**RE & HO Bevan Consulting Pty Ltd**
**Pakistan Veterinary Vaccine Review**
**19th – 30th April 2008**

**Executive Summary**

The vaccine production units at Tando Jam and Lahore were reviewed and comments are presented in this document as presented at the seminar in Islamabad on the 30th April 2008.

The vaccines produced at the government institutes and used in the cattle and buffalo of Pakistan are of questionable quality especially in respect to their sterility status, which can be appropriately addressed with additional formaldehyde and the addition of the preservative Thimerosal.

Efficacy/potency/stability issues also need to be addressed with all vaccines as does the cold chain issue.

In the opinion of the author Foot and Mouth Disease vaccine production in Lahore should be discontinued and a complete review undertaken before re-instituting production as deficiencies in the total production system compromises the quality of the product.

The vaccine production units are dated with respect to design and operation when compared to units found elsewhere in the world. There is a direct need to expose senior staff to contemporary design, engineering, training and operation protocols so that new production units are run using the latest methodologies.

**Introduction:**

Richard Bevan as a director of RE & HO Bevan Consulting Pty Ltd was invited to join and carry out informal audits of selected provisional Pakistani government veterinary vaccine manufacturing plants. This was part of the ASLP Dairy Project which is an Australian Government project [ACIAR; contact Dr Christian Roth] co-ordinated by Charles Sturt University [contact Prof Peter Wynn] and the Livestock and Dairy Development Board [contact Dr Muhammad Afzal, Chief Executive].

The audit was initiated as the vaccines produced by these institutes are used in the field to provide protection against infectious disease caused by various organisms that result in either mortality or more likely morbidity leading to loss of livestock and or production.

Pakistan has approximately 50 million cattle and buffalo providing milk, meat and used as draft animals in farm operations.

The vaccines are supplied by the Pakistan government to farmers at a subsidised rate which is far cheaper than the price for imported vaccines of similar type and composition: these are unaffordable for most of the poorer farmers. Provincial Laboratories exist in all the provinces servicing the needs of the local farmers

Mr David McGill, the Australian project representative in Pakistan, organised the itinerary for this trip, and accompanied me where ever I went. His good company, professional approach to all matters relevant, and ability make things happen made this trip both enjoyable, but more importantly relevant to the health care of the cattle and buffalo of Pakistan.

**Sites Visited.**

Central Veterinary Diagnostic Laboratory, Tando Jam, Sindh. I would like to thank Dr Muhammad Naeem Arain and Dr Jamil Ahmed Shaikh and their staff for open and helpful discussions.

Sindh Poultry Vaccine Centre in Karachi. Dr Shafqat Fatima Rehami, Executive Director and her staff kindly showed David and I her laboratories and discussed her current issues. This laboratory is not part of this report as it was involved in poultry production.

Veterinary Research Institute Lahore where Dr Zafar Jamil Gill, Dr Muhammad Iqbal and Dr Kausar Tasneem facilitated an extensive review of the current vaccines produced in this Institute.

VRI Lahore Foot and Mouth Disease Vaccine Centre where Drs Fayyaz Mahood, Shaukat Ali, Shoab Noor and Talha all facilitated open and frank discussions about their vaccine production.

University of Veterinary and Animal Sciences, Lahore, Quality Control Laboratory WTO where Dr Khusi Muhammad spent the best part of the day showing and explaining the function of the laboratory.

National Veterinary Laboratories NARC, Islamabad where Dr Khurshid Ahmad conducted a tour and fruitful discussions on vaccine testing on behalf of the Pakistani government.

National Reference Laboratory for Poultry Disease, National Agriculture Centre, Islamabad where Dr Khalid Naeem and his staff spent time with David and I talking about the laboratory's function and current research.

