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BIOTECHNOLOGY AND VIRAL DISEASE CONTROL IN LIVESTOCK: a brief review.

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SUMMARY.

Modern biotechnological methods are gaining a tremendous importance in research and in the development of reagents for vaccines and disease control. The advent of restriction endonucleases which can cut DNA molecules into short pieces has enabled scientists to create nucleic acid probes and recombinants.With such advances,it is now assumed that the application of recombinant DNA techniques can result in products that are simple to manufacture,safe and reasonably cheap and of great significance when they become available for controlling and monitoring animal diseases. Therefore,the techniques can be used for inserting the gene coding for the immunizing protein of one virus into the genome of a second avirulent virus (vector) that is safe to be administered as a vaccine. Furthermore,research in recombinant DNA has been conducted with the intentions of obtaining highly specific and sensitive diagnostic probes.In this paper we review briefly the application of genetic engineering in an attempt to create novel vaccines and develop nucleic acid probes for rapid and sensitive diagnostic tools for viral diseases affecting our livestock.

INTRODUCTION.

As early as 1970 scientists found out that certain strains of bacteria produce enzymes that are capable of degrading foreign DNA which enters the bacterial cell, thereby preserving the genome of the bacterium.This phenomenon is termed 'host restriction' and the enzymes responsible for the reductions in bacteriophage infectivity are known as 'restriction enzymes'. Over 200 restriction enzymes are now known,and a standard method of naming them has been adopted. The restriction enzyme binds to specific sequences of nucleotides called recognition sequences, and each enzyme cuts the DNA at a specific cleavage site that is normally located within the recognition sequence.Usually, recognition sequences are palindromes i.e. the sequence of bases in both DNA strands is the same when read from left to right and from right to left. Some common restriction enzymes and their sources are such as EcoRI (*Escherichia coli*),AluI (*Arthrobacter luteus*),and Hind III (*Haemophilus influenzae*). Each restriction enzyme has a specific recognition sequence and a cleavage site. For example, EcoRI recognizes the sequence 3'-C-T-T-A-A-G-5' and cuts between -A- and -G- only (Purchase 1986)

Addition of a restriction enzyme to DNA

molecule causes cleavage of the latter and the DNA is digested at as many positions as there are cleavage sites for that particular enzyme. Normally, such cleavage sites are not positioned at regular intervals along a DNA molecule, and thus the fragments resulting from digestion will differ by their lengths and weights. Such different fragments can be separated according to their sizes by agarose gel electrophoresis. Being negatively charged, the fragments move towards the positive pole, and the largest fragments move slowest. Thus the observed bands (patterns) on an agarose gel after electrophoresis will precisely represent relative positions of the fragments and hence their weights. Comparison of the results of digestions with several different enzymes enables the formulation of a restriction map for the DNA of a particular organism (Nicholas 1987). When the DNA from different strains of viruses or individuals of the same line are treated with restriction enzymes, they yield fragments of different sizes which can be used to distinguish between strains or lines (Kelling et al., 1990, Carrillo et al., 1990).

In order to have a practical value, work on recombinant DNA is essentially aimed at production of an unlimited number of copies of a particular segment of DNA. This process is referred to as gene cloning or DNA cloning. To clone a gene, one attempts to join or to splice a specific segment of DNA (foreign DNA) to a vector (plasmid) which is capable of replication within a particular host. (A vector or plasmid is a circle of double stranded DNA that exists in certain bacterial cells). This final DNA molecule resulting from the joining of the foreign DNA to the vector is the so called recombinant DNA. Presently, it is possible to clone not only a specific fragment of DNA, but also all the DNA molecule of any organism, from microorganisms to large mammals. This allows formation of a gene bank or library for all of the DNA of an organism. The basic processes are indeed more complicated than can be summarised here and are not part of this review. Of particular interest are techniques involving, practical application of recombinant DNA technology in genetic engineering.

