Queensland Parliamentary Inquiry: Hendra virus (HeV) EquiVac® vaccine and its use by veterinary surgeons in QLD

Submission by:

Dr Margaret Williams

Summary of Submission - QLD Parliamentary Inquiry: Hendra (HeV) vaccine

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The approval for the first permit relied on results from 3 trials in HeVsG vaccinated horses challenged with Hendra virus at 28 days after booster vaccination.

Trial 1: Used 2 test (vaccinated) horses and 1 control (unvaccinated) horse (this is the only trial that has used a horse as the control)

Trial 2: Used 3 test horses with 4 guinea pigs as controls, trialled half the vaccine dose of study 1 but went back to 100mcg dose again for study 3; only 1 of 4 guinea pig controls worked, so the validity of this study may be in question

Trial 3: Used 2 test horses and 2 ferrets for controls. Note that ferrets and guinea pigs have different susceptibility characteristics to horses when exposed to Hendra virus. The control ferrets in this test were given a much higher dose of virus (50,000 TCID 50) than was given to vaccinated ferrets in their HeV challenge study (5000 TCID 50). Compared to the test horses in this trial 3, the ferret controls may have been given more than an equivalent dose of virus per kg based on average weight of horses and ferrets. This 3rd trial of 2 horses is the only horse trial that used the changed antigen cell line production system adopted for manufacturing Equivac HeV.

The Ferret HeV trial: 6 ferrets were divided into 3 pairs and given doses of 4, 20 and 100 mcg of HeVsG vaccine respectively, then compared to 2 ferret controls. These doses represent a much higher dose per kg of body mass than what is used to vaccinate a horse (each Equivac HeV dose = 100 mcg of HeVsG, avg wgt of a horse ~500 kg, ferret ~1 kg). Protective antibody levels were generally very high at 21 days post booster challenge in the ferrets (≥ 8192). Significantly, one vaccinated ferret demonstrated evidence of HeV infection with likely replicating virus in nasal swabs, but these levels later settled, probably aided by the very high level of protective HeV antibodies in that ferret.

These appear to be the only HeV challenge trials in HeVsG subunit vaccinated animals, conducted prior to the first permit in late 2012, which was issued before doing the 6 month trials.

The 6 month HeV vaccine trial in horses: 3 test horses and 2 unvaccinated ferrets were used. One of the vaccinated horses was found to have viral genome, indicating replicating virus in its nasal swabs on 4 days. Part of the reason given to encourage Equivac HeV immunizations was due to a potential concern about possible viral shedding in asymptomatic horses. This was based on the finding that viral nasal replication often preceded systemic infection and illness in experimentally infected horses. It has been maintained however that these levels are comparatively low and present a much lower risk for transmission than a very sick or dead horse. The significance of this very same finding of the viral genome in nasal swabs now in a vaccinated horse was minimized. It was said to be transient, at low level, virus was not isolated in the samples and HeV disease or viral genome was not found on post mortem analyses. It was always going to be transient though, as this horse was euthanized the day after a rise in viral genome, even a day before another vaccinated horse that did not have viral material in its nasal swabs. It was said to be low level, but the viral genome levels in this vaccinated horse were higher on some days than in some unvaccinated horses, especially prior to the onset of clinical disease. (The Hendra viral genome levels in nasal swabs of this vaccinated horse were compared with levels in the control horse, from the earlier trial, and to 3 other unvaccinated horses in an earlier study that was done to demonstrate experimental Hendra viral infection in a horse.) One unvaccinated horse didn't have any viral genome in its nasals swabs for several days, and then the level went from zero to rising quickly over the 2 days with onset of fever, illness and systemic spread, prior to euthanasia. This vaccinated horse had

positive viral genome on 3 days that settled to undetectable for 2 days, then rose again, indicating viral replication. Unfortunately the horse was euthanized the next day.

It did not have evidence of Hendra virus disease in its organs after euthanasia, but that would not have been expected before onset of clinical illness and fever, indicating systemic spread and dissemination of the virus. It was destroyed on day 8. The incubation period for Hendra is said to be up to 16 days and there have been reports of longer periods. This horse's protective antibody titres were in the low range at the time of challenge, (HeV antibody titre 16 to 32). In ferret studies, local replication has occurred in vaccinated animals that settled, but these animals had much higher levels of protective antibodies. It would have been good to see what may have happened had the horse lived longer.

It is also maintained that no virus was found in this positive HeV genome vaccinated horse's samples. This result is unremarkable and the comment possibly misleading, as virus was not found on viral isolation in *any* of the samples of the 4 experimentally Hendra virus infected unvaccinated horses prior to euthanasia, not even in very sick horses with high levels of viral genome on PCR. These findings were reported in the results section of the scientific manuscripts for these studies. The technique of virus isolation is just not as sensitive as viral PCR to detect presence of virus. A negative result on viral isolation does not necessarily mean that the virus is not there.

It would be conjecture to say that the viral replication in this vaccinated horse would not have increased or progressed. The infection may well have settled, but if it had have gone on to fulminant disease, this would have had serious implications for the future marketability of the vaccine.

The 12 month trial in horses: This was invalid as the unvaccinated ferrets and vaccinated horses all remained healthy, in other words, the virus inoculum didn't work.

Note that in trials of the closely related Nipah virus (NiV), there was also evidence of viral infection in vaccinated animals; i.e. indications of viral shedding in HeVsG vaccinated cats, and levels of viral genome in the brains of 4 vaccinated cats higher than in the controls. Likewise, in a 12 month NiV ferret study, there was evidence of nasal shedding that settled in one vaccinated ferret and NiV genome was found in a bronchial lymph node of another (vaccinated) ferret.

On the plus side, in reviewing the vaccine trials, HeVsG vaccination appeared to help prevent clinical disease in the time frames of the trials, and in many cases HeV infection. Almost all of the trials, as well as using other species, have other parameters different, such as the vaccine doses used, making absolute comparisons difficult. In many of the trials, challenge is performed at 21-28 days after a booster, when protective antibody levels should be very high which would tend to optimize the outcome. It remains to be seen what the results would be longer term and points out the need for studies of more realistic duration. As the Hendra viral infection is rare and has large annual variability historically, conclusions from field assessment of efficacy would be difficult to make which underlies the importance of viral challenge efficacy studies.

No further vaccine viral challenge efficacy testing in horses has been published, although Zoetis reported intention to do 12 month duration of immunity testing and further viral challenge vaccine trials. They presented tables of serology levels in the press release discussed in this submission and although the sample size was small, over half the horses, before their 6 month booster, had just detectable antibody levels of 8. A second group of horses that were given prime boost then 12 month regimen also resulted in over half of the horses having not detectable or just detectable antibody levels of ≤ 8. Zoetis maintained in that press release "*It is unknown whether horses with undetectable titres can withstand challenge with live Hendra virus".* If it may be that common to have very low HeV antibody levels, shouldn't that be tested to find out? In real world conditions, not all horses will have robust protective antibody levels and there has been no published data on Hendra viral challenge with low or non-detectable antibody level horses. There has been nothing published about these proposed trials that were to be conducted in 2014. APVMA in 2015 has advised "*The approved*

instructions of the product indicate that a booster dose is required every 6 months. This is because a duration of immunity has not been demonstrated for more than 6 months." and *"The potential for a vaccinated horse to pass on the Hendra virus cannot be ruled out. As a precaution, it is recommended people take the same steps to protect vaccinated horses from exposure to infection—and to prevent humans being infected by horses—as are recommended for unvaccinated horses. Personal protective equipment should be worn whenever infection is suspected even in vaccinated horses."*

There have been sweeping generalizations of virtually infallible vaccine efficacy from fairly minimal data with potential red flags in trials that were minimized and to my knowledge not repeated. The practice of applying two different approaches, for instance as regards PPE and the level of care in treating vaccinated and unvaccinated horses, does not reflect the precautionary principle and APVMA advice.

Every medicine or vaccine has the risk of adverse reactions. There have been undeniable and documented serious adverse reactions to the Hendra virus vaccination, including possible death. Serious reactions appear to be uncommon, but then the disease is also rare. It is also likely that adverse reactions have been under reported. Some horses with subsequent vaccinations become hypersensitized and have more severe adverse reactions. This has been acknowledged on the APVMA site. The problem is that the duration of immunity has not been demonstrated to last more than 6 months and this 6 month regimen is causing more reactions in an increasing number of horses.

There have been 3.5 confirmed cases per year of Hendra virus infection in horses in Australia, since the Hendra virus was first described (73 confirmed cases in 21 years). As the disease is relatively rare, with very few cases and none in some years, the risk of serious adverse events from vaccination may weigh against the vaccination having a benefit for the horse in those years. Were Hendra virus disease to be more common, the balance could sway the other way.

There have been 7 human infections with 4 deaths and the vaccine is promoted as reducing the risk of transmission from horse to human. To date, those infections have only occurred in people doing high risk procedures without PPE on an infected HeV horse, or having close contact with secretions or bodily fluids of a dying, moribund horse or dead horse without PPE. Two deaths resulted from infections that occurred before the disease had even been identified and protection with PPE from potentially serious contagious disease was not commonly practiced. Since an increase in awareness of Hendra virus infection and the increased use of PPE, there have been no further infections of humans in almost 7 years, since July 2009. However the potential for transmission exists, as it does for other serious contagious disease and care should be taken. The biggest spike in horse infections occurred before the vaccine became available in 2011 with no resultant human infections, indicating that changes such as PPE may have statistically had the biggest impact in breaking the transmission to humans.

Another option for reducing human risk of the disease would be a human vaccine. This appears to be in development with non-human primate trials having already been conducted. There is also the possibility of inducing post-exposure passive immunity with monoclonal antibody m102.4, which is in clinical trials (and available for compassionate use) and holds much promise as an effective treatment.

Other beneficiaries of the HeV vaccine are Veterinarian surgeons and the Zoetis pharmaceutical company. There is quite a lot of variability in charges for the vaccine by vets ($100-200, consult fees, travel charges, micro-chipping etc). When one considers some vets have reported administering several thousands of vaccinations, it represents an attractive ongoing income stream to add to other general veterinary care income. As this needs to be done 4 times in the first 13 months and every 6 months thereafter, vaccination for Hendra can represent a big expense for owners, especially those that have multiple horses or those struggling already with costs of horse ownership. The cost of Equivac HeV to vets appears to be around $50 and there have been over 400,000 doses sold to date, which means that it has already grossed around $20 million+ for

Zoetis. I have read that the Australian Veterinarian Association recommends all of Australia's horses to be vaccinated. One has to wonder why as there have never been incidents so far outside of northern NSW and Queensland, and the bats that transmit the virus don't occur in all of Australia. Why should one be expected to expose a horse to ongoing 6 monthly vaccinations for its entire life, which has potential side effects, if the risk is, to date, next to zero in those areas?

As to the issue of veterinarians not treating or changing management of a sick horse due to vaccination status, this approach is at the discretion of the vet. The advice in the "Guidelines for Veterinarians handling potential Hendra virus infection in horses" (QLD govt) encourages vets to take appropriate risk assessments in potential Hendra cases and advises no procedures to be conducted by non-veterinarian staff, but does not mention a requirement to treat vaccinated horses differently. If a vet has decided to take a different approach in treating non-vaccinated horses or to deny vaccinated horses treatment at all, the owners need to be advised clearly in advance, so that they can make their own contingency plans. This policy will also be responsible for many horse deaths. I use as an example colic cases. Based on US statistics of colic incidence, there would be potentially 42,000 cases of colic a year in Australia in the domestic horse population versus 3.5 confirmed (or 4.4 confirmed plus suspected but unresolved and untested) Hendra cases, on average per year, since it was discovered. This study reported an 11 % case fatality for colic, even including those colic cases receiving veterinary care, so many horses will die or suffer much morbidity from lack of appropriate treatment. Not all people will vaccinate all horses due to cost, history of adverse reaction, fear of adverse reaction and so on, so this will present a dilemma that is difficult for most vets.

Lastly, the vindictive prosecution of vets by Work Place Health and Safety in Queensland - with threat of fines that could potentially bankrupt a business or individual - are pushing vets to deny or reduce treatment to unvaccinated horses due to fears of being investigated with a Hendra positive horse. This is indirectly enforcing vaccination as owners are finding it increasingly difficult to get veterinary care for their horses. One has to wonder if this is part of an agenda, as there have been no human infections for 7 years since awareness and use of PPE have increased. This could have the effect of causing an irrevocable breakdown in trust between vets and government bodies. Surely, it would be better to use the valuable results of investigation to form workable strategies for future use and instil a spirit of co-operation and approachability to government bodies rather than continuing with this line of fear-based persecution when no harm has been done by actions of these vets, who are just doing their jobs.

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Queensland Parliamentary Inquiry: Hendra virus (HeV) EquiVac® vaccine and its use by veterinary surgeons in Queensland

Submission by:

Dr Margaret Williams BSc, MBBS, FRACGP

[redacted] **Cooroy, QLD, 4563**

[redacted]

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Terms of Reference 1: The development, trials and approval processes of the Hendra Virus Vaccine

Background Development

The Hendra Virus vaccine with trade name "Equivac HeV"(Zoetis) is used to vaccinate horses as an aid to prevent Hendra Virus infection. It is a subunit vaccine containing recombinant G glycoprotein of the Hendra Virus (HeVsG) that can be produced in cell culture. This glycoprotein when injected into an animal stimulates an antibody response specific to that glycoprotein which later helps to provide a neutralizing effect towards a wild or experimental virus antigen challenge.

This vaccine was based on the work pioneered by Christopher Broder Ph.D. of the Uniformed Services University of the Health Sciences (USU) USA and Katharine Bossart, Ph.D., a Uniformed Services University alumna and later assistant professor at Boston University School of Medicine.
These 2 scientists are the co-inventors as on the patent, the assignees are The United States of America as represented by the Department of Health and Human Services and the Hendry M Jackson Foundation for the Advancement of Military Medicine. (Immunization strategies against Hendra Viruses, Broder. C, 2012, see footnote), http://www.eurekalert.org/pub_releases/2012-11/hmjf-hww110112.php

Patents:
US 20130171131 A1 "Soluble Forms of Hendra and Nipah Virus G Glycoprotein"
Australian Patent: 2010241382.
Effective date 7/7/2005. Filing date 11/11/2010
http://pericles.ipaustralia.gov.au/ols/auspat/applicationDetails.do?applicationNo=2010241382&hideNavigation=true
2005327194

This technology was licenced from the Henry M Jackson Foundation for the Advancement of Military Medicine to Pfizer Animal health to develop the vaccine for use in horses. http://equimed.com/news/products/pfizer-licenses-technology-for-hendra-vaccine-for-horses
Pfizer Animal Health later broke away into a separate company from its parent Pfizer and was renamed Zoetis Inc, the current manufacturer of the HeVsG vaccine Equivac HeV. (Wikipedia, Zoetis)

Scientists at CSIRO Australia have contributed to the ongoing progression of this work including vaccine trials in ferrets and later horses amongst other relevant research.

Permits: For vaccination of Australian horses issued by the Australian Pesticides and Veterinary Medicines Authority (APVMA):
Minor use permit PER13510 effective 3/8/12 to 3/8/14
Equivac HeV Launched in QLD 1/11/2012 (AVA website, media release)
Minor use permit PER 14876 for 4/8/14 to 4/8/15
Minor use permit PER 14887 31/3/2015 to 4/8/15
Current Registration: 68996/103910 for 4/8/2015 to 30/6/2016 – (6 month duration of immunizations following booster)
Rejection by APVMA of application by Zoetis for 12 month Duration of Immunization: Announced June 2015 http://www.abc.net.au/news/2015-08-05/apvma-aprroves-hendra-vaccine/6673542 ; APVMA archives- application summary to allow ongoing annual boosters- APVMA application number 105049

Development and Efficacy Trials with the HeVsG Vaccine

Does it Work?

