**Executive Summary**

A very brief summary of the purpose of the project, the research activities, the outputs of the project, and the contribution of the project towards DFID’s development goals. (*Up to 500 words*).

Jembrana disease virus (JDV) causes a potentially fatal infection of Bali cattle (*Bos javanicus*) that is endemic on the island of Bali. Disease control measures are hampered by the current absence of assays for virus detection and *in vitro* culture. Consequently the economic and animal welfare burden within the island is significant, particularly for the subsistence level farmers in the rural communities of the island.

This project had two major objectives 1) to develop an *in vitro*, ELISA assay to detect virus proteins in the blood/serum of infected cattle and 2) to investigate ways to culture the virus in bovine cells *in vitro*.

To achieve objective 1) we had to produce a source of viral protein. We cannot import infectious JDV into the UK therefore molecular techniques were used to clone and express one of the major proteins of the virus, the JDV capsid (CA). This protein was purified and used to raise virus-specific antibodies in mice and rabbits. Different combinations of antibodies were used to optimise an ELISA test to detect very low levels of the recombinant JDV CA protein. We can demonstrate effective detection of CA protein at concentrations of only 3ng/ml. Objective 2) was again limited by our inability to work directly with infectious JDV in the UK. Therefore we used a closely related virus, bovine immunodeficiency virus (BIV). BIV, although endemic in the UK is equally difficult to culture from infected cattle, such that a UK-derived virus hasn’t been isolated to date. To improve the chances of growing virus in culture we produced a specific strain of bovine cells that expressed a JDV protein, tat (transactivator of transcription), that is known to stimulate the expression of viral genes for both BIV and JDV. The assumption to be tested was that stimulation of viral gene expression would increase the rate of virus growth and enhance the chances of isolating the virus in culture. Several lines of bovine MDBK cells that expressed biologically active tat protein were produced. The susceptibility of these cells to infection with BIV was not increased. To date we have been unable to take these cells to Bali to assess the growth of JDV but are maintaining contact with investigators within the collaborating Institution. The same JDV tat construct was then transfected into a more susceptible primary bovine cell line but the viability and passage life of the cells was not sufficient to permit stable cell lines to be isolated and therefore these could not be tested for their ability to support increased viral growth.

As a consequence of these studies we have a cell line expressing JDV tat protein that may stimulate virus growth in culture, but which have yet to be infected with JDV. We also have a robust *in vitro* test which will detect recombinant JDV protein. The potential for this assay to detect viral antigen in samples from infected cattle is very high and is currently being pursued by ongoing collaboration. If proven in the field this assay could form the basis of a virus detection and control strategy that would contribute significantly to the management and prevention of JDV-related disease outbreaks in Indonesia.

**Background**

Information should include a description of the importance of the researchable constraint(s) that the project sought to address and a summary of any significant research previously carried out. Also, some reference to how the demand for the project was identified.

Bali cattle (*Bos javanicus*) are central to the agriculture of Indonesian islands. They provide the draught power to cultivate the narrow and often precipitous rice terraces. They also provide meat and leather. Furthermore, within Balinese culture, they are venerated and many ceremonial festivals are directed at their worship. Over the years, Bali cattle have appeared
relatively resistant to ecto- and endoparasites but in the late 1960’s, a catastrophic disease occurred which, in its first epizootic, was estimated to kill some 60,000 cattle. Since then, further episodes of disease have been recorded and it is now considered endemic within the main island of Bali.

