Proc. 3rd All Africa Conf. Anim. Agric. & 11th Conf. Egyptian Soc. Anim. Prod., Alexandria, Egypt, 6-9 November 2000: 55-59

PROSPECTS FOR BIOTECHNOLOGY IN ANIMAL HEALTH IN AFRICA

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BIOTECHNOLOGY

Strictly speaking biotechnology is defined as the genetic manipulation of micro-organisms for the purposes of production of biological reagents, such as antibiotics or hormones. However more generally it is nowadays taken to mean the application of genomics, that is DNA-based technology, to solve biological problems.

There is no denying the fact that biotechnology is expensive. Molecular biologists require sophisticated and highly specialised training, which takes this takes time and is therefore expensive. The laboratory equipment is highly specialised, which make it which makes it expensive; specialised computing power is required, so the hardware is not the usual mass market PC or file server and is therefore expensive; molecular biological reagents must be of high quality but production levels are low, so again they are often startlingly expensive. Finally, the scientific journals are highly specialised, and are often the most expensive in the library. One frequently hears the question, "Is this technology appropriate for Africa?", and the questioner has usually already assumed that the answer is no. We would like to illustrate why we think that this is an unwise assumption.

The realities of global economics

It is well known that pharmaceutical companies are stopping production of many anti-parasitic drugs because the market is in developing countries which cannot afford to buy them. A Bayer spokesman was quoted as saying, "A company can support some research without being paid off, but not much. Especially with the pressure on shareholder value. There's always the threat of being taken over." So if it's not profitable companies *cannot* get involved, or they risk going out of business. So it is not to the pharmaceutical industry that one can look for the solutions to African animal health problems.

Perhaps it is it cheaper, then, to do without sophisticated biotechnological products and carry on as one did before the biotechnological revolution in the life-sciences? Some recent hard lessons suggest that this is not a viable approach. Not only are outbreaks of contagious animal disease cripplingly expensive in terms of direct losses, but they may lead to the freezing of a country's exports of animal products. Let us note a few examples as quoted in Preslar, 1999.

1- The 1996 outbreak of African swine fever in Cote d'Ivoire cost some US$9.2 million.
2- The same disease in Nigeria in 1998 cost US$8.4 million in six months just in Lagos state.
3- The cost of Taiwan's 1997 epidemic of foot and mouth disease in hogs was reported as US$1.3 billion.
4- In 1999 Malaysia's losses from Nipah virus were reported at US$114 million for the first five months of the epidemic.
5- After an outbreak of contagious bovine pleuropneumonia in Botswana in February 1995 320,000 cattle were slaughtered, at a cost of over US$300 million.
6- Beef exports from Argentina to the United States were re-approved in 1998 after an exclusion period of 65 years, due to outbreaks of foot and mouth disease.

Is there a cheaper way?

Is it necessary to go to the expense of biotechnological involvement in order to solve animal disease problems? Is there not a more appropriate (for "appropriate" read "cheaper") technology for solving African animal health problems? One cannot find a better example of an older, cheaper, technology than the development by Jenner of vaccination against smallpox (Brunham and Coombs, 1998), and his notes make interesting reading today.

"Sarah Nelmes, a dairy maid near this place [Gloucestershire, England], was infected with cowpox from her master's cows in May 1796. A large sore and the usual symptoms were produced. I selected a healthy boy, about eight years old. The matter was taken from the [cowpox] sore on the hand of Sarah Nelmes and it was inserted on 14 May 1796 into the boy by two cuts each about half an inch long. On the seventh day he complained of uneasiness, on the ninth he became a little chilly, lost his appetite and had a slight headache and spent the night with some degree of restlessness, but on the following day he was perfectly well. In order to ascertain that the boy was secure from the contagion of the smallpox, he was inoculated with smallpox matter, but no disease followed. Several months later he was again inoculated with smallpox matter but again no disease followed." (Jenner, E., 1798).