Prior First Permit from APVMA:

Nipah, Hendra and the non-pathogenic Cedar viruses are emergent zoonoses from a newly formed genus Henipavirus. Hendra virus was first identified in 1994. They share some characteristics including the virus envelope glycoprotein G which attaches the virus to the host cell. The genetic coding RNA nucleotide sequences for this glycoprotein G were reported to be around 83% homologous, for Nipah and Hendra viruses, in study by Wang et al, 2001 https://www.deepdyve.com/lp/elsevier/design-and-evaluation-of-consensus-pcr-assays-for-henipaviruses-sg49ztq0V8
These concepts form the basis for using the one subunit vaccine for both species of virus Nipah (NiV) and Hendra (HeV). However, the 2 viruses are not the same, for example they have different host affinities. Nipah can infect pigs and directly infect humans from bats, whereas Hendra virus needs an amplifying intermediary host, for example the horse, in order to infect humans. Intrastrain genetic variability in these viruses is also likely to occur, and has been studied in Nipah and Hendra viruses. (NiV- Bangladesh, NiV- Malaysia, HeV 1994, and HeV Redlands). HeV (Hendra and Nipah viruses: pathogenesis, animal models and recent breakthroughs in vaccination, Weingarti, 2015). Much of the early work with Henipaviruses and vaccine trials were with Nipah virus. This is understandable as Nipah poses a greater human health threat. There have been 477 cases as of 2010 and 252 deaths from Nipah virus in SE Asia with 40% to 70%, and sometimes 100 % case fatality rates depending on the outbreak region. (World Health Organization, SE Asia regional division: Nipah virus)

The Hendra virus vaccine preliminary research data available prior to issue of the original minor use permit for the Hendra vaccine used *other species of host not the horse and another species of virus - the Nipah virus* (not comparing apples with apples so to speak). Some of the early Hendra and Nipah vaccine research used different vaccine components, protocols and in vitro experiments, but were still based on concepts of expression of glycoprotein G (attachment) and or F (fusion) antigens.

An important part of the assessment to determine if a vaccine works is the efficacy viral challenge studies. These studies comprise unvaccinated controls and vaccinated test animals. The controls are used to check for infectivity and check for the virulence of the live Hendra virus challenge. The test animals are vaccinated as per the schedule proposed and at a certain period after the booster dose challenged with live virus. The test and

control animals are all euthanized at the completion of the study and post mortem analysis is conducted to assess for infection and disease in controls, as opposed to vaccinated test animals, to demonstrate that the vaccine works. The animals, if they become sick during the study, are euthanized at a predetermined endpoint for humane reasons.

Some may find these sorts of experiments to be abhorrent, on horses especially, but efficacy studies are done to determine if a vaccine works. They are especially important if the disease under investigation is rare and efficacy can't be assessed in the field. Sadly, horses are not treated as people, for example, 30-40,000 horses are slaughtered and processed for meat each year and 80% of these are from Australia's 1 million domestic horse population, 20% are Brumbies. (Unwanted: the Disposable Horse, J Duckworth, 2010).

To assess efficacy of the current HeVsG vaccine, there was a Hendra virus challenge vaccine study using 8 ferrets (6 test animals and 2 controls) and a series of 3 small Hendra viral challenge studies in horses that had a total of 7 test and 1 control horses prior to release of this vaccine for widespread use in Australia's domestic horse population. As these challenge studies were so small, statistical analyses are limited. I understand that as Hendra is a biosecurity level 4 virus, restrictions on facilities and procedures limited sample sizes.

In these first 3 vaccination challenge trials, the horses were vaccinated, given a booster at day 21 after the first dose, and were administered a challenge dose of Hendra virus a further 28 days later, and euthanized between day 7 and 9 post challenge. (See Public manuscript "Hendra Virus Vaccine, a One Health Approach to Protecting Horse, Human and Environmental Health", D Middleton et al, 2014, for details of these trials)

Initial HeVsG Vaccine Efficacy Trials in Horses

My comments on the horse viral challenge studies:

1. The first study comprised 2 test horses and one control horse. The dose of HeVsG was 100 mcg produced from 293 F Human kidney embryo cell expression system . The control unvaccinated horse after viral challenge did have clinical symptoms and was euthanized at a predetermined humane endpoint and found to have viral genome in several organs. The vaccinated horses remained clinically healthy and did not contain viral genome.

2. For the next trial, the vaccine contained a lower dose of HeVsG antigen (50 mcg) from Human kidney embryo cell line expression with 3 test horses and guinea pigs as unvaccinated controls. The 3 test horses after challenge with Hendra virus did not have viral genome in any tissues or samples and remained healthy until euthanasia between days 7-9. It was reported, however, that only 1 of the 4 unvaccinated control guinea pigs died, (see table 1. Viral infectivity control column, animals died; Guinea Pig -1/4 http://europepmc.org/articles/PMC3944873). In this research paper quoted above, the author mentioned they only expected equal to or more than 25% of guinea pigs to die from the viral infection. I wonder why one would choose such an unreliable control. I note that the horse control was euthanized at a predetermined humane endpoint from the previous study, and note in the ferret studies (J Pallister, 2011) the ferrets were also euthanized at a predetermined humane endpoint, in fact all of the studies I have looked at the sick animals are euthanized or at the end point of the study animals are euthanized. Simply reporting the number of controls that died is not that informative as it fails to distinguish between those euthanized and those that died from the virus. We need to know how many of the control guinea pigs were infected with Hendra virus. It appears from the table the control for viral infectivity worked in only 25% of guinea pigs. The article had very little detail about infection in non-horse controls, but it wasn't clear if all of the guinea pigs became infected or only the one, it would be interesting to see more data. It may be the way this was presented in the manuscript that this is confusing. The relevance of this I believe is that it reduces the quality of the trial as with a different species as control there are different susceptibility characteristics for one.

Controls are used to determine pathogenicity of the virus used to challenge the test animal (or how infective and lethal that virus sample is), it should be from the same sample or batch of virus and during the same experiment, and the control should preferably be from the same species as the test animals. *If the controls didn't work you can't say for sure your experiment worked.* In an earlier study high dose subcutaneous injection of Hendra virus inoculum in guinea pigs induced disease reliably at similar high doses to that used in this study. 14 out of 15 guinea pigs in the higher dose administered subcutaneously became infected. (A Guinea-pig model of Hendra Virus Encephalitis, Williamson, 2001)

I note at this reduced dose of 50 mcg HeVsG one of the horses didn't generate as robust an antibody response, at 28 days post booster the prechallenge antibody titre which should be maximal, was at 128, 256 perhaps this level could drop too much by the 6 month revaccination duration to be as effective. I wonder if that is why the following trial used 100 mcg HeVsG.

3. The third horse study trial: There was a change at this point in the antigen production system from using Human kidney embryo cell lines to produce the glycoprotein HeVsG for the vaccine to Chinese Hamster Ovary cell expression. (This change in the glycoprotein antigen production system was driven by the necessity for higher antigen yields). http://europepmc.org/articles/PMC3944873. Another viral challenge clinical trial would be then needed for efficacy testing with the change in the antigen production system, prior to release of the vaccine. The dose of HeVsG administered was 100 mcg for the vaccine. 2 horses were challenged with Hendra virus at day 28 post booster vaccine, and remained healthy and did not demonstrate viral genome. They both had quite good pre challenge antibody titres of >4096. The controls used were 2 ferrets which succumbed to acute infection with HeV. Again the quality of the study is not as good with another species as the control. Ferrets are thought to be extremely susceptible to Hendra virus and well, they are not horses! Controls are needed to check the virulence of the viral inoculum, amongst other reasons.

   The ferret controls for the last horse study received a dose of Hendra virus of 50 000 TCID50. (Tissue culture infectious dose 50%) Part of a study by Jackie Pallister, 2011, was for Hendra virus ferret model development. In this work 8 ferrets were given a range of doses of Hendra virus oronasally ranging from 50 to 50 000 TCID50 to help derive a challenge dose of HeV for ferrets. All these animals became ill even the ferrets given the lowest dose of Hendra virus ferrets and found no association virus dose and incubation time, clinical signs, time to end point and severity of lesions. My point is firstly horses and ferrets or other animals will have different susceptibility characteristics to becoming infected with Hendra Virus. Secondly ferrets, that weigh .7 to 2 kg received 50 000 TCID50 of virus, a grown horse 400-550 kg and received 2 000 000 TCID 50 of virus in the viral challenge. The 2 control ferrets received roughly 10 times the dose of Hendra virus per kg according to my calculations, if you can validly make the comparison anyway. I feel having another species as a control just adds too many confounding variables and detracts from the potential significance of the results. (Weight of ferrets: Wikipedia; and Merck Vet Manual on Ferrets. http://www.merckvetmanual.com/mvm/exotic_and_laboratory_animals/ferrets/management_of_ferrets.html) What happened to the 3rd horse in the study? There was no mention in the published paper about this. There were 3 horse bays and the controls were ferrets.

HeVsG Vaccine Efficacy Trial in Ferrets

The other animal *viral challenge study* to determine the efficacy of the HeVsG vaccine, prior to the issue of the first permit for use of the Hendra vaccine in horses, was a *ferret study* with 6 vaccinated test animals and 2 unvaccinated controls. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3153950/ The animals were vaccinated with 4 mcg

(2 ferrets), 20 mcg(2 ferrets), and 100 mcg (2 ferrets) doses of the HeVsG vaccine, given a booster dose 20 days later then challenged with Hendra Virus at 5000 TCID50 21 days after the booster. (This was 10 times less viral dose that the dose selected for ferrets in the horse studies). The control ferrets still became unwell and demonstrated viral genome in many tissues, and 4 of the 6 vaccinated ferrets remained well and viral genome or live virus was not detected pre or post euthanasia. One vaccinated ferret became unwell and was euthanized at day 10 post challenge but the illness was not thought to be due to the Hendra virus. *The other vaccinated ferret did have viral genome in nasal washings on day 6, 8 and 10,* but seemed to be self-limiting without evidence of virus in internal organs post euthanasia at day 20. The comment by the author was "this was consistent with viral replication rather than detection of the original inoculum". (J. Pallister et al, 2011)

The viral genome levels in the nasal washings of this vaccinated ferret were noted to be significant at Ct 35.2-37.2 (Cycle threshold (Ct) and PCR- the higher the Ct level the lower the viral genome- under 40 is considered significant ie 35 indicates more viral genetic material than 37. I note this level for the vaccinated ferret is similar to the Ct level in a nasal washings in a Hendra infected gelding on 4 July 2013 on the day of euthanasia of the sick horse at 34.56, ? if these Cts are methodologically comparable?)(Hendra virus infection in a dog, Australia, 2013, Kirkland et al) (Design and evaluation for consensus assays for henipaviruses, K Feldman et al, 2009)

I note that even though the viral genome was found in a low dose 4 mcg vaccine ferret, the ferret produced quite high antibody titres at 1:8192 pre challenge similar to antibody levels in other vaccine dose animals in the trial, which may have been protective. Although the high dose vaccine ferrets were given the same dose as that given to horses, 100 mcg of HeVsG, all the vaccine doses even the lowest dose seem comparatively high to that given to a horse. This may be reflected in the very high neutralizing HeVsG antibody titres in all of the ferrets prior to viral challenge compared with the pre-challenge titres in the horse studies.

J Pallister et al, 2011 noted in a study with cats, HeVsG vaccine and Nipah virus, "*In a similar experiment, cats immunised with 5, 25 and 50 µg HeVsG vaccine and challenged with NiV showed evidence of viral replication with increasing antibody titres post challenge and genome detection in oral swabs, urine and the brains of 4 animals receiving two higher doses of vaccine. The detection of viral genome in the brain of cats with significant antibody levels prior to challenge indicated that a persistent infection might occur despite pre-existing imunity. A farmer infected during the first recorded outbreak of HeV that occurred in Mackay in August 1994 recovered from meningitis only to develop neurologic signs 14 months later and die with HeV present in the brain."* http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3153950/ ; McEachern JA, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine. 2008; 26:3842–52.; Sullivan JD, et al. Fatal encephalitis due to novel paramyxovirus transmitted from horses. Lancet. 1997;349:93–5.

J.Pallister et al, 2011 also maintained HeVsG vaccine *"can prevent clinical HeV disease and in some cases infection"* from the studies to date at that time even though absolute direct comparisons between trials were unable to be made. I think that was reasonable to say, although the HeV trials had been small, and the viral challenges were at 20- 21 days post booster when the immunization should be most effective.

Prior to granting the first minor use permit the HeV viral challenge vaccine efficacy studies included to my knowledge: A clinical viral challenge vaccine study of 2 test horses and one control with viral inoculum at 21 days post vaccine booster. The other 2 studies with horses (3 horses, and 2 horses) used other species for controls, the viral challenge was at 21 days post booster. Obvious issues with not using the same species as controls that I have mentioned above introduce possible confounding variables and reduce the quality of the study when compared to a same species controlled study. I realize that the Australian Animal Health Laboratory (AAHL) have only 3 horse bays suitable for biosecurity level 4 experiments. I see the issues are difficult but feel even with these difficulties that a biosecurity level 4 virus presents, scientific rigor should be maintained.

The other study included 6 vaccinated test and 2 unvaccinated ferrets with comparatively high doses of the vaccine reflected in the high antibody titres at pre viral challenge. I realize a lot of research had gone into the

technology to get to that point but much of it was in other species of animals and in another species of virus, albeit a closely related virus. ***I am amazed that an animal vaccine can be authorized for widespread use without doing further viral challenge clinical trials for more confident in vivo analysis of vaccine efficacy prior to the issue of a permit that enables widespread administration for veterinary use***. Furthermore, one should expect the animals to have robust immunity so soon after immunization at 21 days post booster but that immunity generally wanes with time, so *is* ***it reasonable to issue a permit valid for 2 years, for 6 months duration of immunization, before the 6/12 Hendra vaccine viral challenge study in any animal species was even completed?*** (see permit 13510 APVMA under efficacy)

6 Month HeVsG Vaccine Efficacy Trial in Horses

This trial comprised 3 vaccinated horses and 2 unvaccinated control ferrets. The horses were vaccinated with 100 mcg of HeVsG vaccine, given a booster dose at day 21 then 6 months later challenged with Hendra virus at $2x10^6$ TCID50. The unvaccinated ferret infectivity controls displayed disease and viral genome consistent with acute HeV infection. The vaccinated horses remained clinically healthy in the observation period until euthanasia at from days 7 to 9 post viral challenge. 2 of the 3 test horses were not shown to have any viral RNA in their secretions or samples taken before or after euthanasia. However 1 of the 3 vaccinated test horses (V9) did have viral genome in its nasal swab sample on days 2,3,4 and 7. However no viral genome was found in samples post mortem on day 8 in this horse. See Figure 2 "Hendra Virus Vaccine, a One Health Approach to Protecting Horse, Human and Environmental Health", D Middleton et al, 2014

Figure 2

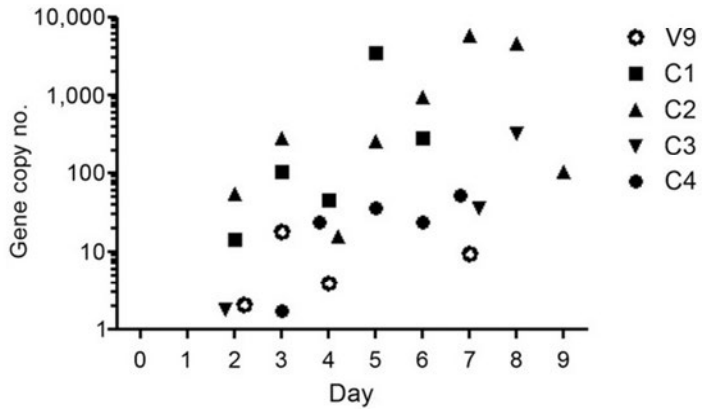


Figure 2: Scatter plot showing quantitation of the Hendra virus N gene in nasal swab samples from 1 vaccinated horse (V9) and 4 control horses (C1–C4); controls were challenged but not vaccinated. Days represent days after challenge.