A seminar was held in Islamabad at the completion of the review in an effort to convey a summary of the findings. At the seminar there were representatives from the laboratories visited as well as those from each province of Pakistan that were unable to be reached within the review schedule. Government officials including the Animal Husbandry Commissioner (Dr Rafiq Usmani) were also present. Professor Dr. Shahana Urooj Kazmi from the Pakistan Agriculture Research Council co-chaired the meeting in Islamabad with Dr Muhammad Afzal.

**Vaccines Manufactured by Pakistan Government Provincial Laboratories Relevant to Buffalo and Cattle.**

1]. Haemorrhagic Septicaemia Vaccine as both Alum and Oil-emulsion presentations [*Pasteurella multocida* Strain 2b].

This is a monovalent vaccine with the majority of use in village animals being the alum presentation. Oil emulsion vaccines are used for larger and more intensive farms. The oil emulsion vaccine produced in Lahore contains lanolin as the oil base resulting in a very viscous vaccine. A suggestion was made to investigate the use of liquid paraffin as an

alternative to produce a thinner emulsion or a double emulsion using gel/ oil based emulsion as an alternative

2]. Blackquarter Vaccine [*Clostridium chauvoei*].

This monovalent Clostridial vaccine against septicaemia in cattle is probably the most refined vaccine produced by VRI Lahore. This vaccine is effective in guinea pigs albeit against the strain of bacterium (*Cl chauvoei)* used for the production of the vaccine. Logically previous experience would suggest that this vaccine will also be efficacious in the field. In Australia this vaccine is combined with one directed to the closely related *Cl septicum* to form a Blackleg vaccine. Similarly other combinations of Clostridial components including *Cl tetani, Cl novyi* and *Cl perfringens* are commonly incorporated into multivalent vaccines in Western nations. The diseases resulting from infections with these organisms would exist in Pakistan and hence a similar combination of components could potentially be incorporated into locally produced vaccines.

3]. Enterotoxaemia B & D Vaccine [*Cl. perfringens* types B and D].

This bivalent vaccine would protect against all disease forming complexes produced by this organism, but there is no reliable testing of this product carried out by VRI Lahore beyond a simple MLD (Maximum Lethal Dose) test for toxin. The product requires further extensive evaluation before being released for commercial use.

4]. Anthrax Vaccine [Sterne 34f2 Strain].

This strain is used world wide and is available from Weybridge free of charge. If a [satisfactory] spore count is present, the vaccine used in conjunction with saponin in glycerol will be effective. VRI Tando Jam needs to qualify this vaccine production with VRI Lahore as does Quetta as their formulation seems different from that used in Lahore.

5]. Foot and Mouth Disease Vaccine [3 serotypes] in Alum.

The vaccine produced at VRI Lahore should be discontinued as the technology is very old, the yield very poor and not cost effective. The production is miniscule in terms of total production required, with only 10 litres of each antigen being produced per week using continuously passaged cells [very poor cytology] and virus [continued passage means attenuation and alteration of virus] with poor contamination control: with fungal contamination most likely be a major problem during the wet season. The inactivant, formaldehyde at 0.1%, will kill the virus but is not as good as BEI (Binary ethyleneimine). The inactivation test is not carried out to a standard methodology and no other preservative is added in the whole process meaning that all batches are most likely contaminated with bacteria, and fungi.

Before resuming vaccine production a suitable internal or external consultant review should be conducted while all the recommendations as found in the OIE Monograph for this vaccine should be incorporated into these protocols.

It would be my opinion that if any vaccine should be produced as a national product then this should be the one targeted, with production preferably being instituted as a collaborative venture with a multinational such as Bayer or Merial. I really think the Tando Jam proposal to

produce this vaccine using the methodology in use at VRI Lahore should be reviewed carefully.

Other vaccines are produced by the provincial laboratories but are not relevant to cattle and buffalo so are not part of this review.