Three main areas can be identified in which recombinat DNA offers substantial prospects. Firstly, recombinant DNA technology offers the possibility to mass produce large quantities of particular polypeptides (proteins). As polypeptides are the translation products of DNA, thus cloned DNA can be engineered to produce its corresponding polypeptide. Many polypeptides can be produced in this way including microbial enzymes, products of higher organisms such as growth hormones, insulin, interferon and also antigenic components of disease causing organisms which can be used as vaccines (Bostock 1990).

Secondly, recombinant DNA technology allows the use of the so-called DNA probes which have a major impact on detection of disease organisms in animals, humans and plants (Gillepsie 1990). This technique, in principle, requires a radioactively-labelled cloned fragment of DNA corresponding to a portion of the DNA or RNA of the pathogen to be mixed with DNA or RNA extracted from tissue of the individual being tested. In this way, DNA probes can identify individuals having particular DNA sequences (genes). It is the objective of immunologists and molecular

genetists to use DNA probes to detect individuals carrying defective genes, or genes for disease resistance (Gwakisa et al.,1990, Gwakisa *et al*, 1991)). In the long term, the technology may enable animal breeders to improve performance traits such as productivity, reproduction or growth rate.

Thirdly, recombinant DNA technology can be used to modify the genetic potential of cells and organisms.It is possible , that microorganisms used widely in beverages , food and antibiotics production and those which operate in symbiosis with animals (e.g.rumen bacteria) could be made more efficient by the insertion of a gene(s) from other strains. It is possible that foreign genes could be introduced into plants or animals (transgenics), either to correct genetic faults or to enhance performance (Rutter 1981). As the application of recombinant DNA show, this technology offers tremendous possibilities to improve the livestock industry. However, there are many technical and basic scientific problems that need to be solved before many of these possibilities become realities. We, here give a brief review of the current information on the use of DNA probes in the diagnosis of viral diseases and give a short outline on vaccine production using recombinant DNA technology.

THE USE OF NUCLEIC ACID PROBES FOR DIAGNOSIS OF VIRAL DISEASES.

The on-going revolution in molecular biology has produced a new generation of exquisitely sensitive tools in the form of nucleic acid probes for diagnosing viral diseases.Two types of probes are used for detecting and characterizing viruses and other parasites. One type is based on deoxyribonucleic acid (DNA) and the other on ribonucleic acid (RNA). The DNA molecule consists of two intertwinned nucleotides bound together on a complementary basis. scientists have known how to denature and separate the two strands either mechanically by boiling or chemically. For a long time it was believed that the process is irreversible but recently it was noted that DNA strands re-formed the double helix if kept for prolonged period at lower temperature. With this background Scientists have been able to isolate the viral genome ,break it into fragments using restriction enzymes and a fragment common to various strains of a virus species selected . This fragment is radio-labelled and employed to detect its complementary copy in a specimen . This process is referred to as hybridization. Although RNA is usually single-stranded its hybridization to DNA in the formation of heterodimers follows the same process.The recent development of a polymerase chain reaction (PCR) makes it easier to amplify a specific nucleic acid fragment to amounts that allow its identification (Wade-Evans et al.,1990).

With the help of nucleic acid probes differentiation of genetically distinct isolates of foot and mouth disease (FMD), African horse sickness(AHS) and blue tongue serotypes and many other viruses have been made possible within a short time. The first three diseases are caused by several serotypes and an animal becomes immune to only one serotype. Therefore, it is important to identify the epizootic serotype as rapidly as possible thereby enabling the correct vaccines to be used (Smitsaart et. al.,1987,Jackwood 1990).