The author has commented on this finding- *"Where evidence of low-level virus replication did occur in secretions, it was transient and unaccompanied by the development of clinical illness, and virus was not isolated from the secretions."* I have a couple of comments on this:

1. In the vaccinated horse V9, there was a finding of viral genome on days 2, 3 and 4, after which there was none found on days 5 and 6, at day 7 viral genome was again found in nasal swabs at a higher level than days 2 and 4. The vaccinated horse (V9) was put down the following day, on day 8, even before a different vaccinated horse (V10), that was euthanized on day 9. The V10 horse did not show evidence of viral genome. (see table 3: re euthanasia dpc is days post challenge) How can you categorically say that the viral replication was transient if the horse has been euthanized? *It was put down the day after a rise in viral genome!* If that horse had have lived longer and had clearly stopped shedding viral genome one could say it was transient but not if the horse was not allowed to live long enough to see what happened.

In an earlier study done prior to the HeV vaccine challenge trials in horses (Experimental Infection of horses with Hendra Virus/ Australia/ Horse/ 2008/ Redlands, G Marsh et al, 2011), 3 horses were successfully experimentally infected with Hendra virus. This historical data is used in the presently considered paper http://europepmc.org/articles/PMC3944873. They are counted as "controls"(C1-3) but they were not viral infectivity controls for any of the vaccine challenge trials, but examples of HeV infection in the horse. The only control horse that was used for the viral challenge in vaccinated horses efficacy study was in the very first vaccine challenge study and was assigned C4, or control horse 4. To clarify, the infection controls in this 6 month study were ferrets.

I tried to compare the levels of viral genome in these earlier horses' nasal swabs to the present vaccinated horse V9. Figure 2 compares the unvaccinated horses C1 –C4 nasal swabs that were successfully infected with Hendra virus with the vaccinated horse V9 that had viral genome in 4 samples of nasal swabs. Unfortunately, I found that the control horses 1-4 in table 2 did not correspond to the C1-4 in figure 2. I calculated the figure for log 10 relative copy number Hendra virus N RNA copy back to a non -log gene copy number for each reading to try and follow the data, this also fits with the different days to euthanasia for the different horses; and linked the same horse data up with figure and table as they seem to have mixed them up: with C1 (figure 2) = 2 (table 2); C2 (figure 2) = 3 (table 2); C3 (figure 2)= 4 (table 2); C4 (figure 2)=1(table 2).The tables I reprinted here for easy reference. Tables and figure from "Hendra Virus Vaccine, a One Health Approach to Protecting Horse, Human and Environmental Health", D Middleton et al, 2014.

Table 2

Quantitative reverse transcription PCR detection of Hendra virus N gene in samples collected daily from control horses*

Horse no., sample	$\log_{10}$ relative copy number of Hendra virus N RNA, dpc									
	0	1	2	3	4	5	6	7	8	9
1†										
Blood	–	–	–	–	–	–0.2	1.4	1.7		
Urine	–	–	–	–	–	–	–0.8	1.1		
Feces	–	–	–	–	–	–	–	–0.7		
Nasal swab	–	–	–0.3	–0.2	1.4	1.6	1.4	1.7		
Oral swab	–	–	–	–	–	–0.1	1.3	1.3	—	—
2‡										
Blood	–	–	–	–	0.5	2.6	3.0			
Urine	–	–	–	–	–	0.3	1.7			
Feces	–	–	–	–	–	–0.05	2.0			
Nasal swab	–	–	1.2	2.0	1.6	3.5	2.5			
Oral swab	–	–	–	–	–	0.4	1.5	—	—	—
3‡										
Blood	–	–	–	–	–	–	1.5	2.8	2.9	3.4
Urine	–	–	–	–	–	–	–	1.8	1.7	3.0
Feces	–	–	–	–	–	–	–	1.5	1.7	2.1
Nasal swab	–	–	1.7	2.5	1.2	2.4	3.0	3.8	3.7	2.0
Oral swab	–	–	–	–	–	–	–	1.9	1.9	2.3
4‡										
Blood	–	–	–	–	–	0.1	1.9	2.5	3.0	
Urine	–	–	–	–	–	–	0.07	0.5	2.1	
Feces	–	–	–	–	–	–	1.3	2.4	2.1	
Nasal swab	–	–	0.3	–	–	–	–	1.6	2.5	
Oral swab	–	–	–	–	–	–	0.2	1.2	1.6	

*Duplicate samples were obtained and tested by reverse transcription PCR. Cycle threshold values were converted to relative copy numbers by using a standard curve of a sample with a known copy number. dpc, days after challenge. – indicates a negative result; blank space indicates no sample was tested.
†N gene data for horse 1 was obtained from the current study.
‡N gene data for horses 2–4 are unpublished data from a previous study (6).

The vaccinated horse V9 challenged with Hendra virus had the viral genome detected on nasal swabs equivalent to the some of the historical data from infected 4 unvaccinated control horses on several days in the pre-symptomatic period. For example, = or more than 2 horses on day 2, day 3 (more than 2 controls horses), day 4 (more than 1 control). On day 7, vaccinated horse V9 demonstrated a rise in viral genome which was less than control horse C3 which was also unmeasurable the previous day.

The following day 8 the nasal swab viral genome in C3 increased substantially, on that day, and V9 was euthanized. The Incubation period for Hendra virus is considered to be between 5-16 days in the horse but could be up to 31 days. https://www.health.qld.gov.au/cdcg/index/hendra.asp# . I expect the nasal replication of HeV often precedes the onset of viremia and viral dissemination around the body of the animal. This horse C3 could just be one day or more ahead in the range of possible incubation periods of the vaccinated horse V9 before disease and virus could spread to the other organs and amplify. By euthanizing the vaccinated horse V9 at day 8 following an increase in nasal viral genome, one will never know if disease would have progressed from local nasal mucosa to systemic infection. If one compares the horse C3 (upturned triangle) in figure 2 it demonstrated very low viral genome at day 2 then nothing until a rise on day 7 and further rise on day 8 prior to euthanasia. Table 2 did not include levels of Hendra virus N RNA in the vaccinated horse V9. Looking at the horse controls the presence of nasal viral genome and an increase often precedes the progression of the disease and presence of genome in other secretions.

I just think this issue is worth mentioning as compared with the transient self-limiting viral replication that later settled in the vaccinated ferret in the study discussed earlier that had very high antibody protective titres of 8192 pre challenge, this vaccinated horse had much lower level antibody titre of 16, and second reading 32, prior to administration of the HeV virus inoculum to try to infect the horse. As the protective antibodies were much lower it may have been more susceptible to developing disease. Weren't they wondering what would happen? Was this a prelude to progression of local infection to generalized viremia and illness, or just transient local viral replication? Why was this horse, V9, euthanized the day before the other vaccinated horse, V10, that did not demonstrate viral RNA in its nasal swabs?

2. The other comment above before the point 1 from the authors of the manuscript in discussion was that virus was not isolated from the secretions, in discussing the vaccinated horse V9 with 4 readings of viral genome in nasal swabs. Virus can be detected by viral PCR or by viral isolation techniques. Viral PCR targets chosen genetic material from a virus (in this case the N gene), and this is expressed quantitatively in a number and is very sensitive. Virus isolation is generally attempted on the identified positive HeV genome tissues found by PCR determination, the tissue is prepared and incubated in culture medium and scores positive if certain characteristic cell changes occur and are present after 6 days of culture. I note that ***no virus was found in any of the pre euthanasia samples in the horses with positive viral genome on PCR be it either the infected unvaccinated controls or the vaccinated horse V9 that had positive viral genome****. The infected unvaccinated horse genome readings are in table 2.* These control horses subsequently progressed clearly to have Hendra virus infection in many organs. This anomaly was discussed in an earlier cat study "Clearly, these data underscore the poor sensitivity of direct virus isolation compared to TaqMan PCR as techniques for virus detection. This anomaly between TaqMan PCR and virus isolation data was most notable in the adrenal samples, which revealed very high relative genome levels but did not enable virus isolation. (Feline Model of Acute Nipah Infection and Protection with a Soluble Glycoprotein-Based Subunit Vaccine, Mungall eta al, 2006). Also a comment from a Hendra Virus infection review: "*Virus isolation can also be attempted in live animals; however, Hendra virus is more likely to be recovered from the tissues after death."(Hendra Virus Infection, The centre for Food Security and Public Health, ISU, last updated Dec 2015)*

3. The 3 of the 4 "control" horses were not infectivity controls as part of this series of vaccination challenge studies, but were used as historical data of infection in a horse as mentioned previously. Concerning these 3 control horses as discussed in an earlier manuscript, (Experimental Infection of Horses with Hendra Virus/ Australia/ Horse/ 2008/ Redlands, Marsh et al, 2011), *"Virus was not reisolated from any sample collected before postmortem examination".* Also, from the presently considered paper which discussed the 1 control horse used in the first vaccination challenge

study quote, "*virus was not reisolated from any sample collected before postmortem examination"* "Hendra Virus Vaccine, a One Health Approach to Protecting Horse, Human and Environmental Health", D Middleton et al, 2014. So the finding that in the horse V9 no virus was found in the positive RNA nasal swab samples was unremarkable, as virus was not found with virus isolation in *any* sample pre euthanasia in the horses, be it horses unvaccinated infected with Hendra virus, or the test vaccinated horse with positive viral genome.

So one could say the vaccine at 6 months after the prime booster regime worked to prevent Hendra virus clinical disease in all 3 horses for 7-9 days post viral challenge, compared to the ferret controls. Viral genome in nasal swabs was found in 1 of 3 horses, on 4 days, likely indicating possible local viral replication in 1 horse. This horse when euthanized at day 8 did not have viral genome in any organs post mortem, but at day 8 this horse was still in the possible incubation period for Hendra virus.

The First 12 Month HeVsG vaccine Efficacy Trial in Horses

The results of this this study were not published in a scientific manuscript, however, the results were made public in the media for the 12 month duration of immunity trial. The horses when challenged with Hendra virus 12 months post booster did not become infected, but the ferret infectivity controls remained healthy also. This indicated the viral inoculum was not virulent. "Dr Middleton says there was a problem with one of the Hendra virus vials" http://www.abc.net.au/pm/content/2013/s3811187.htm
This made the first 12 month duration of immunity in horses trial invalid.

Other Heniparvirus Efficacy Studies using HeVsG Vaccination in other species

12 month Ferret HeVsG Vaccine Trial and Nipah Virus

There were 6 test Ferrets given different doses of the vaccine HeVsG, and viral challenged at 12 months. One of the vaccinated ferrets died prior to the challenge and was excluded from the results. The 5 other ferrets remained well after viral challenge. 2 of the 5 test ferrets were found to have viral genome by PCR. In one 20 ug dose vaccine ferret with pre-challenge antibody titre level of 64, 128, viral genome was recovered from nasal secretions on day 6 and day 8 (with with significant Ct levels at 34.1 and 32.3, see table 2), but not found post mortem tissues. This indicated self-limiting viral replication. The other 20 ug ferret with antibody titres of 16,16, was found to have viral genome in a bronchial lymph node post mortem. (Ct- 33.8). The control ferrets had more extensive disease. (refer: Vaccination of ferrets with a recombinant G glycoprotein subunit vaccine provides protection against Nipah virus disease for over 12 months. J Pallister et al, 2013) Even though the antibody levels may be low ie 16 in the one ferret, the stimulation of immunological memory may be a factor in reducing the course of the disease, as well as the hosts' natural immunity systems. However, a vaccination that delivers rapid access of high levels of antigen into the blood stream to stimulate antibody production may be dealt with differently by the immunological system to low level nasal replication of wild virus. The stimulation of antigenic memory however may take time and at very low levels of antibody titre one can't assume that this would be always protective with a rapid onset of fulminant viral disease.

Again, this study used a different species, the ferret, and in the study discussed below the cat, with a different Henipavirus, the Nipah virus, with different comparative dosing of vaccine, and other experimental variables, to that given in the horse. But as there are such small numbers of HeVsG vaccinated horses that have been experimentally viral challenged with Hendra virus, it is still worth looking at, as these studies

contribute to the basis of experimental body of evidence that the approvals have been based on, but caution should be used in making direct comparisons to the Hendra virus and horse model.

The Cat model and Nipah viral challenge with HeVsG vaccine

A total of 6 cats immunised with 5, 25 and 50 µg HeVsG vaccine were challenged with a lethal dose of NiV. There were 2 control cats. NiV genome was detected from all cats post challenge in oral swabs, viral genome was also found in urine samples of vaccinated cats after Nipah virus challenge, and brains of 4 of the vaccinated cats post-mortem. Note unexpectedly, that the highest levels of viral genome were found in the brain of the vaccinated cats as compared with the controls. Some excerpts are given below from the study *"A Recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats, J McEachern et al, 2008),* see results section:

"NiV genome was detected in oral swabs from all cats post-challenge and results are shown in Fig. 7A. The highest level of apparent shedding occurred on 6 dpi and 8 dpi. Cats 32-25, 33-5 and 34-5 appeared to shed virus at low levels until 21 dpi consistent with post-challenge SNT data, which suggested an increased immune response to replicating virus in these animals. Virus isolation was attempted on all NiV genome positive oral swabs; unfortunately cell toxicity in all samples precluded any successful isolation."; " the detection of NiV genome in urine and in swabs from low dose cats on 21 dpi was perhaps a stronger indication of replicating virus in these animals";

Unexpectedly, cats 29-50, 30-50 and 31-25 demonstrated the **highest level of genome in brain tissues, and levels were significantly higher than those seen in the control cats.** Virus isolation confirmed the presence of NiV in cat 31-25 brain-olfactory whereas for cats 29-50 and 30-50, no virus was isolated" "**Clearly, for all vaccinated animals, systemic NiV-mediated disease and multiple organ system pathology was reduced almost entirely. As an exception, NiV genome was detected in the brain of several animals and the significance of these data remains to be eluci**dated**." "Notably, all the vaccinated cats that demonstrated NiV genome in the brain had significant antibody titres prior to challenge."** (my emphasis added above)

An alternative possible pathway for infection of the Central Nervous System has been proposed since this study was done whereby the Henipavirus could possibly enter the brain via olfactory nerve pathways. The other routes that Hendra virus can spread through the body include respiratory spread and blood born systemic dissemination. Studies in the mouse with Hendra virus (Dups et al, A New Model for Hendra Virus Encephalitis in the Mouse, 2012), and hamster with Nipah virus (Munster et al, Rapid Nipah virus entry into the central nervous system of hamsters via the olfactory route, 2012) further investigated this nerve pathway of experimental Henipaviral infection. Munster described this infection as rapid (4 days) and suggested it may occur simultaneously with systemic viral infection. (I wonder if the reason the **highest dose vaccinated cats in this experiment, were almost clear of multiple organ system disease but had the highest levels of viral genome from the Nipah virus in the brain** might be related to this possible alternative pathway of infection? I also wonder if by this mechanism, the vaccine could be possibly less effective at preventing Nipah viral infection in the brain of the cat,??).