The aetiological agent has been an enigma until recently, although the disease can be readily transmitted to susceptible Bali cattle by the inoculation of blood, spleen or lymph node material from infected cattle. In experimentally infected cattle, the incubation period from infection to the onset of clinical signs varies from 4.5 to 12 days. The major clinical signs are fever, lethargy, anorexia and enlargement of lymph nodes; the pathological changes were lymphoproliferation in lymphoid organs, lymphocytic infiltration into many organs (Dharma et al 1991). A co-ordinated network of researchers from the Bali Cattle Disease Investigation Unit in Bali, the School of Veterinary Studies, Murdoch W.A. and the Royal Veterinary College have been able to identify the agent as a retrovirus with strong resemblances to the bovine immunodeficiency virus (BIV) (Wilcox et al 1992, Kertayadnya et al 1993, Brownlie et al 1995). The agent is now entitled Jembrana disease virus (JDV) and has been shown to have 74% homology to conserved regions of the pol gene of BIV (Chadwick et al 1995).

The diagnosis of Jembrana disease in field cases is still difficult and is based on the description of typical clinical signs. As yet, a cell culture system has not been derived for the growth of JDV and there is presently no means of isolating or quantitating the virus. Current methods for detecting and titrating JDV rely on inoculating Bali cattle; this is unsustainable and a serious constraint on diagnostic and research development. Attempts to develop virus isolation and assay techniques by BCDIU have not been successful. The present proposal provided two alternative approaches to the isolation and quantification of the virus.

References:

Project Purpose
The purpose of the project and how it addressed the identified development opportunity or identified constraint to development.
This projects aims to improve the health and performance of draught animals, critical to the agriculture of Indonesian rural communities, by the development and validation of diagnostic assays for the virus which causes the often fatal Jembrana disease of Bali cattle. Provision of robust assays based on recombinant protein will support Indonesian Animal Health programmes.

Research Activities
This section should include detailed descriptions of all the research activities (research studies, surveys, etc) conducted to achieve the outputs of the project. Information on any facilities, expertise and special resources used to implement the project should also be included. Indicate any modification to the proposed research activities, and whether planned inputs were achieved.
Research Activity 1 (RA1): Development of an antigen-capture ELISA for JDV.
The development of this viral detection assay required the successful completion of several steps: a) the generation of recombinant viral protein antigen (it is not possible to import the virus into the UK), b) the sourcing/generation of JDV-specific antibodies for capture and detection of viral antigen and c) the optimisation of the ELISA test.
The original target had been suggested as being the viral envelope protein but this target was modified as it became apparent that the generation of highly specific reagents to JDV capsid (CA) protein would be more appropriate and achievable during the course of the two year project. Therefore the JDV CA sequences were amplified by RT-PCR, cloned and sequenced prior to recombinant protein expression and purification. Both murine monoclonal antibodies (MAb) and rabbit hyperimmune sera were raised to this recombinant JDV CA protein. The ELISA was developed by testing several formats including direct antigen detection and indirect antigen capture using combinations of murine anti-JDV MAbs, rabbit anti-JDV hyperimmune and bovine anti-BIV hyperimmune or convalescent sera.

Following the optimisation of the assay on recombinant protein products a small number of sera from Bali cattle were made available. Although they were not apparently from diseased animals and had been heat inactivated they were tested in this assay. Not surprisingly, no virus was found.

Research Activity 2 (RA2): In vitro cell culture for JDV.
There is presently no culture system for JDV and this has become a major constraint for research and disease surveillance. Lentiviruses are notoriously difficult to culture in vitro, for example BIV identified in seropositive animals by polymerase chain reaction (PCR) has proved extremely difficult and often impossible to isolate by means of in vitro culture. Replication of a lentivirus such as JDV initially involves trans-activation of the promoter of the integrated provirus by specific cellular nuclear protein factors such as NF-κβ, SP-1 and others. This leads to expression of spliced mRNA species from which viral trans-acting proteins are translated, including Tat and Rev proteins. Tat in particular, is itself a potent trans-activator of the viral promoter, which leads to markedly upregulated transcription and expression of all viral proteins.