There has been much speculation over exactly how the healthy boy was "selected", and what would have happened if he had died of smallpox. It goes without saying that no-one would be allowed to repeat Jenner's experiment today, but these are not the real lessons of this historical tale. The real lesson is that the easy discoveries have been made. Vaccines which can be produced by such simple and dangerous means have been so produced, and the unsolved problems which we are left with are the difficult ones, unsolved simply because they have not yielded to "quick and dirty" empirical techniques. In contrast, the techniques of molecular biology, genomics and biotechnology allow, for the first time in history, a detailed understanding at the molecular level of disease processes, immune responses and parasite behaviour. Such insight holds the promise, in principle, of being able to solve any biological problem which has been refractory to empirical efforts.

INTERNATIONAL SCIENTIFIC COLLABORATION

International collaboration is often seen as an alternative to expensive national scientific commitments, but it is in reality no alternative for national scientific expertise. International collaboration can help to maintain standards and stretch budgets, but it must never be forgotten that collaborators are also competitors. Laboratories and scientists collaborate if they are operating at the same level of expertise, if there are great differences in scientific capability the weaker partner is inevitably forced to adopt a subordinate role, providing biological material but having little say in what experiments are done. Every country which aspires to be independent in support of its agriculture needs the political will to support its own life-sciences infrastructure.

What biotechnology offers

Examples of what may be expected from biotechnological research are new vaccines and new diagnostic tests. New vaccines to control diseases more cheaply and more effectively than antibiotic drugs or acaricide dips, and new and better diagnostic techniques, to improve our epidemiological knowledge and to allow disease control measures to be better planned and implemented. We will look at two specific examples of research into such topics which are ongoing at the Onderstepoort Veterinary Institute (OVI).

A NEW HEARTWATER VACCINE

Heartwater, or cowdriosis, is one of the major African livestock diseases, affecting most of sub-Saharan Africa, as well as certain islands in the Caribbean. It is caused by an obligate intracellular rickettsia, *Cowdria ruminantium*, carried by *Amblyomma* species ticks, and the economic seriousness of the disease is evident from figures from South Africa. It is estimated hat 8.7 million head of livestock are at risk in the small scale farming sector, which threatens the food security of rural people, and 8.8 million head are at risk in the commercial sector, which threatens food destined for the cities and export revenues. Estimates from the Eastern Cape Veterinary Services for 1998 were that US $1 - 4 million had been spent for prophylaxis and vaccination, despite which total stock losses were estimated at US$30 million.

Current control methods include the use of prophylactic antibiotics and tick control with acaricides, but these methods are expensive. In addition there is widespread resistance to acaricides, which are also considered to be a source of environmental chemical contamination. Immunisation is much more cost effective, and an infection and treatment regimen is widely used in southern Africa. This consists of infecting with virulent organisms in sheep blood and then controlling the infection with tetracycline. The disadvantages are that the "vaccine" must be stored and transported at below -40°C, which makes it particularly inappropriate for use in rural areas, and that the animal's temperature must be monitored daily in order to ensure that the infection does not become overwhelming. In addition, the duration of immunity is uncertain, the procedure uses live virulent organisms so it cannot be used in non-endemic areas, and the production and use of the infective blood are both expensive. These factors all translate into a serious need for an improved vaccine.

Possible heartwater vaccines

Several alternative to the existing "vaccine" have been considered or tried. The use of live infective tissue culture material in place of *Cowdria* infective blood was tried at the OVI, but culture and storage problems made this an impossible procedure. An attenuated vaccine would be a possibility (Gueye et al. 1994), but only a few isolates of *Cowdria* have proved amenable to attenuation. Virulent southern African isolates have proven impossible to attenuate, and an attenuated west African isolate does not protect against virulent South African isolates of the parasite (Jongejan et al. 1993). An inactivated tissue culture vaccine is a possibility (Martinez et al. 1994), although the tissue culture of *Cowdria* is expensive and the effectiveness of such a vaccine against virulent southern African isolates has not yet been tested. An inactivated vaccine is currently the subject of research at the OVI and at the Centre de coopération internationale en recherche agronomique pour le développement (CIRAD), Guadeloupe.