In the **2008 Redlands outbreak at a Veterinary clinic**, all 5 horses and 2 humans infected with Hendra Virus primarily presented with neurological disease rather than the previously documented presentations of Hendra Virus infection that had rapid progression of a febrile illness with fulminant respiratory infection and milder neurological signs. ***"The flagrant neurologic features in this outbreak strongly contrast with those of previous cases, in which respiratory disease predominate."***(Hendra Virus Outbreak with Novel Clinical Features, Australia, Field et al, 2010).

Genetic variability has been considered to possibly account for these different disease manifestations, as well that this presentation being simply a part of the possible spectrum. Playford el al, 2010, discussed "the degree of nucleotide sequence variation observed between Hendra virus isolates from this (the Redlands outbreak) and previous outbreaks suggests greater genomic variation than previously assumed", and has a Phylogram showing the relationships between different isolates in the article. (Human Hendra Virus Encephalitis Associated with Equine Outbreak, Australia, 2008, Playford et al, 2010) Marsh et al, 2011, however, in "Experimental Infection of Horses with Hendra Virus/Australia/Horse/2008/Redlands", commented that in the initial experimental infection of

horses with Hendra Virus, that in the Redland's strain, genetic analysis of this isolate showed 99.6% similarity at the amino acid level to the original Hendra Virus isolate from 1994.

If the virus genetics were stable enough since the first recorded case, could it be that this Redland's outbreak with a novel clinical presentation may relate to this newly described possible pathway of CNS infection, resulting in an earlier presentation of neurological disease? I see that one horse presented with bullous keratopathy of the right eye to the vet clinic. (Hendra Outbreak with Novel Clinical Features, Australia., Field et al, 2010). Viral infection such as HSV and others can cause this condition in humans so wonder if the facial nerve paralysis and CNS symptoms seen in this horse, (that he recovered from) could represent an ascending spread of HeV infection that spread from the eye via the Oculomotor nerve. Another horse had a nasal condition that possibly may have made it more susceptible to an olfactory route of spread.

They have used the Redland's isolate in the vaccine challenge horse studies, however, and the clinical illness presentations sound more like the earlier presentations of Hendra virus. There may be other factors at play such as dose and pathway of infection, especially in the wild virus disease. In the review, "The changing face of henipaviruses", (E. Croser, and G Marsh, 2013) mentioned "this alternative pathway for infection of the CNS is a plausible "natural route of infection" and has implications for therapeutic effectiveness".

They recommended future animal model experiments, especially those dealing with therapeutic efficacy, should take the olfactory route into account and challenge accordingly.

I wonder if the horses or ferrets were experimentally challenged with a direct high nasal route (direct to olfactory mucosa) rather than the currently used oronasal (?back of pharynx) which is used for the experimental inoculation of horses with HeV virus, whether there would be a higher incidence of neurological presentations, and if vaccination would be as protective in the proposed alternative pathway of HeV CNS infection. (The olfactory nerve supplies the olfactory mucosa on the upper part of the nasal cavity). I am sure the scientists have considered these issues though. Vaccination didn't seem to stop viral infection in the brains of 4 of the 6 vaccinated cats, despite good levels of anti-Nipah antibodies from the HeVsG vaccine. In the cat study, it was mentioned that it would be impossible to say if that viral CNS infection would go on to encephalitis or not, as the vaccinated animals were clinically well prior to euthanasia. Again, sample size in this cat study is small, there is a different species of host, different adjuvants, different Henipavirus but interesting all the same.

More Recent Henipavirus Trials using African Green Monkeys

African Green Monkey vaccinated with HeVsG and challenged with Hendra Virus

African Green Monkeys (AGM) were vaccinated with 100 mcg of HeVsG, with a prime then boost protocol, and challenged with Hendra Virus (Hendra strain from the '94 outbreak) at 21 days after the booster. The inoculation route for the Hendra virus for all AGMs was intra-tracheal. Then all alive animals were euthanized and examined at the end of the trial which occurred 30 days after the viral challenge. All 8 vaccinated AGM remained healthy and did not shed virus, which is a promising result. There were 4 controls AGMs. One unvaccinated control did not shed virus and was not found to have any disease or virus post mortem and lived until the end of the trial at 30 days. This unvaccinated control AGM seemed to be unwell for a few days during the trial but seemed to recover. The other 3 monkeys were unwell, had disease and viral genome, before and post mortem and were euthanized before the end of the trial as they were unwell. Incidentally the monkeys that weighed 3-6 kg as stated in the study received the same dose of **100 mcg HeVsG vaccine as that given to a 500 kg horse, that is roughly 100 times the dose of HeVsG antigen in the vaccine per kg given to a horse**. It may also be that AGMs need a comparitively higher antigen dose to get a robust antibody response. The HeV antibody titres were good and ranged from 640 to 2580 at the time of challenge. (A Recombinant Hendra Virus G

Glycoprotein Subunit Vaccine Protects Nonhuman Primates against Hendra Virus Challenge, Mire, 2014). This article also maintained that this dose of HeVsG that was used based on the findings from an earlier trial which demonstrated better protective efficacy with the 100 mcg dose, and prolonged level of IgG in vaccinated AGMs compared to those given the 10 or 50 mcg dose in a challenge with NiV.

African Green Monkey vaccinated with HeVsG and challenged with Nipah Virus

In this earlier study also using the AGM animal model, "A Hendra Virus G Subunit Vaccine Protects African Green Monkeys from Nipah Virus Challenge, Bossart et al, 2012, **commented that with low and medium doses of HeVsG (10 and 50 mcg) "IgG antibody titres appeared to wane by 28 days post booster, and weak IgM responses to NiV suggested undetectable amounts of virus were circulating in the host"**. The 6 test vaccinated AGMs were protected however, from NiV infection within the time frames of the study, which was encouraging for the research.

I wonder if the dose in the Equivac of HeVsG at 100 mcg may be too low in the horse to generate and maintain a good level of protective immune titre, compared with the level of doses given to other animals in the experimental studies. Looking at the 6 month titre levels to hand prior to booster in the horse they appear to generally be comparatively low.

Further HeVsG Vaccine Duration of Immunity and Efficacy Challenge trials in the Horse

Zoetis announced on 17 Dec 2013 that Zoetis and CSIRO's Australian Animal Health Laboratory (AAHL) planned to do further 12 month duration of immunity challenge testing with 2 different immunization protocols, one group of horses in early 2014 and the second group in late 2014.
http://www.qldhorsecouncil.com/QldHorseCouncil/media/QHC-Portal/Hendra%20Virus/131217-An-Update-on-Equivac-HeV-DOI-Challenge.pdf The tables below are from this update and reproduced below. Group 1 HeV vaccination schedule: at day 0, 3 weeks later, then 12 months later; Group 2 vaccination schedule: at day 0, 3 weeks later, 6 months later, 12 months later.

They presented Hendra Virus specific antibody profiles as a result of vaccination for 2 groups of horses, each comprising 7 horses. The columns in green indicate antibody levels on the days when a dose of Equivac HeV was administered. The orange columns show the antibody levels at the certain time period after vaccination.

Table 1
Group 1 Data

Animal ID	Day 0	Day 21	Day 42	Day 388
4465	<8	32	1024	8
4783	<8	16	512	8
5991	<8	256	2048	64
6317	<8	16	1024	32
5669	<8	64	1024	8
2500	<8	32	2048	16
9636	<8	16	512	<8

Table 2
Group 2 Data

Animal ID	Day 0	Day 21	Day 42	Day 220	Day 227	Day 577
7403	<8	128	2048	8	4096	>1024
0006	<8	32	2048	8	4096	512
4527	<8	512	1024	16	4096	512
8844	<8	64	2048	32	4096	>1024
5807	<8	16	512	8	2048	256
3654	<8	32	512	16	512	256
6203	<8	8	2048	8	1024	512

Most of the group 1 horses antibody levels were quite low at the one year after booster mark, with some antibody levels ***"at or below the limit of detection (ie 8), as seen in table 1."*** **It was further said** ***"It is unknown whether horses with undetectable titres can withstand challenge with live Hendra virus".*** (Zoetis news release, Dec 2013)

The group 2 horses that had been first administered a 6 month booster at day 220 dose, prior to the 12 month antibody estimation had better antibody levels. Therefore they decided to challenge group 2 horses in early 2014 and conduct a challenge for a different group of horses that followed the same vaccination protocol as group 1 for late 2014. See the above article.

I note that in the group 2 data pre -6 month booster, the horses had low antibody levels pre immunization - four of the seven horses in that group (57%) had levels of 8 (as previously quoted - at the limit of detection) and the highest was at 32. I note that in the 6 month study discussed earlier in this submission one horse with the antibody levels of 16, 32 had evidence of nasal genome in nasal swabs as discussed in the 6th month challenge. In the group 1 horses looking at antibodies at 12 months post booster again 57% or 4/7 horses had antibodies at or below the limit of detection (≤ 8), and the other levels in the remaining horses were not high either. It is unknown whether horses with very low or undetectable levels of antibodies to Hendra virus can withstand infection and disease with the Hendra virus.

In human medicine many diseases that are vaccinated for such as rubella and hepatitis B, can be checked with a blood test to see if the patient has immune titres or levels of the specific antibodies post vaccination. If the disease specific antibody titre is too low, it is reported as non-immune and the patient can be given a booster vaccination. For example in the pathology testing of a vaccinated person to detect the level of protective antibody titres post vaccination, under 10 IU/L in hepatitis B is reported as non-immune and 10-30 if at higher risk of exposure recommendation is to give a booster; in rubella under 30 IU/L is the cut off in the local pathology lab prior to suggesting a booster. These absolute numbers will vary with different measurement methods though. I don't believe that in horses vaccinated for Hendra virus, at this point in time, a specific antibody level has been reliably established that is likely to be protective. Also in some diseases, patients even with antibody levels considered to be protective may still become infected, but that disease may well be reduced or modified. Some patients are poor sero-converters and immunity can wane with time. However even in situations with lower antibody levels it is possible that the stimulation of immunological memory may be a factor in reducing the course of the disease. The host's natural immunity systems will also play a part in the immune defense response. Nevertheless one couldn't assume an individual with a barely or undetectable antibody level will be protected. Also for some diseases the vaccine is not as immunogenic and needs to be revaccinated more frequently to maintain immunity.

I expect a horse with a barely or not detectable HeVsG antibody level of ≤ 8 would have to be considered as not immune from a safety perspective, which is more than half of the horses in the 6 month before booster horse sample in group 2, and more than half of group 1 horses, at 12 months with that proposed protocol.

The significance of this is in real world conditions is that there will be a big variation of likely antibody titres for horses that are vaccinated. This relates to the individual response to the immunization as well as the time from vaccination. For example some horses may be poorer sero-converters that may not generate a good antibody response to the vaccine antigen. This could be more common in horses that are elderly, some medical conditions that may be immune suppressing, nutritionally deficient horses, and there is individual variation.

This has implications in that there is a perception that once a HeV vaccination regime has been started that the horse is going to be immune, which may not always be the case.

I have not found any studies in which a vaccinated animal has been challenged with Hendra (or Nipah) virus with a pre-challenge antibody titre of ≤ 8. Less than 8 is at the same level as unvaccinated horses, see tables at

day 0. The manufacturers were obviously nervous about viral challenging horses with just detectable or not detectable titres of HeVsG antibody, as it may well prove to be too low to provide adequate immunity.

The second trial that was proposed for late 2014 was to follow the group 1 protocol for the 12 month challenge. I hope that the test horses in the new group were not just horses with robust antibody levels, as this group 1 with poorer HeVsG antibody levels were not going to be used for that trial. These trials should have been as representative of the likely antibody level variability in the vaccinated horse population with the vaccination protocol being evaluated as possible, not just doing tests on animals that are likely to succeed. It would also be informative if not already done, to evaluate antibody titres to check maintenance of immunity over time in a large sample, just a blood test required, many thousands have been immunized – it should be possible. I have heard that a specific antibody titre blood test is available from CSIRO for $350. If Zoetis plans to use the data perhaps they could in part subsidize the tests??

Where are the results of the 12 month vaccine challenge studies?

It is now 2016 and I cannot find the results of these 2 studies announced for 2014. I called Zoetis and was not given any useful information as to if they had even been done. On the web, I found reference to the application for "Variation of the label approval to amend the dosing schedule for Equivac HeV to allow ongoing annual booster doses, following the primary vaccination.", this was on the APVMA site archive Application Number 105049, and publicly available. http://archive.apvma.gov.au/application_summaries/105049.pdf . It appears from looking at the description of data titles there has been an interesting timeline with a title likely to be a report after the first DOI 12 month trial on dated 5/6/14, but nothing seems to be publicly available except what I had already seen. I note the entry with data no. 114466, and applicants ref number PAASD10 date 29th Nov 2014, a title **"Equivac HeV Hendra Virus Vaccine for horses PER 14887 and PER 14876. NOTICE- Proposal to refuse application for permit#14887 &Notice- Proposed cancellation of permit PER 14876"**. There were 2 other entries from Zia Hashimi (Zoetis), "Response to Notice of Proposal to Refuse Application No 14887" dated 2014 (Data number 114468); also note "Response to Notice of proposed Cancellation of Permit No. PER 14876" dated 2014. (Data number 114469). It would be interesting to find what that was about and read some of these documents! Might be relevant?? Someone may be able to access this information under a Freedom Of Information request, (RTI- right to information), for this Parliamentary Inquiry all these documents should be made publicly available and assessed.

Full Registration

Even though there has been a paucity of new publicly available information on the research of the Hendra virus vaccine in horses in the last few years, the product has been given full registration. I note that the wording on the APVMA site however has changed from time of the first permit.

The APVMA website states: "*The approved instructions of the product indicate that a booster dose is required every 6 months.* ***This is because a duration of immunity has not been demonstrated for more than 6 months***". (my emphasis). It would be interesting to find out the results of the Duration of Immunity trials that were to be conducted in 2014, I note however, the application for annual boosters for the vaccine was rejected. *"In June, Zoetis' Richard L'Estrange told ABC Rural that the company had requested that the APVMA grant the drug a 12-month duration of immunity, but the move was rejected"*.http://www.abc.net.au/news/2015-08-05/apvma-aprroves-hendra-vaccine/6673542 I looked on the APVMA archive application summary 105049, above, there was still activity on 26/11/2015 on the application to allow ongoing yearly boosters.

Other wording from the APVMA website (Australian Pesticides and Veterinary Medicines Authority) now includes, ***"The potential for a vaccinated horse to pass on the Hendra virus cannot be ruled out. As a precaution, it is recommended people take the same steps to protect vaccinated horses from exposure to infection—and to prevent humans being infected by horses—as are recommended for unvaccinated horses. Personal protective equipment should be worn whenever infection is suspected even in vaccinated horses."***

There have been many vaccinations administered to date. However, the problem with assessing vaccine efficacy in such a rare pathogen such as Hendra Virus, it is more difficult to draw any really reliable conclusions about efficacy of vaccination against wild virus in the field. This underlies the importance of well-designed and scrutinized experimental work.

I hope the regulatory bodies are requesting and analysing all of the raw patient level data from the trials and not just the written submissions and reports by the applicant (Zoetis) to the APVMA. I would like to see more transparency with the approval processes with public access to more of the ongoing research and deliberation not just the rulings.

