorn (Antilocapra americana) fawns and Wapiti (Cervus elaphus nelsoni) calves after experimental inoculation with an orf virus isolated from Rocky Mountain bighorn sheep (30). Serological evidence of orf virus infection has been reported in wild caribou in Alaska (31).

The immediate reaction to the discovery of an orf like disease in red deer in New Zealand was that this was also due to orf virus. If reindeer become infected then why not red deer? Also, because of the high prevalence of the disease in sheep in New Zealand and the recent intensive farming of deer on land frequented by sheep, it seemed that the conditions existed for such an event. However, when the virus isolates were analysed in our laboratory it became clear that the virus was not orf virus. This does not mean that orf virus does not affect red deer but that the two samples sent to us from what were typical cases of the new disease were not orf virus.

The method we used to compare the red deer parapoxvirus isolates with orf virus isolates from sheep is called restriction endonuclease analysis (REA). REA has been used for ten years or so to compare isolates of herpes virus, papilloma virus,

adenovirus, as well as the poxviruses and a large range of bacteria. DNA copies of RNA viral genomes can also be compared with this technique. A variation of the technique, which relies on restriction fragment length polymorphism's or RFLP's, is used to compare the genomes of higher organisms including man. In REA, the DNA of the organism is extracted and digested with a particular restriction endonuclease. Restriction endonucleases of the type called class II restriction endonucleases are enzymes that recognise specific base sequences in DNA and cleave the DNA at that point, or close by. For instance the restriction endonuclease EcoRI cleaves DNA at the sequence GAATTC. The DNA is thus cut up into fragments, the number and size of which can be considered as unique to a particular strain of organism. These fragments can be conveniently displayed by moving them through a gel of agarose under the influence of an electric current. The negatively charged fragments migrate through the gel at a rate proportional to their size and thus a pattern of bands is created in the gel which can be visualised by staining with ethidium bromide and illumination under ultra violet light. Alternatively the fragments can be radioactively labelled and their position determined by the exposure of an x-ray film held in close apposition to the gel.

The pattern of DNA bands produced by two deer parapoxvirus isolates were identical but very different to orf virus patterns (4). We have compared the DNA from some 21 orf viruses isolated in Europe (32), the USA (33), and New Zealand (34) and have found that they show very similar patterns with certain restriction endonucleases. These isolates included a virus that had been isolated from a musk ox in Alaska and had been typed as orf virus on the basis of its ability to produce typical lesions in sheep.

Further evidence that the deer isolates are not orf virus has come from DNA/DNA hybridisation studies. In this technique advantage is taken of the fact that double stranded DNA (which is the form of DNA found in poxviruses) can be separated into two complementary strands by heat or high pH. The two strands will join together again under certain conditions, a process called annealing. However, only DNA strands with a complementary sequence of bases (G,C,A, and T) will anneal, G's pairing up with C's and T's pairing up with A's. Under the more commonly used annealing conditions a stretch of 20 or so bases needs to have a perfect match before annealing will occur. We asked the question, how much of the deer parapoxvirus DNA will anneal with orf DNA? Normally, the DNA of different orf virus isolates will anneal to 95% or more whereas the deer parapoxvirus DNA annealed to only 60% of the orf virus DNA and this annealing was found to be in the central region of the orf genome (A.J Robinson, unpublished data). This is about the same level of relatedness that the DNA of the cattle parapoxviruses, papular stomatitis virus and pseudocowpox virus, have with orf virus DNA.

This raises the question of whether or not the deer virus has arisen from one of the cattle parapoxviruses rather than orf virus. We don't have any DNA/DNA hybridisation data as yet but

if we go back to comparing the deer virus with the cattle viruses by REA it is clear that the deer virus patterns are as different from the cattle virus patterns (32)(35) as they are from orf virus.

Where else might the deer virus have come from? The only other known parapoxviruses are those associated with pox lesions in seals including harbour seals (Phoca vitulina)(36), grey seals (Halichoerus grypus)(37), Californian sea lions (Zalophus californianus)(38), northern fur seals (Callorhinus ursinus)(39), and South American fur seals (Otaria byronia)(40) and one of camels, the latter having been isolated in Khasakhstan from a disease of camels called ausdyk (41). Very little is known about these parapoxvirus strains apart from their morphological appearance and a description of the disease with which they were associated. No one has claimed to have isolated them in cell culture let alone carried out REA on their DNA. The possibility that these viruses could have been transmitted to red deer would seem remote although one should keep an open mind. It seems more likely that the deer virus has been circulating for some time at a low level in red deer and has only become obvious now that deer are being intensively farmed. Serological evidence of this virus in wild red deer populations or, ideally, another isolation of the virus in other parts of the world would strengthen this hypothesis.

Transmission studies to other species will also give clues to the origin of this virus. I understand that other speakers at this conference will be describing their attempts to transmit the deer virus to sheep and orf virus to deer. Attempts to transmit the deer virus to cattle might also be instructive. To date I have not heard of any confirmed case of natural transmission of this virus to man but I suspect it will only be a matter of time before such a transmission occurs. Orf virus (42), including orf viruses from musk oxen and reindeer (43), and mountain goat (44), the cattle parapoxviruses (45)(46), and the parapoxvirus affecting seals (37) have all been recorded as causing pox lesions in man. I would be very interested to receive material from any suspected cases of transmission of the red deer virus to man.

As far as the control of this disease is concerned it should be a relatively simple matter to make a living vaccine similar to that used in sheep for the control of orf although a lot of work would need to be done to get such a vaccine registered for widespread use. It is likely that such a vaccine would perpetuate the virus on a property once it was introduced and, therefore, would only be recommended for use on properties where the disease already existed and was causing a problem. Inactivated poxvirus vaccines have never been successful in the field despite the considerable efforts devoted to this end during the eradication of smallpox (reviewed in 47). It is thus unlikely that an inactivated vaccine would work for the deer virus unless the correct antigens could be defined and presented to the animal such that a protective cell mediated response was elicited.

In conclusion, this virus of red deer appears to be a new parapoxvirus although its characterisation is, as yet, incomplete. REA has been an invaluable tool in unravelling the relationships between various isolates and I am sure that new parapoxviruses which can be considered genetically distinct will be discovered. With this in mind, it would be worthwhile using REA to re-examine isolates of parapoxviruses that have been isolated from other species and which have been assumed to be orf virus.

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