The viral tat gene was amplified by PCR, cloned, sequenced. The construct was then transfected into bovine MDBK cells. Stable transfectants were isolated by limiting dilution and cloning in the presence of zeocin. The biological activity of the tat gene in these cell lines was demonstrated by the stimulation of expression of a chloramphenicol acetyltransferase gene which was expressed from a tat-dependent promoter. Several cell lines were established from these transfectants and tested for their ability to support the replication of the related lentivirus BIV (no JDV can be used in the UK).

Outputs
The research results and products achieved by the project. Were all the anticipated outputs achieved and if not what were the reasons? Research results should be presented as tables, graphs or sketches rather than lengthy writing, and provided in as quantitative a form as far as is possible.

RA1 : In the project proposal we stated that "The most clearly identifiable output will be a viral detection system for Jembrana disease... The detection of virus and viral variants directly from field material will be essential for effective control and eradication measures.". During the period of research the virus antigen detection ELISA has been developed using new reagents created as a direct result of the funding. Originally, the virus envelope protein was the perceived target for this assay but it became apparent that more appropriate reagents, and the one finally used, was the virus capsid protein. Using the experimental and field reagents available we judge this to be a highly sensitive and specific virus detection system. On the basis of work to date we have been able to unequivocally detect recombinant proteins derived from JDV but produced in the lab. The sensitivity of the system developed is great, detecting recombinant protein at a level of only 3 nanograms/ml (see figure 1).

Figure 1: Detection of both JDV- and BIV-derived CA proteins by ELISA.
It has not been possible to import JDV into the UK for direct testing and the political and economic situation which developed in Indonesia made it impractical for the proposed research trip to Bali to take place. However, we are actively pursuing the testing of this assay in ongoing collaborative work. Analysis of a small number of sera from clinically healthy Bali cattle failed to detect viral antigen. Further field testing in Bali would be required to assess its utility for detection of variant viruses that may exist within Indonesia.

RA2: A second major objective at the outset was "... to establish a continuous cell line that is permissive for JDV culture.". The development of a culture system for JDV was initiated as detailed above. MDBK cells (an established bovine cell line) were stably transfected with the JDV tat gene. This gene was shown to be active through its ability to stimulate expression from a plasmid containing the chloramphenicol acetyltransferase gene under the control of the tat dependent promoter. However, the susceptibility of this cell line to infection by BIV was not increased. At this stage it is unclear what is the nature of the molecular block to replication of BIV in these cells. It is still possible that JDV could be cultured in this cell line. This possibility was not tested as the political and economic instability in Indonesia restricted working visits to Bali.

**Contribution of Outputs**

Include how the outputs will contribute towards DFID's developmental goals. The identified promotion pathways to target institutions and beneficiaries. What follow up action/research is necessary to promote the findings of the work to achieve their development benefit? This should include a list of publications, plans for further dissemination, as appropriate. For projects aimed at developing a device, material or process specify:

a) What further market studies need to be done?

It is proposed that the ELISA antibody assay developed for Jembrana disease is available and used within Indonesia. Further market studies should await the outcome of validation of the assay under laboratory conditions in Indonesia.

b) How the outputs will be made available to intended users?
This has already occurred. Professor Brownlie has recently travelled to Inodensia and taken all the developed reagents for use by Indonesian diagnostic services. On that visit, there were discussions and training programmes undertaken.

c) *What further stages will be needed to develop, test and establish manufacture of a product?*

The basic materials (recombinant protein and antisers) have been developed for the ELISA assay; these have been transferred to the Indonesian centre for research on Jembrana disease.

d) *How and by whom, will the further stages be carried out and paid for?*

At present, it is anticipated that the continuance of production of the reagents for the newly-developed assay will be possible by the Indonesian veterinary team. If further help is needed, then we would be willing to help further.

**Annex / Appendix**

Reports, Publications, Thesis outputs etc.

P. Barboni, I. Thompson, J. Brownlie, N. Hartaningsih and M. E. Collins.
Evidence for the presence of two bovine lentiviruses in the cattle population of Bali. Veterinary Microbiology (2001) 80:313-327