Queensland Parliament Hendra Vaccine Inquiry: EquiVac® HeV vaccine and its use by veterinary surgeons in Queensland

1. **Vaccine development**

Background

Hendra virus (HeV) is a highly pathogenic paramyxovirus and is classified as a zoonotic Biosafety Level 4 (BSL4) virus. As result, infectious virus can only be studied in those few laboratories that have the capabilities, expertise and infrastructure to do so. HeV is also among the various pathogenic agents of biodefense concern and is classified as a priority pathogen in category C by the US Centers for Disease Control and Prevention (CDC) and the National Institute of Allergy and Infectious Diseases (NIAID). Category C agents include emerging pathogens that could be engineered for mass dissemination on account of their potential for high morbidity and mortality rates and major health impact.

In Australia, five HeV outbreaks in horses have been associated with transmission of infection to people, and there is a strong epidemiological connection between infection of people and direct contact with sick horses. Six of the seven affected humans have been exposed to the blood or secretions of terminally ill horses or have been contaminated with equine body fluids during post mortem examination of affected animals; three of those people have been veterinarians. In the seventh patient, a veterinary nurse, the risk exposure was assessed to have occurred while performing nasal cavity lavage (for management of another condition) on a horse, which was not known to be infected, during the last three days of its HeV incubation period prior to disease onset[1]. The HeV attack rate for people exposed to potentially infective equine body fluids has been estimated at 10%[1]. HeV infection in people has an incubation period of 9–16 days and causes an influenza-like-illness (ILI) that can progress to encephalitis, which may be fatal. The current human case fatality rate is 57%, with death of one patient attributed to multi-organ failure (with interstitial pneumonia) and the remainder to encephalitis. In one of these patients, the episode of encephalitis that proved fatal had been preceded 13 months earlier by an ILI with meningitis from which he appeared to have made a full recovery[2]. Relapsing encephalitis is also described in people infected with the closely related Nipah (NiV) virus[3], the second of only three viruses known in the genus Henipavirus within the family Paramyxoviridae.

Risk management of HeV infection in people: the rationale for equine vaccination

It is believed that most affected horses acquire HeV infection following direct exposure to infective flying-fox secretions, however the precise way in which this occurs is not known[4,5]. Equine infection

is sporadic and commonly involves only a single horse within a group. However, occasional multi-horse outbreaks have been recorded where there is also evidence of horse-to-horse transmission – most likely via contamination of surfaces or equipment by infectious fluids[6,7,8]. Many of the clinical signs of HeV infection of horses are not specific for that disease and differential diagnosis – especially from more common disorders such as pneumonia, pleuropneumonia, and colic – remains challenging and complex. Although the likelihood of equine and human infection is low, the impacts of infection are potentially catastrophic: the inherent risk of HeV infection is thus significant in both species.

In reviewing the hierarchy of effectiveness of risk controls[9], there is clearly no straightforward means of either eliminating the virus hazard or preventing exposure of horses to HeV shed by flying foxes. Eradication of flying foxes would pose extraordinary operational challenges, notwithstanding their crucial environmental roles in pollination and the attendant ethical issues. Factors influencing interspecies transmission of the virus are also poorly understood, likely to be complex and dependent upon socioeconomic, environmental and ecologic factors[10], and the interface between bats and horses cannot be eliminated within peri-urban and rural communities.

The use of personal protective equipment (PPE) as a risk control is recognised to be one of the least effective in controlling risks to workplace health and safety, and should only be used to supplement higher level control measures or when there are no other practical measures available[11]. In addition to the known limitations of PPE, there are additional challenges to its successful use in the context of HeV. In particular, HeV outbreaks occur in temperate to tropical climates where continual compliance with certain items of PPE will be difficult to achieve; the restrictions it imposes on the wearer add risk in the context of equine veterinary practice, and the goals of protection – especially respiratory protection – are undefined because the occupational exposure limits for the virus by contact and/or inhalation are, and will remain, unknown. It is also important to be mindful that horses may be infective in late in the incubation period and while they still appear to be healthy[1].The emotional attachment of humans to their horses leads to regular close contact between horse and owner; the routine daily wearing of PPE is clearly impractical under such circumstances.