**Production Techniques for Bacterial Vaccines [Nos 1, 2 and 3 above].**

**The methods used are those used in Australia in the 1950-60s with the addition of the minimal use of biohazard/laminar flow units at inoculation.**

The laboratories are very basic in design with little regard to incidental contamination either from the environment or from the workers involved in the production process. The laboratories provide little microbiological safety with open access to outside air, circulating fans in laboratories [dust] and movement of people, who have minimal "protective clothing" causing the rapid spread of microorganisms throughout the premises. The workers are not protected from infection appropriately either. Minimal attention appears to be devoted to cleaning, and general house keeping. Validation of processes are yet to be developed, with Lahore VRI the most advanced.

Many issues can be resolved through the instigation of appropriate validation steps throughout the production process to ensure that vaccines are sterile, pure and safe. Other testing could determine stability and efficacy, although these vaccines in their current form most probably perform to their capability given the compromises to accepted practices used during their manufacture. The Blackquarter vaccine seems to be the "best" vaccine manufactured.

The basic concepts of cGMP [code of Good Manufacturing Practice] could certainly be adopted with appropriate training of staff and the provision of adequate facilities which comply with contemporary industry practice.

**New buildings or modifications to old buildings should be designed with modern GMP uppermost in mind so sterile production and processes can be undertaken from the start of production to finished product distribution. Although capital costs will be considerably higher, the following improvements would result in vaccine production under cGMP conditions:**

- **installation of HEPA air conditioning**
- **properly designed laboratories**
- **proper air locks and balancing of air systems**
- **double door autoclaves**
- **sterile clothing**

**If help is wanted in design then advice can be given by the author of this report as to how this can be achieved.**

**1. Seed storage and Seed lotting for use in vaccine Production:**

This area, one of the most important in vaccine production, appears to be under the control of the staff of the VRI's where the audits occurred. However, this was only checked superficially by the author of this report.

## 2. Antigen Production Techniques:

The production of these vaccines at Tando Jam and Lahore Veterinary Research Laboratories [VRI's] is in 10 Litre flasks containing 6 litres of media which universally seemed to be purchased from Oxoid® originating from the United Kingdom.

It was noticed that some of the media used was Brain Heart Infusion Broth, a highly nutritious broth made from neural tissue, albeit originating from countries free from BSE [most probably Australia or New Zealand]. However this type of media is banned from use in vaccine manufacture in Australia, and most probably all other Western Countries as it may present a very remote chance of TSE transmission. If Pakistan has a concern with BSE or other TSE infections then use of this media use should be reviewed.

The seed material was inoculated under a laminar flow/biohazard cabinet and incubated over night, checked for purity and then inoculated into media that had been pre-incubated [for sterility purposes] either in the incubator or on the bench. Although this method is labour intensive using small volumes of media and low volume production, it is nevertheless a successful technique to produce vaccine. It is not large volume manufacturing using fermentor technology, however this technology will produce vaccine suitable for field use. If realistic costing was applied then this would reveal that this is an expensive manufacturing technique.

The inoculated bottles were incubated and then harvested using 0.5% formaldehyde after randomly checking bottles for purity. Random checks mean that some contaminated bottles may pass through the system undetected. The contamination would be as a result of inoculating these bottles under non-sterile air even though bunsen burners and sterile techniques were applied. It was this method that allowed vaccines to be developed in the first place, so no real criticism is implied here.

The inactivated antigens are tested for inactivation and sterility and cell density for formulation. This is satisfactory as the antigen has been inactivated with 0.5% formaldehyde.

## 3. Vaccine Formulation

Alum vaccines are the predominant vaccines produced by the VRI's. Alum is formulated using potash Alum in either distilled [Tando Jam] or reverse osmosis [Lahore] water and adjusting the pH to form the gel which is then autoclaved.

The gel is added to the antigen in the rough proportions of 4 parts antigen to 3 parts gel to produce batches of approximately 400 Litres. No further formaldehyde is added as preservative.