In Conclusion

The reason that I have written this is not to present a for or against position on the HeVsG vaccine just to highlight that the vaccine although useful does have limitations.

For example: In the 6 month horse vaccination Hendra virus challenge trial 1 of 3 horses (33% of vaccinated horses) Hendra virus genome was found in nasal swabs in 4 days which included the day before euthanasia (antibody titre pre challenge to HeVsG 16,32). In the first ferret study vaccinated with HeVsG and challenged with HeV (Hendra Virus) even with high level HeVsG antibody titres pre challenge of 8192, viral genome was found on 3 days post viral challenge.

As the numbers of horses and trials for Hendra viral challenge in the HeVsG vaccinated horse have been so small, it has been necessary to review research in other HeVsG vaccinated animal models and for challenge with Nipah virus as well as Hendra virus. Of course one cannot make absolute comparisons to the horse animal model. Note there is high level homology between the Hendra Virus RNA and Nipah virus, and there is a proposal to use the one vaccine to vaccinate for both Nipah and Hendra viruses.

In the 12 month ferret study (Nipah virus) viral genome was found in 2 of 5 vaccinated ferrets, one ferret in nasal swabs (titre 64, 128), and one in bronchial lymph nodes post mortem (titre 16,16). In a cat study (Nipah virus) evidence of viral replication was found in vaccinated cats with increasing antibody titres post challenge indicating viral replication and positive viral genome detection in all of the oral swabs, some of the urine samples, and the Hendra virus genome was detected in brains of 4 animals receiving higher doses of the vaccine at levels higher than the control animals. (J Pallister, 2011; J McEachern et al, 2008)

Also not all horses that are vaccinated are likely to be immune in real world conditions as discussed earlier, (Refer my discussion looking at the 2 groups of data for duration of immunity trials). There will be variability in generated disease specific antibody levels even within the suggested vaccination protocol and some horses will have low or very low titres of HeV specific antibody. Even with high titres of protective antibody in other species in vaccine challenge trials evidence of viral genome has also been observed, although this appears to be less common at the higher Hendra specific antibody levels. (Horse HeV 6/12 trial, Ferret HeV, NiV and Cat NiV studies references above). **I would like to highlight the importance of not ruling out the possibility of Hendra Virus in the vaccinated horse as well as the unvaccinated horse.**

The vaccine appeared to protect experimental animals from developing clinical disease (symptoms), and in many cases but not all, of infection with Hendra virus or Nipah virus within the time frames of these studies. However, there are also instances of *unvaccinated* horses having low level symptoms when infected with HeV

such as those having recovered from the Hendra virus, and in asymptomatic horses that have been in contact with a HeV tested positive ill horse, that have gone on to be euthanized after a positive Hendra virus pathological test. Also the case of the HeV positive dog was also asymptomatic. (Hendra Virus Outbreak with Novel Clinical Features, Australia, Field et al, 2010); (Hendra Virus Infection in Dog, Australia, 2013, Kirkland et al, 2015)

Care needs to be taken in both vaccinated and unvaccinated animals alike. However one should remember that transmission to the 7 humans infected with Hendra virus has occurred in people doing high risk procedures (such as endoscopies, nasal lavage or autopsies) or having close contract with dead or dying horses. (The Hendra Virus Report, Queensland Ombudsman, 2011). In these cases there was inadequate personal protective equipment (PPE) used, if any, and were likely to be exposed to much higher levels of virus than in an asymptomatic animal positive for Hendra Virus. At the point when the animal is clinically ill and before doing high risk procedures in any animal vaccinated or not, care should obviously be taken.

Terms of Reference 2: The Incidence and Impact of Adverse Reactions of horses following vaccination and reporting and economic impacts of the HeV vaccine.

Is it safe?

In the decision process to use a vaccine, the owner has to decide whether the benefits of vaccinating outweigh the risks of adverse reactions. Every medication and vaccination has the potential to have adverse side effects.

Vaccine Safety from the Research

Field Trials

There is not much publicly available research documented about the possible side effect profile of the Hendra Virus vaccine from the scientific studies. There is mention of a 34.5% reaction of swelling at the site of the injection with the second injection (3-6 week booster), from the first permit, which resolved within 7 days. I gathered this was based on a 3 month field trial with 29 horses. (D. Middleton, 2014). This number I expect would have included unvaccinated controls. I wonder what other safety parameters apart from the obvious (standing and eating at the end of 3/12?), were measured. I note they mention the horses didn't get colic and only one horse was pyrexic (high temperature), as had one control in this small sample. Were there blood tests for allergy markers, inflammatory markers, full blood counts and kidney and liver function tests as well as HeVsG serology performed? Just how in depth was the safety evaluation pre issue of the first permit? Also, in that initial field trial had the horses had their third vaccination or just the first and a booster? Many of the adverse reactions seem to be more commonly found with subsequent vaccinations. I note in the application for annual boosters from Zoetis to the APVMA, http://archive.apvma.gov.au/application_summaries/105049.pdf , there are titles in submissions from Zoetis that include safety and efficacy, but these are not publicly available to assess what parameters were considered and their results. I wonder also whether this initial 3 month trial was actually adequate not just in what was examined but also a big enough sample size to be able to issue the first permit and allow extensive use of the vaccine, and say it is safe.

Ferrets?

There seemed to be a high attrition rate of HeVsG vaccinated ferrets used in the vaccination challenge studies. In the first study of HeVsG vaccinated ferrets that were challenged with Hendra virus, one ferret was euthanized after viral challenge before the end of the study because it was ill. This was not thought to be attributable to HeV infection. This ferret became clinically unwell 30 days post booster vaccination and was euthanized the following day. This ferret was found to have pale, enlarged kidneys and the stomach contained haemorrhagic fluid and acute tubular necrosis was identified on histology. (J. Pallister, A Recombinant Hendra virus G Glycoprotein-Based Subunit Vaccine Protects Ferrets from Lethal Viral Challenge, 2011). Another study of HeVsG vaccinated ferrets that were challenged with Nipah Virus included 2 arms or experiments. (Vaccination of ferrets with a recombinant G glycoprotein subunit vaccine provides protection against Nipah virus disease for over 12 months, J Pallister et al, 2013). In the first experiment, viral challenge was to occur 20 days post vaccination booster. 2 of the 6 vaccinated ferrets were euthanized after HeVsG vaccination and prior to exposure to the virus and during that 20 day period after the vaccination. It was commented that they were "euthanized for reasons unassociated with the scientific study". The study was to test efficacy or effectiveness of the vaccine in protecting the ferrets from viral challenge not to assess safety of the HeVsG vaccine in ferrets. There was no mention of any assessment in these 2 euthanized ferrets. In the second arm of this study which was to assess 12 month efficacy, one of the 6 vaccinated ferrets also died before exposure to the virus, but it doesn't mention how long after vaccination. That is, 4 of 18 total HeVsG vaccinated ferrets were prematurely euthanized in the period after vaccination, and in 3 of the 4 cases before exposure to the virus. I wondered if the ferrets became ill after the vaccination. The life expectancy of a ferret is 7-10 years (Wikipedia) and I expect for such important studies healthy young ferrets would be selected. Just an observation I noted, there are not many details and it may or may not be relevant.

APVMA Adverse Reactions Tables

The APVMA website lists adverse reactions to the vaccine to mid June 2015. There are many different reported possible and probable adverse reactions listed http://apvma.gov.au/node/15786 .

Swelling at the site of injection is known to be one of the commonest reactions to the vaccine administration – it is the most often sited reaction that vets will advise about. It was reported by first permit to occur in 34.5% of horses after administration of the second vaccine that was given 3 weeks after the primary vaccine. As condition of the minor use permit, vets were obliged to report any adverse reactions.

I went over all the entries looking for the *incidence of injection site swelling* that were categorized as possible or probable from the APVMA website as above. They have tabulated reaction incidence % based on 369,759 doses administered until June 2015. Looking at the table and the preceding table of absolute horse numbers with the adverse reaction I found: 1 horse had a probable *injection site swelling* in history to June 2015 reported, this was also reported as <0.001%, actually is 0.00027%. Other possible categories that this reaction may fit into included *injection site reaction* 0.18% (but this may also include infection or abscess formation at the site of injection?), *site reaction* -3 horses in history, or <0.001 % I calculated actually 0.0008 % (**not sure how *site reaction* is different to *injection site reaction*- did anyone at APVMA check this table!**), also swelling local 0.03%, and lump local 0.02% (this may also indicate a persisting lump perhaps). Even if we lump all of these reactions together that would include swelling at the injection site, transient or persistent, that may be an immunological reaction, infection at the injection site, abscess formation that may require vet attention and a persistent lump the total would be 0.231%. That is 2.3 horses per thousand horses injected. Does this mean that a vet has only seen or heard from owners about some kind of injection site swelling or infection or

any other reaction at the injection site serious, or transient swelling only twice after injecting 1000 horses with equivac HeV? The next most commonly reported adverse reaction was oedema. I didn't include this in my cumulative possible estimation for injection site swelling because oedema could be local, peripheral or generalized. Oedema is reported at 0.11%. Is there overlap in the categories? Can one adverse reaction be listed in two (or more?) categories such as injection site reaction and oedema, for example? So as you can see, trying to figure out something as simple as injection site swelling is not really informative from the tabulated adverse reactions in the APVMA adverse experience report tables.

From these tables, even adding the % incidence of adverse reactions from several different categories (that could possibly include injection site swelling amongst other reactions), together into one figure, I see the incidence of this adverse reaction is over 100 times less common in the APVMA reported tables, than previously reported from early research. (34.5% injection site swelling at second vaccination- Permit 13510) We know that injection site swelling is much more common in subsequent vaccinations. Many vets say injection site swelling is the "norm" and do not see it as an adverse reaction, and therefore don't report it. I expect many vets will only report what they consider to be a more serious reaction.

How can you calculate reliable statistics that will be used and quoted on the adverse reactions with undefined adverse reaction categories, and under reporting of adverse reactions? Under reporting of injection site swelling is an obvious example of the gap between reporting and reality.

The under-reporting of adverse reactions to human medicines is also very common in human medicine, however reporting of post market experience is very important. Sometimes a medication may have its indications restricted, or withdrawn completely from the market as a result of adverse reactions found from post market surveillance after the medication has been released to the market. Of course a human medication or vaccine is subject to much more expensive and extensive research than an animal medication to get to market. Conversely, Equivac HeV was launched under a minor use permit for this reason, until enough data from research became available that would satisfy the regulators that it fulfilled requirements for full registration. As a part of the APVMA minor permit conditions of the first permit, vets administering the vaccine were required to report any adverse reactions "*12. Adverse reactions, including lack of efficacy, resulting from the use of the vaccine, must be reported to Pfizer Animal Health as soon as possible."*(Permit 13510**).**

This post market surveillance has contributed to the basis for the full registration of Equivac HeV and perceived safety profile for the vaccine, so the reporting of adverse reactions were imperative to critical appraisal.

Under-reporting of Adverse Events

A systematic review of under-reporting of adverse drug reactions to regulatory authorities was made for human medications and vaccines, over 37 studies in 12 countries with large numbers of patients, by the Drug Safety Research Unit, Southhampton, UK. (Under- Reporting of Adverse Drug Reactions, A Systematic Review, L Hazell and S Shakir, in publication Drug Safety,2006). **They found the median under-reporting rate across 37 studies in 12 countries was 94%. That means a median of only 6% of adverse drug reactions were reported.** The median under-reporting rate for serious adverse drug reactions across 19 studies that examined serious reactions was 85%. Interestingly, in another study with a serious drug reaction- Idiopathic thrombocytopenic purpura (ITP) with the MMR vaccine was found to be 82% under-reported. (Under- Reporting of Adverse Drug Reactions, A Systematic Review, L Hazell and S Shakir, 2006).

There are many possible reasons for under-reporting. In the case of vets and human doctors, often the practitioner has to make the decision that there is a vaccine reaction, and **may not see the link between the vaccination and the adverse reaction**. One vet said to me the reports of reactions seen are often anecdotal. The problem is if the vet does not report what he or she believes is probably not a vaccine reaction, there may

be a lack of evidence to support inquiry into an adverse reaction cluster, and that reaction may not otherwise be picked up. In contrast to human medicine, especially with "Medicare" and free or subsidized pathology service, many vague ill health symptoms, or even serious disease in a horse may go by not investigated or elucidated.

Another important aspect that can contribute to under reporting is **workload**. The extra paperwork at the end of a long busy day doesn't always get addressed; days can become weeks, then forgotten. I read in the PER 14876 issued Aug 2014 to Aug 2015, that there was a requirement for veterinary surgeons to "report to Zoetis any adverse reactions including lack of efficacy, resulting from the use of The Product *within 48 hours* of either observing or being notified of an adverse reaction." The time permitted to report an adverse reaction was "as soon as possible" in the first permit not 2 days. It is no wonder that the reporting incidence of adverse reactions to Equivac HeV in 2015 dropped to under half that of 2014, and almost half for 2013, even when you take into account that 2015 reporting on the APVMA site was for 6 months. 2 days can go past in no time and why would a vet report an adverse reaction late and risk reprimand. Vets are becoming increasingly nervous of the increasingly unpredictable authority meddling, with fines and their concerns for their very registration to practice, when they are just doing their jobs.

The other issue is, has Zoetis and formerly Pfizer Animal Health been passing on all the adverse reaction material? There is **risk of conflict of interest** with Zoetis managing all the adverse reaction information and investigations. Has this flow of information been *completely* transparent and satisfactory? Perhaps even the vets themselves in some cases are subject to reporting bias about the possibility of vaccine adverse reactions after reading the educational resources from the pharmaceutical company and elsewhere and **under-report where there is no described association to vaccination.** Their own fears of contracting the Hendra virus, and individual attitudes to the vaccine may also potentially play a role in incidence of reporting.

I hope this inquiry presents a voice to those owners, in the cases where the vets have decided that the clinical condition that they perceive was not causally related to the Equivac HeV vaccine, and their cases were not reported. It may also facilitate exploration of possible disease clusters that may be more common than in the average horse population, disease that may have a temporal relationship to the vaccine administration.

Notes on a couple of Adverse Reactions:

Death

There have been 77 possible adverse reactions listed for the Hendra virus vaccine on the APVMA website. I have made some notes on - horse deaths, laminitis, and an example of an increase in severity of an adverse reaction with repeated immunizations.

There have been **7 possible, but 0 probable horse deaths** reported on the APVMA site. It doesn't say what the cause of death of these horses were, but it would be useful to know.

I note from media/social media reports that the condition of ITP (idiopathic thrombocytopenic purpura) as a diagnosis in the cause of death that was mentioned in 2 vaccinated horses. I don't know if the APVMA found these horses' deaths to be possibly related to the vaccine. However I write about ITP as an example of a possible serious adverse vaccine reaction. ITP is a recognised possible vaccine related adverse reaction in humans with an immunological basis. Here is an excerpt from an article "*Do childhood vaccines cause thrombocytopenia?"* (L Sauve et al Paediatric Child Health, 2009.), *"Thrombocytopenia is a rare but important adverse event following vaccination; its clinical presentation and management are similar to that of ITP. Clinicians should seek a history of recent vaccination in children presenting with apparent ITP. VATP (Vaccine acquired thrombocytopenia) is usually self-limiting, but there is a small risk of severe bleeding-related complications."* (ITP is idiopathic = unknown cause of low platelets causing a tendency to bleed).