A more reliable control for managing the risk of transmission of HeV to people is equine vaccination in order to reduce or eliminate viral shedding by horses, thus providing a higher level of health and safety protection[12,13]. An added benefit is that this will not only reduce the risk of human infection by HeV, but also protect the health of horses. In summary, the overall aim of equine vaccination is to suppress virus replication in horses in the event that they are exposed to HeV in the field.

Suppression of virus replication will prevent the development of the acutely ill horse, which is the animal recognised to be responsible for the chain of onward transmission of infection to people.

The science behind EquiVac® HeV

For paramyxoviruses like HeV, it is the envelope glycoproteins which elicit the majority of virus neutralizing antibody in an infected host[14] that is responsible for virus clearance from the body. It is also known that neutralizing antibodies are the key vaccine-induced protective mechanisms in the case of well-known paramyxovirus diseases of humans (mumps and measles viruses) [15, 16]. HeV encodes two envelope glycoproteins: an attachment glycoprotein (G) and a fusion glycoprotein. The G glycoprotein is a type II transmembrane protein and it plays a critical role in initiating infection by binding to the receptor molecule ephrin B2[17], which is expressed on neurons, smooth muscle cells, and endothelial cells lining small arteries in mammalian hosts.

It has been shown by passive immunotherapy that antibody raised against either the G or F glycoprotein of HeV or Nipah virus alone can prevent the development of fulminating infection[18]: G glycoprotein–specific human monoclonal antibody prevented Nipah virus disease in ferrets[19] and HeV infection in African green monkeys[20], and F or G glycoprotein–specific monoclonal or polyclonal antibodies prevented HeV and Nipah virus disease in hamsters[21-23].

In deciding upon a platform technology for the HeV vaccine, it was recognised that live-attenuated vaccines - as commonly employed against important human viral pathogens - would not be likely to be approved for any BSL4 virus. In view of the key role in HeV protection played by antibody to envelope glycoprotein, a subunit vaccine incorporating a recombinant immunogen represented the most viable alternative. Subunit vaccines involve traditional and comparatively straightforward methods of vaccine production. Moreover, as they do not involve live virus in their manufacture, they can be administered without risk of infection.

Using data generated by Yu *et al* (1998)[24] and Wang *et al* (2000)[25] on the HeV and glycoprotein G open reading frames, Bossart and co-authors (2005)[26] reported the development of a recombinant soluble HeV G (sG) that elicited potent virus neutralizing antibodies in rabbits. These findings indicated that the recombinant sG preserved key functional and antigen characteristics including the ability to bind virus receptor, block virus infection, and elicit robust polyclonal neutralising antibody responses. Accordingly, sG was identified as a potentially useful component for vaccine development.

2. Vaccine trials

Proof-of-concept vaccine efficacy studies in non-target laboratory mammals

All animal studies were endorsed by the CSIRO Australian Animal Health Laboratory Animal Ethics Committee.

The proof-of-concept efficacy studies for a subunit HeV vaccine were initiated in the context of research into a human vaccine against the closely related Nipah virus. As there is powerful cross-neutralisation of Nipah virus by antibodies to HeV, a subunit HeV vaccine is applicable to the prevention of infection with either virus. By its very nature, vaccine development for BSL 4 agents typically has humans as the ultimate target and so conventional clinical efficacy trials are not possible. Accordingly, FDA implemented an Animal Efficacy Rule in 2002 that permitted the evaluation of vaccine efficacy in humans by using data generated from animal infection models that faithfully recapitulate the features of the human disease: the relevance and application of data from non-target animal studies is an accepted principle in the evaluation of potential countermeasures to BSL 4 pathogens. The approach has also been endorsed by Queensland Health in administration of the monoclonal antibody m102.4 to people as a post-exposure therapeutic for HeV under a compassionate-use protocol, using efficacy data supplied from ferret studies with Nipah virus[19] and non-human primate studies with HeV[20].

The first vaccine efficacy study where HeV sG was incorporated as the vaccine antigen was conducted in cats[27], a species which is highly susceptible to both Nipah and HeV infection and develops similar clinical and pathological features to the human and equine diseases. The subunit vaccine was formulated using 100ug of recombinant sG with CSIRO triple adjuvant, and Nipah virus was used as the challenge virus. Vaccinated animals showed no signs of disease following exposure to what would otherwise have been expected to be a lethal dose of virus; there was no evidence of virus replication or shedding at any time; and no features of infection were detected at post mortem examination of animals which had been vaccinated.