Additional formalin could be added at a rate of 0.2% v/v [800mLs/400Litres] which would act as a preservative, with no adverse safety/efficacy issues. However a much better preservative [Thimerosal] should be added as a final addition to the vaccine prior to filling at the rate of 0.1mg/mL, providing a self-sterilising mechanism which would overcome the non-sterile filling operation and provide an added assurance for sterility even after multi penetrations that will occur in the field.

### 4. Sterilisation of Bottles and Stoppers

The bottles are sanitised after washing with a cresol solution and drained before filling. The stoppers are sanitised with a cresol solution. The bottles can apparently be sterilised by autoclaving without any undue effect, so consideration should be given to sterilising the bottles this way as with the stoppers.

### 5. Filling Rooms:

At both sites the vaccines were filled in rooms that were totally unsuitable for the sterile task undertaken. Apparent problems were:

- Non-sterile dirty rooms
- Free access to all personnel
- Ceiling fans circulating non-sterile air to keep non sterile gowned staff cool under oppressive heat whilst filling the sterile product under open conditions
- Equipment that had been sanitised but not sterilised.

**Protection of the product** should be the first consideration. Under the conditions currently used to package the products there is no way that either mass contamination or incidental contamination can be avoided. Therefore every batch produced would be contaminated either grossly or the odd bottle may be contaminated and would not be detected by random sterility tests on the final product.

**Solution:**

**Step 1**: Assume that the initial formaldehyde added at inactivation of the antigen is absorbed by the antigen and is thus chiefly unavailable to inactivate any further incidental contamination which will occur during the production process.

By simple mathematics, if 0.5% formaldehyde is added at inactivation and then the antigen diluted by the addition of the gel then the final concentration will be approximately 0.28% in a 400Litre batch.

Therefore we should add an extra 0.25% formaldehyde to the batch before filling. This will increase the concentration to a theoretical level of just over 0.5%, but if measured will be in the vicinity of 0.3%. That is we should add 1.0Litre formaldehyde to a 400Litre batch to protect the product more effectively. This will have no effect on safety or efficacy and will help with the sterility status of the product immensely.

**Step 2.** Pakistani laboratories purchase Thimerosal [Thimersal] CAS Number: 54-64-8 and adds this to vaccines at the rate of 0.1mg/mL which would equate to 40grams per 400Litre batch. This product is used by all vaccine manufacturers in the Western World as a bacteriostat for the production of multi-dose products

Thimerosal can be purchased from major Chemical suppliers such as Sigma Aldrich or Merck or other reputable fine chemical suppliers but I would recommend Gihon Laboratories Quimicos SRL in Argentina but serviced by:

Ms. Katja Wilfert
**Cfm Oskar Tropitzsch**
Waldershofer Str. 49-51
D-95615 Marktredwitz
Germany
Telefon: +49-(0)9231-9619-28
Fax: +49-(0)9231-9619-60
Email: katja.wilfert@cfmot.de
Homepage: http://www.cfmot.de

Quote:
5 kg Thimerosal USP/BP/EP
Price: 800 USD/kg (without any commission, without any licenses, legalization and special documents)
CIP air Lahore or Karachi
Delivery time: to be agreed
Packing: 1 UN box with 5 x 1 kg tins
Prepayment Origin: Argentina

If each Laboratory purchased a kilogram of Thimerosal then effectively this would assure sterility both from the manufacturing process but also from repeated penetrations into the bottle to withdraw vaccine for inoculation. This one kilogram would be able to be used for a total of 6500 litres of vaccine at a cost of approximately USD$0.12 [12 cents per litre or 3.5 cents per bottle or 0.06 cents per dose]. This small cost can ensure sterility and prevent potential problems.

**In summary the industry should protect the products by the addition of an extra litre of formaldehyde per 400Litre batch and add Thimerosal at the rate of 0.1mg/mL to ensure sterility of the product.**

**Expiry:**

All products which leave the provincial laboratories carry a 6 months expiry date. This is probably a good practice as the “cold chain” in Pakistan is poor. However it is strongly recommended that stability testing is carried out on both HS and Blackquarter vaccines with product held at 2-8$^0$C and also under conditions applicable in the field at 40$^0$C for times zero, 3 months, 9 months and 12 months. Sufficient vaccine should also be retained to extend this time further if shown to be successful. This will then give a better indication of the durability of the product.