In a large study in the USA of 1.8 million children, funded by the FDA (USA), **exploring ITP as an adverse vaccine reaction to vaccination, statically significant associations were made with several vaccines** in different age groups, not just live attenuated types of vaccine. Known rare complications of ITP include intracranial haemorrhage and severe bleeding. I note that for part of the study, the exposed period from vaccination was 42 days, but other parts of the study considered cases without the definite time frame. In this study possible confounds such as chronic conditions known to cause ITP, amongst other possible confounds were eliminated for their statistical analysis, which reduced cases from 698 potential cases to 197, to explore the link between vaccination and the incidence of ITP in a more meaningful way. After this elimination there were found to be 197 chart confirmed cases of ITP in 1.8 million children. The incidence of possible vaccine associated ITP was low but important to be aware of, serious sequellae in these cases analysed were found to be uncommon, and the disease was usually self-limiting. (The Risk of Immune Thrombocytopenic Purpura After Vaccination in Children and Adolescents, S O'Leary et al. 2012). **When I translate this to our horses with suspected ITP as a result of vaccination, I see the problems for the investigators to list this as probable reaction, as in life there are so many variables, but nevertheless it is plausible that ITP could well have been a possible adverse reaction to Equivac HeV.**

There have been further reports of horse deaths apart from the 7 stated, but these may have been categorized as having an unknown causal link or unlikely. There may be others where no relationship was suspected or just not reported or investigated. Someone has to pay for the autopsy of a dead horse and many owners may elect not to. **The likelihood of possible death from the Equivac HeV vaccine was reported as 0.002% of vaccinations given, that is 2 horses per 100 000 immunized.** (Look at APVMA website for table and definitions of possible and probable, probable indicates no other alternative explanation amongst other criteria). I think this figure for the reasons outlined earlier in the submission could be an underestimate, but nevertheless death is recorded as a rare *possible* adverse reaction.

The Hendra virus infection though is also a rare disease. Australia was thought to have a horse population of about *1 million domestic horses* from Australian Horse Council figures (Unwanted: The Disposable Horse, J Duckworth, 2010). I am not sure what proportion of domestic horses inhabits Queensland and NSW, where there is risk of Hendra virus transmission from bats. In both 2012 and 2013, there were 10 and 8 Hendra cases in Australia. If I guessed that there are roughly 400,000 domesticated horses in NSW and Queensland together, the risk of a horse in this region developing Hendra Virus disease was 0.0025% and 0.002% respectively, in each of those 2 years. I note from human population data that QLD and NSW comprise 52% of the human population of Australia. (Ranked list of States and Territories of Australia, 2015, Wikipedia). There were 5 cases of Hendra virus disease in horses in 2014 or 0.0012% of the horse population based on my assumption of 400 000 horses in Qld and NSW. Last year there was only 1 case in Queensland, 3 in total in Australia, which with my calculation for Q and NSW is 0.00075%. Conversely in 2011, the year prior to vaccine launch, there were the most cases in history in one year. There were 23 horses in Australia infected, (by my calculation for QLD and NSW), that is 0.0057% at the highest incidence in history, and in 2010 there was only 1 case in all of Australia. The percentages I calculated rely on my guestimate of the number of horses in QLD and NSW. If I used Australia's total horse population, the incidence % would be much lower. There has been considerable annual variability in the incidence of Hendra Virus infection since it was first discovered in 1994. (Annual Hendra Virus Disease case numbers from Wikipedia, List of Australian Hendra Outbreaks, also Croser et al, 2013.)

Death as an adverse reaction to the Equivac HeV vaccine is extreme, but there are many other adverse reactions from common and mild to more serious and rare to consider. An example of another adverse reaction is colic, which was listed at 0.01 % (that is 1 in 10,000 horses) of the reported possible or probable adverse reactions to the vaccine. I have read that post vaccination colic preceded the death in another horse, however there is not much that is publically accessible to substantiate what the diagnoses were with those 7 horses whose deaths were possibly linked to the vaccine.

As Hendra virus infection is a rare disease, the risk of also rare serious vaccination adverse events becomes more important than if the disease was common, in considering the balance of benefits of vaccination to the risks of adverse reactions from that vaccination in the decision making process for one's horse.

A problem with the statistics for horse deaths from Hendra virus is that once a horse has been found to be positive for Hendra Virus, it is euthanized regardless of health status. Due to this policy, **all horse infections have been counted as horse deaths**. I have seen videos of asymptomatic healthy horses at early seminars on Hendra Virus that we were told were positive for Hendra and later euthanized. I read that one horse in the Hendra outbreak at Redlands Vet clinic outbreak recovered, but was euthanized anyway (Hendra Virus Outbreak with Novel Clinical Features, Australia, Field et al, 2010). There are several other examples in the Ombudsman report on Hendra Virus. *"Approximately 75% of Hendra virus cases in horses are fatal. Often, horses that survive show very mild signs of the virus for a few days and then appear to recover. There is a national policy that any horses that recover from Hendra virus are destroyed, because of a concern about recrudescence."* (The Hendra Virus Report, Queensland Ombudsman, 2011). So 25 % of Hendra horse cases may recover from the disease. I am unsure as to the extent of research to come to these figures though.

A recent review, however, states that **persistent infection in horses has not been identified**.(Hendra and Nipah Viruses: pathogenesis, animal models and recent breakthroughs in vaccination, H. Weingarti, 2015). Hopefully now the decision for mandatory euthanasia of HeV PCR positive horses can be reassessed.

Laminitis

Laminitis is another condition that some owners, and even farriers I have talked to, suspect could be occasionally linked to the vaccination. Laminitis as a possible or probable adverse reaction to the Equivac HeV vaccine has a reported incidence of 0.002% in the APVMA records. As many vets or owners do not see the possible relationship, it may be under-reported. Also, as the aetiology of laminitis is likely to be multifactorial, with diet, trimming, obesity and many other factors influencing an individual case, it would be very difficult to investigate whether there actually was a link in many cases to the Equivac HeV vaccination.

The following study explored the link between chronic laminitis horses and hyperreactivity after antigenic stimulation, (vaccination is an example of antigenic stimulation), and also comments to be aware of the possibility of the presentation of acute case of laminitis following vaccination. This study "Evaluation of systemic Immunologic hyperreactivity after intradermal testing in horses with chronic laminitis", (Wagner et al, American Journal of Veterinary Research, 2003) demonstrated results that supported *"**the hypothesis that CL*** **(chronic laminitic)** ***horses develop hyperreactivity to various antigenic stimuli**, compared with non laminitic horses. Therefore, the possibility of clinical signs of laminitis should be discussed with horse owners. Chronic laminitis should also be a consideration when a horse becomes lame following antigenic challenges."* And ***"when clinical signs of laminitis appear suddenly after administration of routine vaccinations radiographic evaluations of the feet should be completed to validate any pre-existing laminitic condition"***(Wagner et al, 2003).

Since so many of our domesticated horses suffer with subclinical laminitis (subclinical= not lame, not showing overt symptoms), these points could be relevant, perhaps some cases of lameness and laminitis that have not even been considered as a vaccine reaction may be in part related, as above, and should be considered. Subclinical laminitis is common: "*Sub clinical laminitis, is what we as natural hoof care practitioners see every day we trim horses. A horse with a stretched white line, seedy toe, reoccurring abscessing, annoying thrush that won't go away, dropped flat sole, small reoccurring pit in the centre of the sole at the toe, chronic cracks and stringy, tattered white line….*" (Anne Riddell, 2006, American Hoof Association)
http://www.americanhoofassociation.org/article-nonstructural.html

An example of a case of Hyperreactivity with subsequent boosters of Equivac HeV

"It is not unusual for booster shots for any vaccine to result in more severe reactions… In some individuals a particular type of antibody is produced in excess, which produces a marked response by immune cells the next time the body is exposed to the same antigen—such as a booster vaccine. In these individuals, the normal course of vaccination may lead to an exaggerated immune response to vaccination. This is known as a hypersensitivity reaction and each subsequent exposure can result in a similar or more severe reaction in the individual." (APVMA, website). http://apvma.gov.au/node/12881

The same site also warns: *the product should not be used in sick or immunocompromised horses*

Many owners, but not all, seem to be finding more intense adverse reactions occur with subsequent boosters of Equivac HeV. Some have stopped vaccinating due to these sorts of issues. Even large stables claim to have stopped vaccinating due to worries about increased risk of adverse side effects of the vaccine.(Such as Heath Ryans stable in the media)

I have a friend Lee C. who has one horse called "Little Girl". Her experience in part demonstrates this issue. Lee was one of the most ardent supporters of the Hendra vaccine and commenced the usual Equivac HeV schedule in 2013, with follow up 6 monthly boosters. She is happy to provide further personal details if required for the inquiry.

At the time of her 4th vaccination, Lee moved to a new area and found a new vet for Little Girl and Lee's dogs. Little Girl at her 4th vaccine booster developed a worse than usual swelling at the injection site, high temperature and temporary malaise. She was told it was a normal reaction but this worried Lee as the reaction was much worse than previously experienced. The day after the vet administered the 5th booster of Equivac HeV, Lee found her horse standing with her head lowered, panting, with her tongue hanging out, covered in sweat, febrile, and with a massive swelling on her neck. She said this neck swelling was deep and over 12 inches in diameter, and she was stiff in the neck. In Lee's opinion she was very ill. Lee rang the vet and was referred to the another vet clinic that was covering for Lee's vet, as he was away, who recommended hosing the horse and icing the horses neck and body to cool Little Girl down, and re-evaluating temp, counting her respiratory rate periodically, and administering Bute that she happened to have at home. The vet kept in communication throughout the day with Lee over the phone, as the vet was unable to attend straight away. After several hours the horse improved and the respiratory rate settled. She kept up her care of little girl for several days as she remained sick and pyrexic but not to the same degree. It was summer and warm weather may have exacerbated the adverse reaction to the vaccine. She believed the swelling on the horse's neck lasted about 1 -2 weeks. The extent of the post immunization symptoms frightened Lee, she still fully believes if she wasn't home to help Little Girl she would have died.

The 5th booster Equivac HeV may have caused a hyperimmunization state with higher than normal numbers of antibodies in Little Girl. This creates a state of immunity that is greater than normal, and immune system overactivity has been associated with a more pronounced or hypersensitivity reaction to the vaccine. Lee's horse Little Girl was also potentially at risk as she has a documented past history of chronic laminitis since 2013, which took one year of specialist veterinary and farrier involvement, and of course a strict diet and care regime before it settled. Even though the laminitis did not appear to be active and her hooves looked fine at the time of the 5th vaccine she may have been more hyperreactive/hypersensitive. (See comments and reference in section on hypersensitivity in chronic laminitis horses).

She said she has had one horse die from snake bite (the dead snake was found next to the dead horse), and she thought there was more chance of Little Girl dying from a vaccine reaction with the next vaccination than the Hendra Virus. **No adverse reaction was reported by a vet.**

Her vet when back from leave, said for with his own herd of 11 horses he would pre-medicate all of his horses with Bute for 3 days prior to vaccination with Equivac HeV to reduce these sorts of side effects of the vaccine he also would continue the Bute for a couple of days or until the symptoms settled. He recommended this approach if Lee wanted to vaccinate again, but she has decided not to. She later rang Zoetis to find out information about dates of her immunizations and the person she talked to also recommended Bute and continuing vaccination as one option. She refuses to further vaccinate because of safety concerns.

Bute (phenylbutazone) is an NSAID an analgesic and anti-inflammatory agent used in horses. (NSAID= non-steroidal anti-inflammatory drug). It may contribute to reducing or treating an inflammatory response but may potentially result in a lower sero-conversion level which by vaccination one is trying to promote to boost antibodies that may be protective against Hendra virus. It is a non-specific COX 1 and COX 2 inhibitor, (Beretta, 2005). http://www.ncbi.nlm.nih.gov/pubmed/15939622 . Several studies have found that Cox 2 is crucial for optimal antibody production. These studies have found that the use of NSAIDs may significantly impair antibody production to vaccination and certain infections. For example, in one study using HPV 16 vaccinated mice challenged with antigen, Cox 2 deficient mice produced 10 fold less neutralizing antibody compared with wild type mice in response to antigen stimulation. In another experiment related in the same article using vaccinated human sera found similar reductions in immune response after COX 2 inhibition. (Non-steroidal anti-inflammatory drugs reduce optimal antibody production in response to vaccination and vaccinia virus infection, Phipps et al, 2011; Cyclooxygenase-2 inhibition Attenuates Antibody Responses against Human Papillomavirus- Like Particles, Ryan et al, 2006; Chronic Inhibition of Cyclooxygenase-2 Attenuates Antibody Responses against Vaccinia Infection , Bernard et al, 2010) In a horse study investigating the concurrent administration of an NSAID with the equine influenza vaccine commented- ***The study authors emphasized that veterinarians should take care when administering NSAIDs with vaccines, as they can reduce a vaccine's efficacy at stimulating antibody and CMI*** **(Cell Mediated Immunity*) responses**. *As a result, horses with reduced immune responses might need to be vaccinated more frequently.* http://www.thehorse.com/articles/33253/nsaids-might-impair-horses-response-to-influenza-vaccines . In human medicine there has been a move away from giving analgesia prior or concurrently with a vaccination, even for paracetamol which is not an NSAID, however analgesics are still used to treat adverse reactions post vaccination. *October 16, 2009 — Prophylactic administration of acetaminophen (paracetamol) to reduce fever or febrile convulsions after vaccination in infants actually results in reduced immunogenicity and should not be routinely recommended, according to a new study published in the October 17 issue of The Lancet.* http://www.medscape.com/viewarticle/710788

Although Bute may help alleviate Lee's horse's inflammatory hyperreactivity to the vaccine, it is another drug with other side effects and can potentially reduce the effectiveness of the vaccine. Should vets and Zoetis recommend using an NSAID as a matter of routine in a horse to pre-medicate prior to vaccination, and to recommend continuing to vaccinate a horse that is potentially or perceived to be high risk for adverse reactions with further boosters?

Lee did not continue vaccinate Little Girl with Equivac HeV. She continued using her vet who she liked, as per usual for spaying 2 dogs, for dog immunizations, and for her horse. She received emails from Zoetis just before 12 months after the last booster reminding her to vaccinate, but she had made the decision not to continue.

When Little Girl was found only 1 month after this 12 month reminder after the 5th booster, with a swollen tightly closed eye, with copious purulent discharge in her eye and streaming down her cheek, and in a lot of pain, not just a conjunctivitis that every horse owner has seen from time to time, she contacted her vet, who refused to see her horse as her Hendra vaccination had lapsed. I believe this presentation virtually equalled an ophthalmic emergency to save the horse's eye. I discuss this further in terms of reference 5 "Veterinarians applying a policy not to treat unvaccinated horses".

In Conclusion

As every medication and vaccine has the potential to have adverse side effects, I can't see the benefits in vaccinating if the horse is not in a Hendra risk area. If one lives in a Hendra risk area, a choice needs to be made considering the potential benefits of vaccination to risk of adverse vaccine events. Hendra virus infection is serious, but still remains a rare disease. As the Hendra vaccine has not yet been demonstrated to provide immunity in horses for more than 6 months (APVMA), boosters are still required every 6 months. The APVMA has acknowledged the risk of more severe adverse reactions from hypersensitization that occurs in some horses with subsequent booster immunizations. Some horses may suffer an adverse reaction even on the first injection, but this seems to be less common. It seems that to achieve a higher protective level of HeV antibodies at this stage, a 6 month immunization schedule is required, but in continuing this protocol some horses may be hyperimmunized and or hypersensitive and suffer adverse consequences from immune overactivity. In my opinion, whether to vaccinate with the Hendra Virus vaccine or not should remain a personal choice, there are risks and benefits in both vaccinating and not vaccinating.

