Executive summaries

of

RMRD FUNDED

Research Projects

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1 ASF in soft Tampans

By Dr A Pretorius (previously Dr L Heath)

Research Institute: ARC-Onderstepoort Veterinary Institute (OVI)

Industry Sector: PORK

Focus Area: Animal Health and Welfare (3)

Contract dates: 01/01/2005 to 30/06/2007

Total Funding: R 109 600.00

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In recent years South Africa has experienced a drastic expansion of game farming and related ecotourism activities. This has meant that several species of game have been reintroduced into areas that have previously been used for commercial farming. The increased likelihood that contact between wild life and domesticated animal may occur in these region represents a significant threat of disease transmission to naïve domestic livestock.

Of particular interest to the pig producers in South Africa is threat that African swine fever may be introduced into areas that have previously been free of the disease. ASF is currently restricted to the far-northern parts of South Africa where it is maintained in three epidemiological cycles. The sylvatic cycle involves wild suids, and soft ticks belonging to the Ornithodoros moubata complex. As the natural arthropod host of ASFV soft ticks represent the link between wild suids and domestic pigs. In areas where domestic pigs are kept within the home range of wild suids, such as warthogs, the spread of ASFV to pigs are often facilitated by soft ticks. Once established in domestic pig populations the virus can be maintained independently of the wild suids and ticks.

Anecdotal evidence has suggested that the distribution of warthogs and soft ticks associated with the species is slowly expanding southwards. Concerns have been raised that this expansion may have resulted in the dissemination of ASF beyond the current ASF control zone. In response, the RMRDT and the Department of Agriculture have initiated surveillance projects aimed at determining whether the disease and its associated vectors are present south of the current control zone. The results of the surveillance can potentially be used as a guideline for the possible re-demarcation of the ASFV control zone borders.

The previously described PCR methodology for the detection of ASF in soft tick was modified in line with standard operating procedures implemented at ARC-Onderstepoort Veterinary Institute, Transboundary Animal Diseases Programme.. Modifications included the use of an alternative DNA polymerase reaction mixture and minimal adjustments to the thermo-cycling conditions as recommended by the manufacturer. It was further demonstrated that the sensitivity of the nested PCR assay exceeds that of the conventional diagnostic PCR for detection ASFV in tick homogenates.

To monitor the effect of inhibitors in the tick homogenates, a control template (CT) was constructed by cloning the target region into a suitable bacterial plasmid. All PCR reactions were carried out in duplicate both without CT and using the smallest concentration of CT that proved to be consistently amplified. The results of PCR reactions that failed to yield the expected fragments when the CT was included were disregarded.

Ticks collected from various locations in Gauteng south of the current ASF control zone in were submitted to TADP by the provisional veterinary services. Of the 17 batches submitted for testing, 5 tested positive for ASF genetic material by PCR (Annex 1, Table 1). Virus could only be isolated from 1 batch originating from Mangena Nature Reserve. It should however be noted that the Mangena Nature Reserve is situated a considerable distance south of the ASF control line. Phylogenetic analysis using the p72 gene region of various South African isolates indicated that these viruses fall within the known genotypes occurring in the region. (Annex 1, Fig. 2)

In conclusion, some evidence exists suggesting that the distribution of soft ticks belonging to the Ornithodoros moubata complex extends further south than previously reported. Furthermore, the detection of ASFV in these ticks may represent a significant threat of ASF beyond the current ASF control zone. However, more research is necessary to establish the level of risk along the entire ASF control line. A detailed study into the role of climate change on the tick distribution, changes in distributions of warthogs due to increased wildlife farming and risk of contact with domestic pig should be conducted before recommendation on the alteration of the current ASF control zone can be considered.

2 Genetic Predictions for Beef Cattle

By Dr A Maiwashe

Research Institute: **ARC – Animal Production Institute (ARC-API)**

Industry Sector: **Cattle/Beef**

Focus Area: **Livestock production with global competitiveness (2)**

Contract dates: **3/1/2007 to 21/06/2009**

Total Funding: **R 326 102.00**

Final report approved: **17 Mar 2010**

Continuous improvement of methods to estimate breeding values is a necessary for sustainable genetic improvement. In the current project we conducted a research to develop a new breeding value for tolerance to ticks. We also investigated the feasibility of implementing more accurate methods for genetic analysis of stayability and post-weaning performance in intensively fed beef bulls.