For example, FMD virus consists of seven distinct serotype A,O,C, SAT 1,2,3, and ASIA 1 and within each serotype several strains have been identified (Stave *et al*,1988). The serotypes have usually been characterized and identified by immunological and serological tests such as complement binding and virus neutralization methods but these methods are tedious and time consuming, and have low sensitivity (Meloen & Briaire 1980, Westbury et. al.,1988). Beck and his colleagues (1987) developed a method for determining the nucleotide sequence of the FMD capsid protein (VP1).The synthetic oligonucleotide complementary to the preserved areas of the VP1 was hybridized to the viral RNA. From these primers, and with the use of reverse transcriptase which was able to write from an RNA matrix, a cDNA strand was produced. The cDNA was then sequenced and compared to the nucleotide sequence of the suspected isolate from an outbreak. Similarity in the DNA sequence confirms with a high degree of certainity that the isolate was the cause of the outbreak. Also, Rossi and his co-workers (1988) were able to detect the presence of non-infective viral mutants of FMD virus in the oesophageal-pharyngeal fluids of carrier cattle 180 and 560 days post-infection using dot blot-hybridization techniques in which cDNA probes for the three types A,O,C were used. It was not possible to recover infective virus from these samples by cell culture.

Similarly, Bremer and his colleagues (1990) studied the dsRNA profiles of all nine African horse sickness virus serotypes using agarose gel electrophoresis. Although the agarose profiles were identical, there was a different profile for each serotype by polyacrylamide gel electrophoresis. These differences were then used to identify the causative serotype during an outbreak.

Previously in 1985, Roy and his colleagues created a DNA copy of the segment 3-RNA of bluetongue virus serotype 17 by sequencing homologous segments of 19 other serotypes. The BTV-17 DNA probe developed was used to detect acute and inapparent bluetongue infections in animals cause by serotype 17. More recently,Wade-Evans and his colleagues (1990) reported the development of a PCR for the detection of bluetongue virus RNA in suspected specimens.

They were able to develop a PCR-amplified DNA which contained an identical region to the genome 7 of the bluetongue virus. Using these segment 7 oligonucleotides it was not only possible to detect routinely as few as 6 molecules of segment 7 dsRNA per sample but also to detect purified dsRNAs from isolates of other BTV serotypes.Likewise, Schoep *et al*,(1990) were able to use molecular hybridization techniques to detect and survey bluetongue virus serotype 17 in the insect vector,the *Culicoides variipennis*. These hybridization techniques provided an alternative sensitive method to virus isolation for the surveillance of BTV in the vector population.

Belak and Linne (1988) developed a specific pseudorabies virus probe which can detect pseudorabies virus in tissue samples or swabs from infected pigs. The probe is a cloned biotin or 32-P-labelled viral DNA which can easily be detected by straptavidin or by autoradiography respectively. Many more probes are being developed everyday such as one for infectious bursal disease viruses (Jackwood 1990),and avian infectious

bronchitis (Collison et al., 1990).
Similarly, polyacrylamide gel electrophoresis (PAGE) of rotavirus RNA has been developed to provide diagnostically and epidemiologically useful information about avian and mammalian rotavirus infections in livestock (Todd *et al*,1980, Herring *et al*,1982, Rodger *et al*, 1981, Schnagl *et al*,1981, Follett and Desselberger 1981, Snodgrass *et al*,1984).

NOVEL VACCINE PRODUCTION.

Since the advent of viral vaccines many major epidemics and pandemics have been prevented for example, smallpox (Fenner et al,. 1988). Live virus vaccines are more effective as they elicit both humoral and cell-mediated immune responses.The classical method of producing live vaccine involves serial passage in cell cultures or several live animals to attenuate the virus. With the advent of recombinant DNA techniques the genes coding for the immunogenic antigen of a virulent virus can be isolated and inserted into the genome of a second avirulent virus such as adeno- herpes- polio- and pox viruses, that can then be administered as a vaccine (Bostock 1990).