In a second vaccine study[28], also carried out in cats, the HeV sG subunit vaccine was formulated using 5ug to 50ug of recombinant sG with an adjuvant suitable for human use; Nipah virus was used as the challenge virus. Vaccination prevented the development of clinical disease, no virus was detected in blood and there was no evidence of systemic spread of infection at post mortem examination of vaccinated animals. A rise in antibody level was recorded in two clinically healthy animals after virus challenge, consistent with low level and self-limiting virus replication: virus was not re-isolated from any secretions from these animals.

A third vaccine study[29] was conducted in ferrets, another laboratory animal species which recapitulates the salient clinical and pathological features of both human and equine henipavirus infection. The animals were vaccinated with a HeV sG subunit vaccine formulated with an adjuvant suitable for human use. Various doses of HeV sG ranging from 4ug to 100ug were incorporated into the vaccine formulations and HeV was employed as the challenge virus. All vaccinated ferrets remained free of clinical signs of HeV infection following exposure to live HeV. In addition, there was no evidence of virus or viral genome in any tissues or body fluids of animals vaccinated with 100 and 20 µg doses of sG antigen. Genome – but not virus - was detected in the nasal washes of one animal only in the group receiving the lowest dose of antigen (4ug). Considered together, these findings indicate that 100 µg or 20 µg doses of HeV sG vaccine can completely prevent a productive HeV infection in the ferret, suggesting that the goal of vaccination of horses to prevent infection or to reduce shedding of HeV was feasible.

The outcomes of the proof-of-concept vaccine studies in non-target laboratory animal species encouraged and justified translation of vaccine efficacy studies into the horse. The findings from a more recent study using HeV sG subunit vaccination of non-human primates later exposed to HeV[30], wherein vaccinated animals showed no signs of illness and no viral genome was recovered from any sample including blood, swabs, or post mortem tissues, further supported this decision.

Vaccine efficacy studies in the target species (horse)

Vaccine efficacy studies in the horse were conducted in collaboration with *Pfizer Animal Health*, now *Zoetis.* CSIRO provided the capability and know-how for the BSL4 animal studies and *Pfizer Animal Health* contributed the vaccine formulations and guidelines for the vaccination regimes. All animal studies were endorsed by the CSIRO Australian Animal Health Laboratory Animal Ethics Committee.

The HeV equine infection model: Experimental exposure of four unvaccinated horses to a HeV isolate (Hendra virus/Australia/Horse/2008/ Redlands) from a field disease outbreak was carried out under BSL4 conditions at the CSIRO Australian Animal Health Laboratory, Geelong, Victoria, Australia[31,32]. Virus was administered oronasally at a dose of 2×10^6 50% tissue culture infectious doses. This route of exposure was selected as it reflected a plausible natural route of field infection; the challenge dose was chosen as it was known to induce fatal disease in experimental horses with the Hendra virus/Australia/ Horse/1994/Hendra isolate. Clinical signs and pathological findings which developed in each horse recapitulated those recorded for field spill-over events, as well as the observations made during earlier experimental studies conducted using the first HeV isolate (Hendra virus/Australia/ Horse/1994/Hendra).

In naïve horses exposed to HeV, viral genetic material was recovered during the preclinical stage of infection on nasal swabs as soon as two days post-exposure to virus[31,32]. HeV gene copy numbers in nasal secretions steadily increased through incubation period and into the clinical phase of infection, consistent with local replication in the upper respiratory tract or nasopharynx. Viral genome was recovered from at least one and typically more of the clinical samples from each horse on or before day five after exposure to HeV. Viremia then ensued, followed rapidly by the onset of fever; soon afterwards viral genome was also recovered from oral secretions and urine. Signs of systemic illness developed shortly thereafter as HeV replication became more widely established in tissues and organs. Once disease was apparent, all clinical samples were positive for HeV genome. The clinical signs of disease progressed over 24 to 48 hrs, with animals reaching their predetermined humane endpoints for euthanasia between days 6 and 9 post-exposure to HeV. At post mortem examination, all tissues from infected horses were positive for HeV genome, characteristic histopathological lesions were present, and virus was reisolated from target tissues.