Terms of Reference 3: Who bears the risks of HeV infection and who incurs the costs and receives the benefits of each risk mitigation option?

Who is at risk and what is the risk?

To evaluate this further I have reproduced a table with the historical incidence of Hendra cases, horse and human.

Table 1
Summary of Hendra virus disease events in Australia.

Year	No of disease events	Horse cases	Human cases	Human deaths
1994	2	22	3	2
1999	1	1	–	–
2004	2	2	1	–
2006	2	2	–	–
2007	2	2	–	–
2008	2	8	2	1
2009	2	6	1	1
2010	1	1	–	–
2011	18	23	–	–
2012	8	10	–	–
2013	8	8	–	–
2014	5	5	_	_
2015	3	3	_	_

Data and table from *The changing face of Heniparviruses* E Croser, 2013, modified to be updated to 2015

Incidence of Hendra Virus Infection in the Horse

There have been 93 reported cases of Hendra Virus infection in horses since its discovery in 1994, in 21 years. This table above and the total figure of 93 cases *include* 20 unresolved or untested horses, thought to have had Hendra virus infection.

There have been 73 confirmed cases of Hendra virus in horses in history since 1994 to the present. This information can be found in a table on a Queensland Government website (I added one extra case as it

occurred after the table was prepared last year.)Refer to table -Summary of Hendra virus incidents in horses. Queensland Government. https://www.business.qld.gov.au/industry/service-industries/veterinary-surgeons/guidelines-hendra/incident-summary

That works out to 4.4 cases a year per million horses, including untested/ unresolved cases. (The population of domesticated horses in Australia is approximately 1 million). (Australian Horse Council figures source; Unwanted: The Disposable Horse, J Duckworth, 2010). Of the confirmed cases it works out to 3.5 cases on average a year, in Australia's horse population.

So far Hendra has only been found to occur in horses in QLD and northern NSW. The human population of QLD and NSW comprises 52% of the total population in Australia. (Ranked list of States and Territories of Australia, 2015, Wikipedia). Let us guess that there may be 400 000 horses in the risk area. Then that figure would be approximately 1.1 Hendra virus horse cases in 100 000 horses per year in the risk area since it was discovered in 1994. Even if horse population in the area demonstrated to be at higher risk is lower at 300 000 to be conservative, the figure is 1.46 in 100 000 horses per year, for the higher risk area. (That is using the total including the 20 suspected to have had Hendra also.)

Let us compare statistics for Hendra virus infection in the horse to that of colic, to put this incidence into a perspective. A large USDA National Animal Health Monitoring System Equine 1998 study was the first very large scale formal initiative to estimate the incidence of equine colic in the continental United States. Horses housed at racetracks were excluded from the study as a possible confound. The incidence of colic was found to be 4.2 events per 100 horses per year. The case fatality rate was 11 %. (Incidence of Colic in U. S. Horses, Info Sheet Veterinary Services, United Department of Agriculture, Oct 2001). A few calculations extrapolating that US data would indicate there were 4200 colic events per 100 000 horses per year and 11 % of these died- **462 horses died per 100 000 horses per year from a colic event**. If we compare the historical average of Hendra virus horse cases per year (cases= deaths as they all euthanized recovered or not), for all of Australia to reliable US data estimates of incidence of colic deaths, we see that there was **on average 4.4 horse Hendra cases in Australia per million per year compared to 4620 colic deaths per million horses per year, those colic deaths were from 42 000 colic cases per million per year.**

Looking at the table one can see 2 peaks one when it was first discovered in 1994 of 22 horses, with the incident at Hendra, and Mackay. This was the year when the Hendra virus was first identified. There were 7 confirmed cases and 13 untested/ unresolved horse cases, that died or were euthanized at Hendra that year and 2 confirmed horse Hendra cases in Mackay. The second peak in incidence of Hendra cases with an expectantly high number of cases, 23, was reported in 2011.

Multiple horses were infected in HeV events where horses were stabled. The first episode in 1994 was at a racing stable, and again in the stables of Redlands Vet clinic incident of 2008. Also in Cawarral, 2009 with multiple small paddocks, multiple horses were infected.
It mentioned in the Ombudsman report that in the situation where horses are stabled it appears that HeV has the potential to spread to other horses either through close direct contact with infectious body fluids or excreta, or through indirect contact via contaminated fomites, including human-assisted transfer. It also mentioned that all these events appear to have arisen from a horse initially becoming infected in a paddock or outside yard. It also remarked that "In a paddock situation, HeV disease in horses is more likely to occur as a single sick or dead horse. In paddock situations to date, the majority have involved one infected horse that went on to die without any companion horses becoming infected. However, on three occasions, one or more companion horses have become infected with HeV after close contact with the index case prior to or at the time of death." In Hendra horse cases often there seems to be mention of a proximity to flying fox colonies in the Ombudsman report. (The Hendra Virus Report, Queensland Ombudsman, 2011).

In 2011 there were an increased number of outbreaks with smaller numbers of horses affected in each outbreak, with the highest historical peak in cases. It remains unclear what the cause of the peak from only 1

horse infection in the 2010, to 23 horse infections in 2011 may have been due to. Suggestions of flood effects from earlier in the year and late in the previous year have been proposed but the reasons appear to be unknown to date.

There have been an increase in horse cases in the last 10 years, compared to the previous year's overall, possibly due to increased awareness of Hendra virus infection and inclusion in a differential diagnosis, leading to more HeV testing. It would be hard say from these statistics if the vaccination program that was launched in November 2012 if having an effect, as the disease incidence seems similar to the few years before the uncharacteristic 2011 peak. As maintained by Zahoor and Mudie 2015, in discussion the prevention strategy for HeV in Australia includes "the equine vaccine whose efficacy remains to be tested given the low prevalence of HeV". (The Imperative to develop a human vaccine for the Hendra virus in Australia, Zahoor et al, 2015)

Risk of Hendra Viral Infection in a Horse

Hendra Virus Infection is a very serious disease as we all realize in the horse. There have been estimates of a 75% case fatality rate. From information about the confirmed cases to date, approximately 25% of horses can survive acute infection. (Ombudsman as above). I note reading the horse cases from this report that some were euthanized early prior to developing disease, others recovered, but were then destroyed due to Hendra positive horse euthanasia policy, so the figure would be an estimate. I note there also have been issues with different labs getting different PCR results on one horse, one positive and one negative (Ombudsman, Peachester, 2007). From perspective of risk for the individual horse, diagnostic testing was a risk as if it yielded a positive result the horse was euthanized regardless of health status. I think this policy of mandatory euthanasia of HeV PCR positive horses is being revised. In a 2015 review article the author stated that persistent infection in horses has not been identified. (Hendra and Nipah Viruses: pathogenesis, animal models and recent breakthroughs in vaccination, H. Weingarti, 2015).

Hendra Virus Infection in Humans- the Incidence

There have been **7 known human infections, including 4 deaths**. Hendra virus infection is one of the rarest human diseases in the world. As one can see from the table above there have been no further human infections since 2009. There have been 51 Hendra positive horse cases since 1/9/2009 when there was the last human fatality, and 2 asymptomatic HeV positive dogs, which were also euthanized, which is over half the animal cases in history. (Pre and including 2009 until the death of the last human case- 42 HeV horse cases with 7 human infections; after 1/9/2009 there was one further horse case in 2009. Since the last human fatality there were 51 horse cases and 2 dog cases and **zero** human infections). **Despite the increase in HeV animal case numbers in the period since 2009 there have been no further human HeV infections.**

I suspect the increased awareness and employment of such disease infection control strategies as using Personal Protective Equipment (PPE) for those at risk has been a key factor in prevention of transmission from horses to humans.

I will describe briefly the historical human Hendra Virus cases to demonstrate that the humans infected with Hendra virus were performing high risk procedures and or had close contact with a moribund horse.

Human Hendra Virus human infection- a description of cases

1.& 2. 1994 Hendra- 13 horses died- 2 people infected with HeV - horse trainer Vic Rail(*died*) **nursed dying horse-** tried to force feed dying horse – putting hands with food down throat, index horse "Drama Series", Mr Rail died of respiratory and renal failure, source of virus was most likely frothy nasal discharge from index case.

- stable worker Ray Unwin -influenza like illness *survived*; **nursed dying horse** and also tried to force feed dying horse. Horse index case "Drama Series",

"*We believe that it was because of the very close, intimate contact of Vic Rail and Ray Unwin with the infected horses".* www.abc.net.au/radionational/programs/backgroundbriefing/outbreak-at-victory-lodge/3564868#transcript
No PPE was worn

3. 1995 Mackay – Mark Preston horse owner assisted his wife veterinarian to do **autopsies** on 2 horses later found for be positive for HeV -*died* 13 months later of relapsing Hendra virus encephalitis, after recovering from initial infection

4. 2004- North Queensland- Veterinary surgeon – **autopsy** on horse – developed mild influenza like illness 7 days later, (*recovered*), (deceased horse assumed to have Hendra virus) No PPE
https://www.mja.com.au/journal/2006/185/10/hendra-virus-infection-veterinarian

5.&6. 2008 -Redlands 2 people infected - Veterinary surgeon Dr Cunneen- influenza like illness followed by encephalitis. Performed **nasal cavity lavage** on Hendra infected horse **and autopsy** on Hendra infected horse- (*died*)

- Veterinary nurse, Natalie Beohm, assisted in **nasal cavity lavage "**on seemingly well horse", (survived), suffered encephalitis

7. 2009 Cawarral – Dr Rodgers-Veterinarian- **endoscopy** on sick horse "Steggles" horse thought to be suffering from a snakebite (*died*) 1/9/2009

(Further comments on the Redlands 2008 Hendra incident- 20 staff reported contact with potentially infected equine body fluids, 14 identified high risk contact like unprotected mucous membrane, broken skin, or respiratory route; self- reported use of PPE was low only 7% for the 14 staff high risk exposure; High-risk exposures identified in the infected human patients included performing nasal cavity lavage during the last 3 days of the incubation period of 1 infected horse (both patients) and necropsy of another infected horse (Dr Cunneen). (Human Hendra Virus Encephalitis Associated with Equine Outbreak, Australia, 2008).
http://www.couriermail.com.au/news/queensland/vet-faces-legal-action/story-e6freoof-1111117207688 some comments from a horse owner re hygiene used.

The veterinary nurse, Natalie Beohm, in the Redlands incident is often quoted as being an example of human transmission of HeV virus from a "seemingly well" horse. Firstly the nurse was exposed to infected nasal secretions from what is considered to be a high risk procedure, nasal lavage, without PPE for 3 days until the horse became quite unwell thought to be late in the incubation period. I doubt that you can say a horse is clinically well if the horse is requiring regular nasal lavage. I think today in doing a high risk procedures vets and assistants would use adequate PPE. Secondly this assistant may have had other exposures as well, as swabs taken from 3 stalls were found to have positive tests for Hendra viral PCR. (Hendra Virus Outbreak with Novel Clinical Features, Australia, 2010). This nurse also assisted in moving a dead horse. From the report investigating the Redlands incident, it does not say she used PPE, it does say the other staff member who moved the horse used some PPE , but no mention was made of this nurse using PPE. In another section of that report it mentioned that a staff member "that may have been Natalie Beohm" was seen to be collecting urine that had blood in it from one of the horses that later died with Hendra virus infection, with her bare hands. Investigation report Redlands Veterinary Clinic, DLA Phillips Fox, Dec 2008. This investigation was instigated by the Veterinary Surgeons Board of QLD, http://www.vsb.qld.gov.au/RVC-Investigation-Report.pdf

I note from reading the articles that deal with human events of HeV infection that many people have had high risk exposure and not succumbed or even tested positive for the disease. In 2 cases that have recovered have been found to shed virus for a 5-6 weeks then stop shedding virus. This (No evidence of prolonged Hendra virus

shedding by 2 patients, Australia, Taylor et al, 2012). The first surviving case according to the interview above wasn't followed up.

In horses infected with Hendra virus, and transmission risk is highest during terminal stages of the disease and is maximally infectious at necropsy. All cases had exposure either during necropsy of infected horses or from close contact with respiratory secretions and or blood from terminally infected horses, or during high risk procedures. (The changing face of Henipaviruses, Croser and Marsh, 2013) Other sources "The natural history of Hendra and Nipah Viruses, Field et al, 2001; The Hendra Virus Report, Queensland Ombudsman, 2011; Wikipedia, Henipaviruses; and those listed above)

Who is at Risk?

Veterinary Care Workers risk for a Hendra Virus infection

To put it simply the risk based on the evidence from historical horse human transmission events involves close contact with secretions from a dying horse or doing high risk procedures without adequate PPE.

It would seem logical to use some level of graded PPE for veterinary health care workers for all high risk procedures, in any animal, and use PPE for the care of a terminally ill horse. I would add in line with the APVMA recommendations that these measures should also include vaccinated horses. Vaccinated horses and animals may still become infected with HeV. (See terms of reference 1). So those who are at risk based on the cases so far include those people who have had the kind of exposures described without use of PPE.

There are other risks that veterinary health care workers are exposed to such as other zoonoses. A serological survey of Australian veterinarians from 1975 to 1982 determined that the most common zoonotic infections were brucellosis, toxoplasmosis, and Q-fever. (Occupational health hazards in veterinary medicine: Zoonoses and other biological hazards, Epp et al 2012) There are other examples of horse to human zoonoses, so the use of PPE for in any risk situation is warranted not just for the unvaccinated Hendra horse. http://veterinarynews.dvm360.com/zoonotic-diseases-horses-humans?id=&sk=&date=&pageID=2 .

Furthermore serious injuries to Australian veterinarians were studied and found *2188 serious injuries reported in the HRAV study, 1583 (72·3 per cent) were associated with animals, and of these, 453 (28·6 per cent) involved horses. The most common serious injury was fracture, and nearly 20% of all serious injuries resulted in hospital admission*.(Injuries to Australian Veterinarians working with horses, Lucas et al, 2009) What I am trying to demonstrate is that Australian veterinarians have more common workplace serious health risks apart from Hendra Virus.

The risk however for those in the horse and veterinary health care industry is present and a low risk, but something to be aware. Since there has been more awareness and use of PPE in appropriate situations there have zero deaths for over 6 years from this disease. No horse dentist, chiropractor, farrier or horse physical therapist has ever been infected with Hendra virus. Of course interaction such as routine dental work should be delayed on a febrile or sick horse.

Risks Owners and Riders risk in contracting Hendra Virus disease

No owner, or rider has been infected with Hendra virus with one exception: in 1994 of the one owner assisting his veterinarian wife with autopsies of 2 dead Hendra Virus positive horses before Hendra was known about. This was actually the first case of infection of Hendra virus.

I refer to the photos of a child kissing the horse that I have seen used engender guilt and fear for the promotion of Hendra Vaccine in its marketing. This risk profile has not been backed by the evidence of transmission events so far, unless that child assists with an autopsy or high risk veterinary procedure without PPE. One can "never say never", but that is what the evidence to hand indicates, but care should still be taken. That child is more likely to die from being run over by or falling off that horse than by Hendra virus infection when looking at risk statistics from horse injuries. Approximately 20 people a year are killed riding horses in Australia with peaks in young females and older males. (ref: paragraph below)- Head injury is a common cause of mortality in horse accidents, yet on the Queensland Horse Council Website, which promotes the Hendra vaccine for the safety of everyone, has a picture of a happy group of horse riders without helmets on the front page of their website. I think they do a great job and have been to one of their very good large animal rescue courses before, but think that people get tunnel visioned about Hendra Virus, when other more evidence based risks to owners and riders are being overlooked. These risks should be put into a realistic perspective.