The development of a new breeding value for tolerance to ticks was conducted using tick count data collected by ten seedstock Bonsmara farmers. The development of the new breeding value was carried out in two steps. In the first step, environmental and genetic factors that underly the differences in tolerance to ticks among animals were identified using statistical methods. This step is commonly known among academic animal breeders as the "model development step". The environmental factors found to be important were the farm and date on which tick load was assessed, sex and age of the animal. Only one genetic factor was found to be important. This genetic factor is due to genes influencing tolerance to ticks that are passed on to the offspring and is technically called direct additive genetic factor. In the second step, the heritability for tolerance to ticks were estimated using the factors identified in step one. The heritabilities found in the current study indicate that 13% of the differences in tolerance to ticks in the Bonsmara breed are attributable to differences among breeding values.

The feasibility of using more accurate methods for estimation of breeding values for stayability and post-weaning weekly feed intake and weight gain were conducted separately. For stayability calving records on Angus cows participating in the National Beef Improvement Scheme were considered. In this study we used a statistical method called the "threshold model" to analyse stayability and heritabilities found in the current study are similar to those reported in similar research from other countries.

The Bonsmara data was used for post-weaning weekly feed intake and weight gain in intensively fed beef bulls. Genetic analysis of these traits was conducted using a statistical method called Random Regression Model. We successfully implemented the Random Regression model in the current

study. The heritabilities obtained in the current study were similar to those reported in countries were this method has already been implemented.

Practical implications:

A method to estimate breeding values for tolerance to ticks was developed. This new breeding value provides an opportunity to Bonsmara beef producers to improve tolerance to ticks through selection. Improved tolerance to ticks will lead to reduction in costs associated with use of acaricides to control ticks. The method developed in this study can be adapted for other beef cattle breeds. Successful implementation of methods for estimation of breeding values for stayability and post-weaning feed intake and weight gain indicates the feasibility of using these methods in South Africa. Implementation of these methods in the routine estimation of breeding value should be preceded by education of farmers in using breeding values from these methods.

3 Recombinant heartwater vaccine for cattle, sheep and goats

By Dr M van Kleef and co-researcher Dion du Plessis

Research Institute: **ARC – Animal Production Institute (ARC-API)**

Industry Sector: **Cattle/Beef**

Focus Area: **Animal Health and Welfare (3)**

Contract dates: **01/10/2005 to 30/09/2007**

Total Funding: **R 340 778.00**

Final report approved: **17 Mar 2010**

Heartwater is a tick-borne disease of domestic and some wild ruminants caused by E. ruminantium. Cell mediated immune responses accompanied by IFN-γ production is required for protection against heartwater and proteins that can induce such responses may be good vaccine candidates. It has been shown that low molecular weight proteins of E. ruminantium induce CD4+ cells to proliferate and produce IFN-γ. Therefore, in an attempt to develop a DNA vaccine for heartwater this project focused on low molecular weight proteins as potential vaccine candidates. The aim was initially to obtain the amino acid sequence of selected proteins by mass spectrometry and screen the E. ruminantium complete genome sequence to determine the full DNA sequences encoding the low molecular weight proteins. The low molecular weight proteins were separated by 2-dimensional electrophoresis and three protein spots were prepared for mass spectrometry analysis. However, no mass spectrometry data could be obtained due to the low protein concentration. This method was therefore abandoned and low molecular weight proteins were alternatively selected from the E. ruminantium genome using Bioinformatic tools. Five proteins were identified and were successfully expressed in a bacterial expression system. Their ability to induce recall T-cell responses as well as IFN-γ production was evaluated in vitro using lymphocyte proliferation assays (LPA) and ELISPOT respectively. All the recombinant proteins induced T-cell proliferative responses as well as IFN-γ production by PBMC from immune sheep. The corresponding five ORFs were then incorporated into a pCMViUBs vaccine vector and tested as a cocktail in outbred sheep using the DNA prime–protein boost immunization regimen. The cocktail of the five DNA constructs only provided 20% protection against a virulent E. ruminantium (Welgevonden) needle challenge. The sheep vaccinated with the cocktail DNA vaccine showed increased T-cell proliferative responses and IFN-γ production before challenge. However, this response decreased after challenge in sheep that succumbed and increased in the sheep that survived challenge. Further evaluation of the five open reading frames (OFRs) individually and vaccine dose optimisation is necessary in order to determine which ORF/s was/were responsible for the partial protection. Inclusion of the ORF/s in a vaccine

cocktail together with the four 1H12 ORFs previously identified may improve the protective efficacy in an outbred population of ruminants when tick challenged in the field.