The dynamics of HeV infection were highly consistent between individual control horses, and the equine infection model was also shown to be stable over time as evidenced by the naive cohort data having been generated from two separate studies two years apart. As a result, the data met the requirements of the Australian Pesticides and Veterinary Medicines Authority (APVMA) for defining the horse HeV infection model at BSL4, and allowed the responses of vaccine cohorts to be evaluated by comparison with findings in naïve horses under the equivalent conditions of HeV exposure.

The most informative test for detection of HeV replication in vaccinated horses is post mortem examination during the time of putative active virus replication, namely between day seven to nine post-exposure to HeV. Post mortem examination enables an extensive and detailed search for evidence of virus genetic material, whole virus particles, and viral proteins; a wide range of tissues may be sampled and examined by PCR, histology and immunohistology to determine the presence and extent of any infection. In the event that vaccination does not induce sterilising immunity, we also know that the dynamics of any low level infection is the same as is found in unvaccinated animals[28, 29, 33] and in animals with passive immune protection[19].

The sampling strategy developed for vaccine cohorts also accommodated the practical limitations of being able to house only three horses at a time in the BSL4 animal facility, the period of time for which animals can be safely handled under these containment conditions, and being able to test only one horse per day at post mortem for operational reasons.

The principles above formed the basis of the experimental readouts used for assessment of the responses of vaccinated horses to exposure to HeV.

Vaccine formulation: For use in the horse, a subunit vaccine containing HeV sG recombinant protein as the antigen was specifically formulated in a proprietary adjuvant (*Zoetis*) already approved for use in that species. The HeV sG glycoprotein was produced by using a Chinese hamster ovary (CHO) or a 293F human embryonic kidney cell expression system[29] with one of two different HeVsG glycoprotein preparations: 1) affinity-purified sG glycoprotein (293F cells) or 2) clarified sG containing cell culture supernatant (CHO cells). Vaccines for initial efficacy studies in horses were formulated with 50 µg or 100 µg of affinity-purified sG glycoprotein. All subsequent vaccines were formulated with clarified CHO cell culture supernatant that was then gamma irradiated. The change of the expression system from 293F cells to CHO cells was driven by the need for higher antigen yields, and bioequivalence was supported by laboratory analysis of the expressed antigens from the two systems and a comparison immunogenicity study in ferrets.

All immunizations comprised two 1-mL doses administered intramuscularly three weeks apart. Seven horses received vaccine containing 100 µg of HeV sG glycoprotein/dose and three horses received 50 µg of HeVsG glycoprotein/dose[32]. Seven horses were exposed to HeV 28 days after the second vaccination, and the remaining three horses (each vaccinated with 100ug HeV sG) were challenged with virus 194 days after the second vaccination. Each BSL4 vaccine efficacy study in horses included a mammalian pathogenicity control for the virus inoculum, provided variously by horse, ferret or guinea pig species; a principle which has been validated within the peer-reviewed scientific literature[34].

Outcomes of exposure of vaccinated horses to HeV: In contrast to unvaccinated control horses, all vaccinated horses remained clinically healthy during the observation period after exposure to an otherwise lethal dose of HeV. Following elective euthanasia of vaccinated horses at the time of predicted peak viral replication (should it have occurred), there was no gross or histologic evidence of HeV infection in vaccinated horses; all tissues examined were negative for viral antigen by immunohistochemistry; and viral genome (HeV N gene) was not recovered from any tissue, including nasal turbinates, pharynx, and guttural pouch, as well as adrenal gland, bladder, brain (including olfactory pole), cerebrospinal fluid, heart, kidney, large intestine, liver, lung, lymph nodes (bronchial, inguinal, intermandibular, mandibular, renal), meninges, ovaries and uterus (where present), small intestine, spinal cord, spleen, sympathetic nerve, and trigeminal ganglion. For nine of 10 vaccinated horses, viral RNA was not detected in any of the daily nasal, oral, or rectal swab specimens or from blood, urine, or feces samples collected before euthanasia, and virus was not reisolated from any of

these clinical samples. For one of three horses exposed to HeV six months after completing the prime-boost vaccination course, low viral gene copy numbers (compared to control horses) were detected in nasal swab samples collected on post-exposure days two, three, four and seven. Virus was not reisolated from these samples, and the horse was negative for viral genome at post mortem on day eight after exposure to virus. Overall, the findings in this animal are consistent with transient, low level, local viral replication, and the horse did not meet the epidemiological criteria associated with transmission of infection to people.