**Anthrax Vaccine:**

I did not review this in production but rather reviewed the production technique presented by a production scientist at Tando Jam. As stated before this vaccine is fairly straight forward and provided the seed was pure and the spore count was correct then there would not be a problem. However with the current production techniques *Bacillus sp* contamination would be a problem and be difficult to distinguish from the *B.anthracis* antigen. Again this vaccine can be problematic especially in yields and needs to be carefully controlled.

**Code of Good Manufacturing Practice as adopted by Pakistan Government Vaccine Facilities.**

1. Documentation is not developed as it should be. Full standard Operating Procedures for Purchasing, Production, Quality Control and dispatch of goods should be prepared by the appropriate staff and be retained as the approved documentation. If these procedures are not recorded then they will not be adhered to.

2. Validation of the equipment has not been carried out. Examples of equipment/procedures requiring validation include autoclaves and sterilisation protocols, volumetric measurements for fill volumes, balances, laminar flows and related equipment.

3. Training of senior staff in modern vaccine technology would be highly recommended as there is little knowledge of the latest techniques in sterile vaccine production in high volumes.

4. Training of senior staff in the needs of a modern factory and for suitable engineering staff and preventative maintenance is mandatory.

5. Training of staff in GMP especially in cleaning techniques, sterile techniques, sterile clothing and operating in a sterile manner.

END

# Appendix

## Vaccine Seminar Review

## Wednesday, April 30, 2008

Prepared by David McGill

## List of Participants

| # | Name | Designation |
|---|---|---|
| 1 | Prof. Dr Shahana U. Kazmi | Member for Animal Sciences PARC |
| 2 | Dr R. H. Usmani | Animal Husbandry Commissioner, MINFAL |
| 3 | Ghulam Rasool Dutani | Deputy Director General, Health |
| 4 | Dr Jamil A. Shaikh | Project Director, Vaccine Production Unit Tando Jam |
| 5 | Dr Syed Imam Shah | Senior Scientific Officer, VQC, National Veterinary Laboratories Islamabad |
| 6 | Zafar Jamil Gill | Director General Research, LDDDept. |
| 7 | Khalid Naeem | Chief Scientific Officer, Animal Health, NARC |
| 8 | M. Qasim Khan | Principle Scientific Officer (PSO), Animal Helth, NARC |
| 9 | Prof. Dr. Iftikhar Hussan | Dept of Microbiology, UAF |
| 10 | Dr Nasir Hussan Shah | Director, VRI, Peshawar |
| 11 | Dr Khurshid Ahmad | Principle Scientific Officer, National Veterinary Laboratories Islamabad |
| 12 | Shafqat Fatima Rehman | Sindh Poultry Vaccine Centre, Karachi |
| 13 | Dr M Iqbal | Director, VRI, Lahore |
| 14 | Dr Shakeel Babar | Director, Centre for Advanced Studies in Vaccinology & Biotech, Quetta |
| 15 | Dr Sulman Hameed | Assistant Animal Husbandry Commissioner, MINFAL |
| 16 | Dr Mudasser Habib | Nuclear Institute for Agriculture and Biology, Faisalabad |
| 17 | Mrs Anwar Begum | Director NCLB, Ministry of Health |
| 18 | Dr Shaheena Hafez | Principle Scientific Officer, Animal Sciences, PARC |
| 19 | Richard Bevan | Vaccine Consultant, Australia |
| 20 | David McGill | Project Manager, ASLP Dairy Project |
| 21 | Dr Muhammad Afzal | CEO, LDDB |
| 22 | Shoaib Ahmad Siddique | Secretary Livestock Sindh |
| 23 | Prof. Dr Kushi Muhammad | Director, Diagnostic Laboratories, UVAS Lahore |
| 24 | Azra Barias | Deputy Director, Fisheries, PARC |
| 25 | Dr Ikram Ullah Majeed | Deputy Director, Animal Health PARC |
| 26 | Rab Nawaz Khan | Deputy Director (Animal Production), PARC |
| 27 | Dr Aama Bin Zaluez | Senior Scientific Officer, Animal Sciences, NARC |