4 A study of bovine malignant catarrhal fever (MCF) in South Africa “Snotsiekte”

By C V Vroon

Research Institute: **ARC Onderstepoort Veterinary Institute (OVI)**

Industry Sector: **Cattle/Beef**

Focus Area: **Animal Health and Welfare (3)**

Contract dates: **02/01/2000 to 31/12/2008**

Total Funding: **R 521 509.00**

Final report approved: **17 Mar 2010**

The ARC-OVI and UP research teams set out to investigate various aspects of the epidemiology of MCF in South Africa, to develop rapid diagnostic procedures for the detection virus-specific antibodies, and to lay the groundwork for the production of new generation vaccines.

OvHV-2 was indicated in 77% of the four sheep breeds tested. This could have practical implications for farmers.

In the blood of hybrid wildebeest, AlHV-1 could be detected in 23/25 animals and also in various organs. In a small study to determine whether Gedoelstia hässleri spp. collected from the heads of these hybrid wildebeest could play a role as a vector of AlHV-1, the presence of AlHV-1 could not be detected by nested PCR.

A study was done by a team from the university of Pretoria UP team led by Professor Armanda Bastos, on the variation in the fine structure of the genetic material of wildebeest-associated virus isolates from different geographical regions of South Africa. Initially three genes were targeted and in a follow-up study five genes. Two distinct evolutionary groups of viruses that cause "snotsiekte" in South Africa were identified in both studies. As levels of variation are high in the virus it is essential that many genes be typed in order to find epidemiological links between different MCF cases. No geographical structuring was indicated implicating that at the moment ica the virus probably spreads due to intervention by man in South Africa.

The prevalence of bovine lymphotropic herpesvirus (BLHV) was determined in the blood of 74 bovines infected with AlHV-1, in order to determine whether this virus may play a role in the pathogenesis of MCF. A one tube nested PCR was developed and BLHV DNA was identified in 13% of the bovines. Bovine lymphotropic virus probably does not play a role in the development of MCF.

Various attempts to develop a competition ELISA using phage display technologies were unsuccessful. In an alternative approach an indirect ELISA was developed using a recombinant virus protein expressed in bacteria. The specificity of this antigen was determined using a panel of positive and negative sera that had been analysed previously with the commercial BMCFV antibody detection kit (VMRD, Inc. Pullman, WA, USA). Initial indications are that this ELISA can discriminate between antibodies induced by AlHV-1 and OvHV-2. Such an ELISA has the potential to be used in legal cases e.g. when a game farm is introduced in the vicinity of a cattle farm. The spread of AlHV-1 to the cattle herd due to the introduction of wildebeest can be monitored. It can also be used as a complementary testing procedure when diagnostic PCR is negative and all clinical indications are that an animal had contracted MCF. It is more cost effective to use ELISA than PCR for large-scale screening of herds of cattle to determine their exposure to AlHV-1 and their possible carrier status. Furthermore, in wildebeest blood the levels of AlHV-1 are often very low and undetectable by PCR. The ELISA can be used a complementary diagnostic test to determine their carrier status.

In an attempt to identify immunologically important AlHV-1 epitopes which could give an indication of which proteins to use as vaccine candidates, an AlHV-1 genome specific library was developed. Although such a library has the potential to provide valuable information, so far no epitopes could be identified from the different sera tested. The problem may be that too stringent washing conditions had been used. Under alternative conditions important epitopes may be identified.

Virus structural proteins on the surface of the virus envelope and the virus capsid were targeted as potential vaccine candidates. The first candidate vaccine is a recombinant DNA vector containing the gpB gene encoding a virus envelope protein. The second was the protein product of the ORF65 gene expressed in bacteria. These can now be tested as candidate vaccines. A preliminary experiment was done in rabbits, a suitable laboratory model for MCF, to determine an optimal concentration of virus to be used as intranasal inoculum (the natural route of infection) when testing the protective potential of the candidate vaccines. No clear clinical signs of MCF were observed in the 15 inoculated rabbits although histopathological lesions characteristic of MCF was observed in one rabbit which died two weeks after inoculation of the virus. To obtain the required result higher virus concentrations and alternative methods of inoculation of animals seem to be necessary.

Practical implications:

Information has become available that in South Africa a high percentage of sheep are carriers of OvHV-2.

The indirect ELISA has the potential to be further developed and validated for use as a complementary diagnostic tool and also for epidemiological studies.

It was shown that the spread of AlHV-1 strains can be monitored using bioinformatic procedures. These procedures also have the potential to be used in legal cases where it could possiby be used to give an indication of the origin of AlHV-1 strains found in cattle with MCF.

Should candidate vaccines be tested in rabbits/cattle their protective ability can be determined. Development of the candidate vaccines can be done. For this additional funding will be required.