Virus neutralization antibody titres in vaccinated horses prior to HeV exposure ranged from 128/256 (test replicate) to >4,096 for those challenged 28 days after the second vaccination and from 16 to 32 for horses challenged 194 days after the second vaccination. At the time of euthanasia following exposure to HeV, no rise in antibody titre was detected in any vaccinated horse. Failure to observe a rise in neutralising antibody titre in vaccinated animals after exposure to either Nipah virus or HeV is regularly described in non-human primates[30], ferrets[29,35] and cats[28] and is more commonly recorded than a rise in antibody titre. The most plausible explanation for these observations is that, even in horses with the lowest level of neutralising antibody (1:16) at the time of virus exposure, there was sufficient protection against infection of host cells to prevent initiation of a detectable anamnestic immune response.

Summary: Vaccination completely prevented HeV infection of nine out of 10 horses when they were exposed to a dose of virus that would otherwise have been expected to be lethal. The last horse also remained clinically well, did not develop systemic infection, and showed evidence only of self-limiting virus replication in the nasal cavity which was at a low level compared to unvaccinated horses. It is worth bearing in mind that all horses had been exposed to an amount of virus which, based on shedding rates from the reservoir host, we estimate is about a million-fold higher than they would receive in the field. As a result, experimental efficacy tests are likely to under, rather than over, estimate the benefit of vaccination in suppressing virus replication.

There are substantial limitations posed by BSL4 conditions on the acquisition of experimental data from animal species of high individual value[30, 36], such as the horse. In spite of this, we successfully generated internally consistent data sets from naïve horses, vaccinated horses, and virus-controls (variably horse, ferret and guinea pig), using the same HeV isolate with the same passage history, at constant species-specific doses, administered on each occasion by the same operator.

The launch of the HeV vaccine for horses was heralded internationally as an outstanding example of the “One Health” approach for control of a major public health threat. In recognition of the research

that underpinned this achievement, The Hendra Virus Research Team (Australian Animal Health Laboratory) was awarded the 2013 CSIRO Chairman's Medal, honouring the very best in CSIRO research which is of national or international importance. In the following year, the CSIRO Team was also awarded the 2014 Australian Infectious Diseases Research Centre Eureka Prize for Infectious Diseases Research - one of the country's most comprehensive national science awards. This award also recognised the achievement of the provision, through the vaccine, to Australia and the world of a targeted tool to protect people and animals against this deadly virus.

There is unarguable evidence in both target (horse) and non-target species that vaccination using sG HeV antigen reliably induces virus neutralising antibody. It is a biological inevitability that virus replication will be less efficient in any animal which has either circulating neutralising antibody to the homologous virus, or possesses immunological memory generated through prior vaccination, compared to the same individual exposed to virus but without these defences. Immunisation of horses with vaccine formulations that deliver such outcomes clearly contributes to reducing their likelihood of developing HeV infection with overt clinical disease, and thus potentially reduces the risk of onward transmission of infection to people.

The uniformly favourable clinical outcomes of the equine HeV vaccine studies are not unexpected: we know from other work carried out over the years within CSIRO and also from reference to the literature that surface glycoprotein vaccination which induces detectable neutralising antibody against henipaviruses is repeatedly, consistently and reliably protective against clinical disease in hamsters, cats, ferrets, and non-human primates. Following natural infection, the development of neutralising antibody is temporally and causally associated with virus clearance from the infected animal, and it is also well known that most neutralising antibodies to enveloped viruses are directed against the surface glycoproteins (G protein in the case of HeV). As also observed in the equine studies, neutralising antibody titres that have afforded clinical protection against experimental henipavirus challenge in non-target animals (ferrets) have been as low as 1:16 [35].

In addition to providing protection from disease for horses, immunising horses against HeV using vaccine formulated with sG antigen meets the requirements of a higher order control for reducing the risk of transmission of HeV. By eliminating the potential for shedding of HeV by horses after their exposure to field virus, or by reducing virus replication in them to the point where clinical illness does not occur, the proximal cause of human infection is removed from the landscape.

3. Approvals process

The data generated from the vaccine efficacy studies described above were provided to *Pfizer Animal Health* (now *Zoetis*). They contributed to the portfolio submitted by *Zoetis* to APVMA in support of release of Equivac® HeV to the horse-owning community under Minor Use Permit in 2012 and full registration of the product in 2015.

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