### Overview of Seminar

The aim of the seminar was to get all of the major personnel related to animal vaccines, both in policy and technical applications, together to talk about the issues of vaccine production and distribution within Pakistan. It was also a chance for Richard Bevan (vaccine production consultant for the ASLP Dairy Project) to share his experiences and recommendations from his trip to Pakistan and review of some vaccine production units. The seminar was organised by PARC with the help of LDDB and the ASLP dairy project team.

The seminar was started by introductions of the participants, the basic issues of veterinary vaccines in Pakistan, the ASLP Project and Richard Bevan's trip. These introductions were carried out by Dr Shahana Kazmi, Dr Tusneem (Chairman PARC) and Dr Muhammad Afzal.

The major producers and researchers of vaccines in Pakistan each gave a presentation on their institutes and some of the problems/successes that they have had. The presentations were by:

- Dr Iqbal – VRI Lahore, Punjab
- Dr Nasir – VRI Peshawar, NWFP
- Dr Habib – NIAB Faisalabad, Punjab
- Dr Shakeel – VRI/research Quetta, Balochistan
- Dr Shafqat – Poultry Vaccine Centre, Karachi, Sindh
- Dr Jameel – VRI Tando Jam, Sindh
- Dr Kushi – UVAS Lahore, Punjab

Some of the good points brought up from these presentations were that they could see the need for a central facility for producing vaccines and that there is also a need to develop standard operating procedures to be used across the country.

Richard was the last speaker of the day. He gave a 45 minute presentation covering all the major problems and recommendations from what he discovered in his 10 day visit to Pakistan. During his trip to Pakistan he reviewed the laboratories and work at:

- VRI Tando Jam
- Poultry Vaccine Centre Karachi
- VRI Lahore (and FMD section)
- Diagnostic Laboratories, UVAS Lahore
- National Veterinary Laboratories, Islamabad

Some of the main points that Richard covered in his presentation were:

- Vaccine Production Units are not research facilities, they are factories.
- There is a need for engineers/maintenance personnel at vaccine units to keep all equipment in safe and working order.
- If a procedure is not recorded it may as well have not been done. Records are required for quality assurance, precision and safety.
- Production of FMD is important and expensive. It should be carried out at one site in Pakistan and distributed to the other provinces of the country.
- The techniques used currently at the vaccine production units are old, but still effective. However, there are numerous steps that can be taken to decrease contamination.

## Discussion

After Richard's presentation there was a general discussion with the entire group. The aim of this discussion was to answer any questions from the participants and to come up with some possible areas where policy personnel or the technical personnel could focus their attention to make some improvements. Some if these points are listed below:

- The Secretary of Livestock, Sindh started discussions about linkages and collaborations between vaccine units to learn from each other and refine skills and techniques.

- There is a need of a consortium of leaders to check each of the vaccine units. This is because there are tests and GMP available in Pakistan, but they are seldom adhered to. The Animal Husbandry Commissioner (Dr Usmani) and the Ministry of Health (who are required to police the production of veterinary vaccines) came to an in principle agreement to follow up on the policy issue relating to this.
- Vaccine units and technical personnel will work on the areas of:
  - Staff training
  - Cleanliness
  - Sterilisation of product
- Vaccine units now have a copy of GMP from Richard Bevan which will be utilised in any future facility developments.

**Photo: Participants of the ASLP Dairy Project Vaccine Production Seminar in front of the PARC building Islamabad on the 30th of April 2008**