5 Response to balanced protein by pigs

By Prof R M Gous

Research Institute: **University of KwaZulu-Natal (UKZN)**

Industry Sector: **Pork**

Focus Area: **Red Meat Safety, Nutritional Quality and Value (5)**

Contract dates: **01/04/2008 to 01/10/2009**

Total Funding: **R 120 000.00**

Final report approved: **24 Feb 2010**

Pig producers would like to be able to formulate a set of feeds that will ensure that the profitability of their enterprise is maximised. To achieve this, one needs to know about the genotype being used, the cost and availability of ingredients, the revenue derived for different grades at the abattoir, and the environment in which the pigs are to be housed. A model is now available that can achieve this goal, and consequently it is worth improving the description of genotypes that are used in the commercial pig industry so that accurate predictions can be made of voluntary food intake, which leads on to being able to optimise the feeding strategy used.

Modern pig strains have been selected for improved growth and feed efficiency using high protein feeds, and such selection has resulted in leaner carcasses and perhaps a reduced ability to fatten when faced with feeds deficient in an essential nutrient. Two trials were conducted using five local strains to measure this ability in the early growing period. Four levels of balanced dietary protein were fed and growth rate, food intake and feed efficiency were measured. At the end of the trial the carcasses of all pigs were analysed for water, protein and lipid.

Feed intake was unaffected by feed protein content in both trials, while both body weight gain and feed efficiency increased with dietary protein content, although not significantly. Body protein content increased significantly with feed protein content in two strains, and body water increased and body lipid decreased with feed protein content in the others. Had more pigs been used in each treatment these differences would have shown up more clearly, and this was a definite limitation in this exercise as only four males and four females of each strain could be used at each dietary protein level.

The results of this trial have demonstrated that pigs respond differently to dietary protein, which means that there is a protein level that will maximise profitability for the producer. The question is how to determine what this level is. For the commercial pig producer models are now available that make it possible to change the emphasis when choosing nutrient specifications for formulating

feeds from ‘least cost’ to ‘maximise profitability subject to the constraints which act on the business’. Because economic circumstances change periodically, different nutritional strategies will be needed to maximise margins. Instead of sticking rigidly to a set of nutrient specifications it is possible, with the use of simulation models, to respond immediately to those changed circumstances, ensuring that the profitability of the enterprise is maximised under all economic circumstances. Such is the way that pigs should be fed in the future.

6 NFSC010 - Beef tenderness model (Phase 2)

By Dr L. Frylinck

Research Institute: **ARC Animal Products Institute**

Industry Sector: **Beef**

Focus Area: **Animal Products Quality and Value-adding (4)**

Contract dates: **01/04/2004 to 31/13/2008**

Total Funding: **R 1 379 200.00**

Final report approved: **17 Mar 2010**

The aim of this study was to evaluate the meat quality differences (colour of meat and subcutaneous fat, drip loss, water holding capacity, tenderness and other sensory attributes such as juiciness and aroma) of A-age (feedlot and pasture) animals, AB-age (feedlot and pasture) animals, and B-age (pasture) animals under the determined ideal slaughter conditions for South African crossbred beef breeds.

Each age-feed group consisted of 10 animals of each cross breed (Brahman-X, Simmental-X, Nguni-X). Feedlot animals were raised until required age-classes (A-age (zero permanent incisors) and AB-age (one-two incisors)). These test groups will be revered to as AF and ABF. The pasture animals were introduced to pasture after weaning until required age-classes (A-, AB- and B-age (three - six permanent incisor)). These three test groups will be referred to as AP, ABP, and BP. The animals were slaughtered according to normal South African slaughter procedures and the carcasses were electrically stimulated for 15 sec (400 V peak, 5 ms pulses at 15 pulses per sec). Carcasses were chilled directly after dressing at room temperature before loading at 0 – 4 °C.

Depending on the production system, careful controlled slaughter conditions and breed type, Warner Bratzler shear force and sensory judged first bite, tenderness, and residue results showed that older animals can produce more tender meat than younger animals. On average the AB-age feedlot produced animals were the most tender followed by the B-age veld produced animals, then the A-age feedlot animals similar to the AB-age veld animals, with the A-age veld animals producing the least tender meat.

Differently from the results from Phase 1, the Simmentaler-cross animals produced the most tender meat compared to that of the Brahman-crosses and Nguni-crosses as should be expected from a Bos taurus breed. This indicates to the importance of choosing the right agent to source animals from a bonafidé source according to stipulated specifications and that not all Simmentaler-crossings

are beneficial to meat tenderness (i.e. Simmentaler – Brahman crosses). Breeders should be made aware of the genetic consequences on all levels including meat quality characteristics.