In p: .iple a recombinant vaccine for heartwater could be cheap to buy and effective to use, but we need to identify *Cowdria* genes coding for antigens which stimulate the protective response. In heartwater this is predominantly T-cell mediated {Du Plessis, Berche, et al. 1991 #2030} and there are no known directed strategies for locating such genes. The research therefore requires a detailed knowledge of the organism at the molecular genetic level.

Studying the molecular genetics of *Cowdria ruminantium*

The organism is difficult to study because it is obligately intracellular and the tissue culture system is fragile and demanding. The elementary bodies (*Cowdria* cells) need to be purified from bovine host cell nuclei in order that the *Cowdria* DNA prepared from them should contain the minimum of contaminating bovine DNA. Prior to beginning this work nothing was known about the size, structure or organization of the *Cowdria* genome, and very few *Cowdria* genes had been identified. We decided that we should sequence the whole *Cowdria ruminantium* genome.

The rationale for genome sequencing

Judging from the experiences of others who work with difficult organisms we believed that whole genome sequencing would provide the quickest route to the genetic information we needed. After the completion of the *Treponema pallidum* genome sequence in 1998 it was said "Because the organism is so difficult to work with, it is one that should have been sequenced first." (Lukehart, 1998). This applies with equal force to *Cowdria ruminantium*.

We were also attracted by the extensive "fringe benefits" which a genome sequencing project brings, especially to a laboratory in the developing world. These include maintaining a strong infrastructure for biological research, stimulating complementary work in other areas of biology, installing a capability for the use of the latest technologies, and encouraging the development of scientific talent (Bevan 2000).

The *Cowdria* genome consortium

An international consortium was formed, consisting of the following organisations: the OVI, CIRAD-EMVT, France, the University of Stellenbosch (South Africa), Utrecht University (the Netherlands) and the Sanger Centre (Cambridge, England). The funding was supplied by South Africa, France, the European Union, and the Netherlands. Twenty seven complete bacterial genomes are known at present, but as far as we know the *Cowdria ruminantium* project is the only bacterial genome sequencing project underway in Africa.

The groundwork

A great deal of preparatory research had to be undertaken. A well characterised *Cowdria ruminantium* isolate was chosen, the organism was purified and intact *Cowdria* DNA was prepared (de Villiers et al. 1998), genomic libraries were constructed (Brayton et al. 1997), the genome size was determined and a physical and genetic genome map was constructed (De Villiers *et al.*, 2000). These essential preliminaries to the project took seven years to complete.

Strategy

We have two libraries constructed in phage lambda, a ëZAPII library consisting of 10^4 genomes with inserts averaging 2kb, and a and a ëGEM11 library consisting of 10^3 genomes with inserts of 12-22kb. We shall obtain the bulk of the data by shotgun sequencing of 10,000-15,000 clones from the ëZAPII library, and gaps will be filled using clones from the ëGEM-11 library. The full raw genome data should be available by mid 2001, and annotation will take 6-12 months depending upon the availability of resources.

How we will use the data

At present there is no way of identifying which genes are likely to be useful as vaccine candidates for a T-cell mediated protective response, so we shall use the complete genome data to identify a manageable number of *Cowdria* genes which may be likely vaccine candidates. Initially we shall compare the *Cowdria* genome to the known complete genomes of non-virulent bacteria and rule out orthologues as being "probably benign". The remaining genes are likely to be "enriched" for vaccine candidates. We will then use the genome data to prepare micro-arrays which recognise all *Cowdria ruminantium* genes to test for expression under different controlled circumstances. It is known, for instance, that *Cowdria ruminantium* loses its virulence to ruminants after culture in some "unnatural" host cells, although infection still appear to occur. In other "unnatural" host cells the ability to infect is also lost. By comparing expression in these cells to expression in "natural" cells it should be possible to pinpoint genes required for infection as well as genes involved with virulence.

The objective is to identify less than 100 genes which are likely to be suitable as vaccine candidates, and then to test these *in vivo* , individually or in groups, for their ability to protect against virulent heartwater challenge.