Vaccinia virus has proved to be of immense value as a vector for expressing foreign genes derived from a variety of disease agents as well as showing how such recombinant viruses may be used as potential vaccines (Mackett and Smith 1986, Moss and Flexner 1987, Hruby 1990). Examples of such vaccines are the recombinant vaccinia virus carrying and expressing the rabies glycoprotein gene(Blancou et al., 1986,Pastorect et al., 1988), rinderpest virus F protein gene or its haemagglutinin gene (Yilma et al., 1988, Belsham et al., 1989),and FMD virus protein genes (Vlak and Roosein 1988, King et al., 1988).There are many more being studied. One such possibility is creating a recombinant vaccinia virus carrying and expressing African swine fever virus genes. A marketable novel vaccine currently in use is the vaccinia-rabies recombinant vaccine for oral vaccination of foxes against rabies in Belgium (Brochier et al.,1990).

However, some scientists believe that the widespread use of recombinant vaccinia vaccines may results in the virus reverting to virulence in some host species. Therefore the use of alternative viruses, such as other pox viruses is now being investigated (Williamson*et al*, 1990). The main disadvantage with poxviruses is that their use is restricted to particular target animal groups e.g. fowl pox virus as a vector system for avian species (Harley *et al*, 1990,Boursnell*et al*,1990); swine pox virus for swine (Schnitzein and Tripathy 1991) and capripox virus for sheep,goat and cattle (Gershon and Black 1989).

Boursnell and his group (1990) have managed to develop and test a fowl pox virus vector system. In their work the fusion gene of NDV was inserted into the non-essential region within the terminal inverted repeats of the fowl pox virus and this recombinant was then inoculated into chicken. The results were quite encouraging for they indicated that the recombinant had the ability to protect the chickens against challenge by a virulent strain of NDV by eliciting the formation of anti-fusion protein antibodies.Another group in Ontario Veterinary College, Guelph,Canada,with which one of us was involved (Nagy *et al*, 1990b) attempting to

develop a multivalent fowl pox vaccine for poultry. They have managed to isolate the HN gene of NDV and have managed to insert it successfully into the polyhedrin gene of baculovirus. The baculovirus-HN recombinant expressed haemagglutinin which were antigenically similar to the haemagglutinin produced by NDV (Nagy et. al.,1990a).

CONCLUSION.

These new biotechnologies will definitely lead to improved disease diagnosis and control,and identification of animals with desired disease resistance and high productivity.

Disease diagnosis will be improved through preparation of better immunogens, specific antibodies, the use of DNA probes, and development of simple , sensitive and rapid tests. These quick tests will be used to detect antigens in minute quantities and the presence of infection earlier thus allow more time for countermeasures. Tests for antibody will continue to indicate the health and immune status of the flock.

Disease resistance will be improved through development of effective vaccines against the major viral diseases and other parasites. Using the molecular techniques, livestock will be bred for their ability to respond immunologically to and resist the effects of disease agents. In addition, breeds with resistance trait to a particular disease e.g. N'dama and Muturu to trypanosomiasis, will be identified and this can be amplified and introduced into other breeds which are more productive but susceptible to the disease (Wakelin 1978, Lewin 1989).

Productivity will be improved by engineering of the genes that control productivity traits and optimization of hormones involved in growth and reproduction. This will lead to development of transgenic animals and chimeras as bioreactors for high productivity in milk or meat (Crittenden 1986, Gwakisa *et al*,1990).

We are now in the biotechnological era in the livestock industry whereby techniques for diagnosis, prophylaxis and breeding will be greatly increased in efficiency and effectiveness while at the same time being enormously simplified.

Researchers are not only developing and applying recombinant DNA technology for better detection of disease causing organisms such as FMD , rotaviruses but also in human medicine for diagnosing genetic diseases, for screening blood to be used in transfusions, for characterising tissue samples for graft and transplant operations and DNA fingerprinting for use at the witness stand. These diverse applications and the promise of many more are causing an explosion of interest in the recombinant DNA technology.

It is pertinent, however to point out that not all potentials of these technologies are currently applicable. But the encouraging fact is that despite the void that exists in our current understanding, the gap in our knowledge is rapidly narrowing.

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