The Nguni-crosses produced the most tender meat when produced from AB-age feedlot and B-age veld production systems showing that they adapt better to these production systems.

The genetic expressed calpain proteolytic ageing system plays a pivotal role in determining the ultimate meat tenderness, but although connective tissue becomes less soluble the older the animal it did not play an important role in determining tenderness in the age-groups A, AB and B.

What did seem to play an important role in the tenderness outcome of the age-feed groups seemed to be the % intra muscular fat (i.e. marbling) thus the fat condition of the animal at slaughter. The Nguni-crosses seemed to marble well at the AB age group that gave it the competitive edge in this production group.

The Nguni-crosses also seemed to produce the juiciest and most flavoursome meat with the best aroma according to the sensory panel. The older animals seemed to produce the most flavoursome meat; feed regime can change the flavour of the meat.

The energy status of the muscle of the animal at slaughter influences the biochemical mechanisms that have an effect on meat tenderness, juiciness and flavour. The energy levels should be controlled by means of optimum feeding conditions and avoiding unnecessary stress at slaughter. The Nguni-type is more prone to stress than the other breeds.

Judging eating quality on the grounds of visual subcutaneous fat colour is not reliable because carcasses from older animals and certain production systems produce yellower fat, but is not necessarily tougher. The South African classification system and resultant remuneration should be revisited and consumers should be educated accordingly. Production systems should be optimised to provide optimum pre- and post slaughter conditions and adapted to accommodate different breed-types.

7 Attenuated vaccine against heartwater

By Dr E P Zweygarth (previously dr Mirinda van Kleef)

Research Institute: **ARC Onderstepoort Veterinary Institute (OVI)**

Industry Sector: **Small Stock**

Focus Area: **Animal Health and Welfare (3)**

Contract dates: **01/02/2005 to 31/13/2008**

Total Funding: **R 205 807.00**

Final report approved: **17 Mar 2010**

Heartwater, or cowdriosis, is an infectious, non-contagious, tick-borne disease, caused by the intracellular rickettsial agent Ehrlichia ruminantium. The disease affects cattle, sheep and goats, and also some wild ruminants, and is transmitted by ticks of the genus Amblyomma. Heartwater is usually an acute disease and may be fatal within days of the onset of clinical signs. Field observations and experiments under laboratory conditions have shown that infected animals are capable of developing a protective immunity against heartwater after surviving a virulent infection. At present, the only commercially available immunization involves infecting animals with cryopreserved sheep blood containing virulent E. ruminantium organisms, followed by chemotherapeutic treatment when fever develops. We previously reported that intravenous immunization with an attenuated E. ruminantium (Welgevonden) stock provided full protection against a virulent homologous needle challenge in Merino sheep and Boer goats. Further experiments showed that cryopreserved stabilates were similarly effective. Vaccination did not produce disease in any of the animals and upon challenge with the virulent homologous stock all animals were fully protected. When sheep were challenged 6 months after i.v. immunization they all were fully protected whereas the vaccination protected all except one animal in the group challenged 12 months after immunization. The subcutaneous and the intramuscular routes of vaccine application were tested as more convenient alternatives to the intravenous. Protection in animals injected subcutaneously was not as pronounced as if the animals were injected intravenously. When the vaccine was applied by the intramuscular route, 4 out of 5 animals were protected against a lethal needle challenge with the virulent homologous stock. The efficacy of the attenuated vaccine was also evaluated in Angora goats, which are highly susceptible to E. ruminantium infection. Three groups of Angora goats each were inoculated i.v. with various vaccine doses. Upon immunization, all animals showed a rise in body temperature. The group receiving the highest dose required chemotherapeutic intervention to prevent losses; of the remaining goats only one animal in the group receiving the lowest dose had to be treated. All immunized Angora goats, including those which were treated, were subsequently found to be fully protected against a lethal needle challenge with the virulent homologous stock and none of the animals showed any challenge

reaction. The attenuated vaccine still showed an unexpectedly high degree of virulence in Angora goats. In Friesian cattle vaccination did not produce clinical disease in any of the animals and no rise in body temperature was observed. Upon challenge with a virulent E. ruminantium stock all but one animal were fully protected.

The attenuated Welgevonden stock vaccine has shown great potential as a possible new interim vaccine against heartwater until better alternatives may become available. A cryopreserved attenuated vaccine could be distributed like the Ball3 vaccine, and could become available for broader use in large scale field experiments. Effective logistic back-up and sustained financial support will be required to develop a marketable product.