DISEASE DIAGNOSIS AND EPIDEMIOLOGY

Highly contagious animal diseases can devastate the agricultural economy of any country, and foot and mouth disease (FMD) is a good example of such a disease. For effective control measures to be instituted it is essential to be able to reach a quick diagnosis, to characterize the pathogen, and to determine its origin and relationship with related pathogens. This then facilitates the identification of its mode of transmission or introduction. However, in the case of FMD traditional serology cannot satisfy these requirements.

FMD in southern Africa

There are 7 serotypes of FMD in the world, of which six occur in Africa. There are only three serotypes in southern Africa, these are the southern African territories (SAT) types 1, 2 and 3. The SAT type viruses are carried by the African buffalo (*Syncerus caffer*); live virus can be maintained in a single animal for up to five years and in an isolated herd of buffalo for more than 24 years (Condy *et al.*, 1985). Carrier buffalo therefore pose a threat of infection to all susceptible cloven-hoofed species and the presence of carrier buffalo in large parts of sub-Saharan Africa complicates control. In South Africa the country has been zoned according to OIE regulations into an FMD-free zone without vaccination, and an FMD endemic area that includes the Kruger National Park (KNP). There are two zones of control around the KNP; firstly a vaccination zone, within which livestock are vaccinated twice annually, and secondly a surveillance zone, within which all livestock are subject to regular veterinary inspection. In order to maintain the status of the rest of the country as FMD-free without vaccination strict movement control is maintained.

Phylogenetic analysis and the geographical origin of FMD viruses

PCR amplification and sequencing of the 1D gene that encodes for the major antigenic determinant of the virus (VP1) reveals geographically distinct 'topotypes'. These are clusters of viruses that appear to be evolving separately in the buffalo populations of southern Africa (Bastos *et al.*, submitted). The individual topotypes are restricted to distinct geographic areas and it has been shown that viruses from the different topotypes differ serologically to such an extent that vaccine strains need to be chosen from each topotype to ensure proper control of the disease (J. J. Esterhuysen, *pers. comm.*).

Since FMD is such a threat to the economy of a country, it is necessary to diagnose the disease promptly and accurately. FMD can be diagnosed within 5 hours of receiving clinical material by two different PCR assays. One of these is a nested PCR assay that targets the 3D gene which encodes for the virus polymerase (Bastos, 1998) and the other uses serotype specific primers that target the 1D gene. The latter assay is used to identify the FMD serotype in the event an outbreak, the PCR fragment is sequenced and the sequence subjected to phylogenetic analysis, which allows the origin of the virus can be determined within 48 hours.

Two practical applications show the value of this technology. It has always been assumed that African buffalo are the source of infection for other species, although the evidence was only circumstantial. Phylogenetic analysis has now conclusively demonstrated that buffalo are indeed the source of infection for impala in the KNP (Bastos *et al.*, 2000) which contributes to a better understanding of the epidemiology of FMD.

During September 2000 there was an outbreak of FMD in South Africa, which is ongoing at the time of writing. This outbreak was found to be caused by a type O virus never before seen in the country. It was a member of the "Pan Pacific" lineage and its origin must therefore have been south-east Asia or the Middle East (Sangare *et al.*, submitted). This information was vital for pinpointing the source of the outbreak and demonstrating that it was not spill-over from infected buffalo in the KNP.

The financial value of FMD identification

Understanding and controlling FMD is vital to South Africa. It threatens food security and also affects the country's animal export status, thereby impinging on all sectors of the population. This is a vivid demonstration of how a country's capabilities for biotechnological research, and capacity to apply the results, determines its international agricultural status.

CONCLUSIONS

Biotechnology is scientifically exciting, since for the first time in history we can actually read the genomes which drive life. Over and above this, however, it is of immense practical value in allowing us to understand animal diseases more completely, and providing us with new tools for animal disease control. Biotechnology is an intellectual challenge for the 21st century; the discipline is rigorous, the intellectual rewards are great, and it provides an absorbing career for young university graduates at the forefront of the life sciences. We believe that all governments need to encourage and nurture this technology, for the good of all their citizens.

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