Some interesting information from "Horse related injury in Australia,(Cripps, Australian Injury Prevention Bulletin ,2000): "*Estimates of injury rates based on exposure (riding hours or horse riding participation) among all classes of horse riders combined are generally of the order of one injury per 1000 riding hours. This rate suggests horse riding is more dangerous than motorcycle riding and automobile racing"* Also*… "young females and older males are particularly at risk of sustaining fatal head injuries, with most of these deaths occurring in young, female, amateur riders who fall off their horses, followed by male professional riders aged 30 to 50 years."* Further injuries that happen on the ground were not included in this study, and they used data from horse riding injuries from hospital presentation, some less serious accidents may not report to hospital.

Costs and Benefits of Risk Mitigation Options

Vaccination of Horses

1. The Horse's Benefit?

The horses may benefit from Equivac HeV vaccination as an **aid in preventing clinical Hendra disease** in the horse. This has to be weighed up against the **risk of adverse reactions**, in considering the risk benefit balance for the horse's health.

In considering that the Hendra virus disease as rare, a suitable vaccine would also have to have an even more rare adverse event profile for this balance to weigh in the favour of vaccination in considering solely the horses benefit.

The Hendra virus infection is rare, has high mortality in the infected horse but low to no morbidity (sickness without dying) as the disease events are low, and there has been a policy to destroy all HeV infection positive horses. When considering adverse events from the HeV vaccination the morbidity (not dead but sick) is higher and possible mortality events have been reported, and should be considered. In considering this balance if the disease was common the balance would weigh more favourably toward vaccination. As the disease is still rare, even rare adverse events are much more important, especially if they are serious. If the situation changes one could re-evaluate.

I discussed this further in the terms of reference 2 on adverse events, and 1 on efficacy and about the limitations of the vaccine.

There may be a lower incidence of Hendra virus events in the last year since there has been an uptake in vaccination, but it's hard to know as the disease is rare and with large natural annual variations in incidence of equine HeV events. With this increase in vaccination at the same time there is an increase in those that have had sometimes serious adverse reactions. For a horse owner concerned with the health of their horse and living in an area where Hendra virus does occur the decision may be still be difficult. I do understand and see both arguments for and against about whether to vaccinate or not. People love their animals and want what they believe is best for them.

I see the **biggest risk to the horse's health through not vaccinating is not being able to rely on veterinary care**, with the current situation, and that is probably causing more deaths and illness than the protection from Hendra virus disease. I know of 2 colic cases personally in friend's horses and one case of a critical ophthalmic (eye) condition where there have been delays causing suboptimal treatment in all 3 cases, one may have been contributory to a death for a colic horse.

There is no benefit for "herd immunity" with vaccination of horses for Hendra Virus as the reservoir for Hendra Virus are bats.

2. Veterinary Surgeon Benefits?

For the vets there is an increased risk for zoonotic transmission in general from animals due to the nature of their work so for them vaccinating horses may be seen as a plus to **reduce the risk of transmission of HeV** in the vets. This is understandable.

The recommendation from the Australian Veterinary Association (AVA) on a policy briefing states "*it is strongly recommended that all horses in Australia are vaccinated against Hendra virus to protect humans from its potentially fatal outcome."*
www.ava.com.au/sites/default/files/AVA_website/pdfs/Equivac%20HeV%20fact%20sheet_about%20Hendra%20virus%5B1%5D.pdf
Hendra virus disease so far only occurs in Northern NSW and Queensland. Every medication or vaccine has risk of adverse side effects. Not all of Australia has the types of bats that spread the disease.

Also for vets the vaccination of all the horses in the practice would **add significantly to the income stream** of vets. An injection is an easy consult and every 6 months is a boost to the income. However, on the other hand with the whole **HeV vaccination agenda**, work place health and safety heavy handed approach, and policies of not treating sick unvaccinated animals until a blood test is completed and so on, is causing much stress to many vets and I am sure ill health from **anxiety and depression** in some. I know of one vet who has moved from large animal medicine because of this stress.

However there is a perceived idea that a vaccinated horse can't become HeV infected, or shed virus, even amongst the vets. There is an annoyance that people won't vaccinate their horses so the vets **can stop worrying about PPE,** for example, I have seen that written by a vet. It is a difficult situation for them.

However these ideas and policies go against the APVMA guidelines that state "*the potential for a vaccinated horse to pass on the Hendra virus cannot be ruled out. As a precaution, it is recommended people take the **same steps** to protect vaccinated horses from exposure to infection—and to prevent humans being infected by horses—as are recommended for unvaccinated horses. Personal protective equipment should be worn whenever infection is suspected even in vaccinated horses".* (my emphasis)

I see it as a problem that vets are **not protecting themselves to the same extent in a vaccinated horse**, and may open themselves to HeV or other infection anyway. As I have cited in terms of ref 1, in reviewing animal studies the HeVsG vaccine does not always completely protect experimental animals from HeV or

NiV infection, although is generally protective for clinically apparent disease in the time frames of the studies. It may reduce incidence of viral replication, but even in one of the 3 vaccinated horses challenged with HeV at 6 months post booster, there was evidence of viral genome in nasal secretions and that horse was euthanized in the middle of the possible incubation period, before one could assess the significance of this finding. Similar results were found in the ferret and HeV challenge studies. In one cat study the highest HeVsG dosed vaccinated cats challenged with Nipah virus had much higher levels of viral genome in the brains than the control unvaccinated cats. Other issues I have pointed to include the variation of HeVsG titres which over half of the horses prior to the 6 month booster, sample had barely detectable antibody levels. Zoetis was unsure if these levels (8) would have been protective. I wonder if Zoetis will ever do viral challenge studies on horses with that level of antibody which may represent the variation in real world conditions, or just select horses for their trials with higher antibody titres with a better chance of success. (My references are in Terms of ref 1)

So vets should not completely rely on the HeV vaccine to protect them, it may well be an aid, but the awareness, PPE and hygiene changes have probably made the biggest contribution to the lack of transmission of the Hendra virus disease from horses to humans since 2009.

3. Benefits to Owners?

Owners are divided on whether to vaccinate with Equivac HeV often passionately so. However the **availability of Equivac HeV gives them that option** should they choose to.

Some owners feel that they benefit as feel they are protecting themselves and their horses from Hendra virus disease, and are happier vaccinating. Very few would question that this may not be 100%. (see terms of reference 1). It may be pertinent to mention that no owner or child has contracted Hendra virus from their horses, except the 1995 case where an owner assisted his veterinary wife with necropsies on 2 Hendra virus positive dead horses.

Other owners, and we have all talked with them, have been relieved at the arrival of the HeV vaccine, been big advocates and ended up getting adverse reactions, and becoming the other voice against the vaccine. Probably the majority haven't had problems.

On the minus side is the **cost**, if you have many horses the cost may be prohibitive, and owning horses is an expensive exercise anyway for most people before adding other expenses. One friend is starting to vaccinate for endurance and we worked out the first 13 months will be $1720 for 3 horses, she won't do all her horses as it is just too expensive, and the cost will be ongoing. She is more worried about side effects as knows people that have had horses suffer adverse reactions to the vaccine. She will not be able to compete unless she vaccinates with the new regulations being introduced for "vaccination only" events. She says she wouldn't vaccinate otherwise.

The problem is once one **starts vaccinating and gets a severe adverse reaction** and wants to **stop vaccinating some vets just drop the client** without notice, often at a critical time, refusing to attend. So the situation is stressful for many horse owners at the present.

4. Benefits for Zoetis?

Of course Zoetis who claim to be the largest global animal health company has the licence to manufacture and market Equivac HeV vaccine. There have been over 400 000 doses sold for Australian horses so I believe since it became available in November 2012. It is an expensive vaccine for the vets to buy that cost

them around $50- so expect the gross since it became available to be over $20 million, but out of that one would have to deduct production costs etc. It wouldn't be their most profitable product but would certainly contribute to the portfolio.

If it could be mandated to be used over all of Australia profits would really improve, and if it were also indicated for dogs and cats! I note that the Australian Veterinary Association recommends all of the horses in Australia be vaccinated with Equivac HeV. Great potential for market expansion!

5. Benefits for the Owners of the Technology?

The inventors of the technology behind the HeVsG vaccine were scientists K. Bossart and C. Broder as assignees for **The United States of America as represented by the Department of Health and Human Services and the Hendry M Jackson Foundation for the Advancement of Military Medicine**. (Immunization strategies against Hendra Viruses, Broder. C, 2012, see footnote; *"Conflict of Interest C.C.B is a United States federal employee; C.C.B is coinventor on patents relating to human monoclonal antibodies against Hendra and Nipah viruses and C.C.B and K.N.B are coinventors on patents relating to soluble forms of Hendra and Nipah envelope glycoproteins and vaccines; assignees are The United States of America as represented by the Department of Health and Human Services (Washington, DC), and the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc. (Bethesda, MD).")*, (also see owners of technology as assignee in patents, numbers on page 1)

An assignee is a person appointed to act for another or a person to whom a right or property is legally transferred.

This technology was licenced from the Henry M Jackson Foundation for the Advancement of Military Medicine, USA to Pfizer Animal health to develop the vaccine for use in horses.
http://equimed.com/news/products/pfizer-licenses-technology-for-hendra-vaccine-for-horses
http://www.eurekalert.org/pub_releases/2012-11/hmjf-hww110112.php

There would be a financial benefit to the owner of the technology in awarding the licence of this technology to Pfizer animal health, as well as profit to Pfizer Animal Health (now Zoetis). However there are further potential benefits in a marketed vaccine for HeV administered to a large mammalian animal population for the owners of the technology. This could go towards building a safety profile for the vaccine. As Hendra virus is considered to be a biosafety level 4 pathogen, the development for a human therapeutic approach such as a vaccine requires the establishment of at least 2 well researched animal models. (see for explanation Animal Rule, FDA).

Also HeV and NiV were identified by the National Institute of Allergy and infectious Diseases (NIAID) as select pathogens of concern. Here is an excerpt from the 2006 patent for this technology - *"Soluable Forms of Hendra and Nipah Virus G Glycoprotein"* which is the underlying patent for the HeV vaccine. *"[0005] Nipah and Hendra virus are NIAID select, category C viruses and possess several features which make them highly adaptable for use as biowarfare agents. For example, both readily grow in cell culture or embryonated chicken eggs, produce high concentrated titers near $1x10^8$ TCID50/ml, are highly infectious and transmitted by the respiratory tract, and can be amplified and spread in livestock serving as a source for transmission to humans. Recent evidence also indicates that nosocomial transmissibility of NiV from patients with encephalitis to healthcare workers is possible."* (Patent WO 2006/085979 A2) So another motive for the development of a human vaccine would be biodefense.

As the pathogen is so dangerous in humans it cannot obviously be ethically trialled in humans experimentally. The experimental trials with animals are both costly and trial animal numbers are limited due to the pathogen being a biosecurity level 4 pathogen. It is often quoted in the pharmaceutical industry that it costs between 70 and 90 million to get a medicine to market. As 90% of products trialled

for that one condition never makes it to market through the 3 phases of clinical trials, the often quoted real figure of the cost of getting a new drug to market is actually around 500 million. However in a Medicines Australia source I found that to be a gross underestimate *"The process of bringing new medicines and vaccines to market is expensive, time consuming and incredibly risky. On average, the cost of bringing a new medicine or vaccine to market is approximately US$2.6 billion (including the cost of failed research and development projects), and it can take between 10 to 15 years to complete the process."* *(Medicines Australia Facts Book, 4th Edition, 2013* https://medicinesaustralia.com.au/wp-content/uploads/sites/52/2010/11/MAFactsBook4_update2015.pdf *).* The cost to get a new vaccine such as one for HeV to market is likely to be as comparatively expensive. Other vaccine candidates have already been trialled but not continued. The yearly cost for say an influenza vaccine is much less as the structures are already in place.

Principles of what I have just discussed are often mentioned in articles here are a couple of examples: *"Guidelines for human vaccine development encompass principles of cost, efficacy, and safety. Guidelines of animal vaccine development are less stringent. The development of a human HeV vaccine can be time-consuming and prohibitively costly at the current stage."* (The imperative to develop a human vaccine for the Hendra virus in Australia, Zahoor, 2015.)

"Given the speed and lost cost of veterinary vaccine development, some interventions based on the immunization of animals could lead to rapid and relatively inexpensive advances in public health. Opportunities for vaccine-based approaches to preventing zoonotic and emerging diseases that integrate veterinary and human medicine (the One Health paradigm) are emphasized." (*"Vaccines against diseases transmitted from animals to humans: a one health paradigm."* Monath, 2013)

The newly discovered Henipaviruses include: the pathogenic Nipah virus (discovered 1999) that has been responsible for over 250 human deaths (WHO, 2010) with high case fatality, in Malaysia, Singapore, India and Bangladesh; Nipah or Nipah like virus has recently been identified to be the cause of deaths of 9 people in the Phillipines, in 2014 (Weingarti,2015); Hendra Virus (discovered 1994) caused 4 deaths and 7 infections in Australia; and non-pathogenic recently discovered Cedar virus in Australia. There has been found to be evidence for the presence of henipa or henipa- like viruses in bats across Central and South America, Africa , Asia, and Oceania. (The Changing face of the henipaviruses, Croser, 2013; Hendra and Nipah viruses: pathogenesis, animal models and recent breakthroughs in vaccination, Weingarti, 2015)

High level homology nucleotide sequences coding for glycoprotein G of the henipaviruses, Nipah and Hendra are the basis of using the one subunit vaccine for both viruses, HeV and NiV. So the large mammalian animal field trial in horses, and the small efficacy trials in horses, will contribute towards the data and research base towards development and approval for a human vaccine for Henipaviruses, potentially at a faster rate than otherwise, and also possibly at a significantly reduced cost.

Vaccination of Humans

Further research has been underway with a primate animal model, the African Green Monkey,(AGM) which would represent the best animal model for human Henipavirus infection, and would be required before the FDA would license the HeVsG vaccine for human use.
http://www.medicinenet.com/script/main/art.asp?articlekey=161210

I have discussed two AGM trials using the HeVsG subunit vaccine in terms of reference 1. Note there are differences in the vaccines intended for use in humans as opposed to other animals such as horses. In one of these trials different adjuvants were being assessed for efficacy for the proposed human vaccine. I also

note that the Equivac HeV for horses contains thiomersal, a mercury based preservative that is being phased out of human vaccines.

With the ongoing research towards a human henipavirus vaccine, it will be a matter of time before it will become available, it may not be that far away. Once a human vaccine is available vets and concerned health care workers will have a choice themselves whether to use a Hendra Virus vaccine.

Passive Immunity

A human monoclonal antibody treatment, m102.4, has also been developed for post exposure treatment for Henipavirus infection. This antibody therapy targets a G protein receptor binding site on the virus and is based on the same subunit G technology. "US Hendra virus expert Professor Chris Broder developed the antibody, which binds to a protein on the surface of virus particles, blocking entry to healthy human cells. It is hoped that this will then allow the immune system to fight off the virus." http://www.aibn.uq.edu.au/hendra-virus-facts ; https://www.usuhs.edu/content/human-clinical-trials-begin-deadly-hendra-virus-therapy

This immunoglobulin post exposure treatment has been available for compassionate use in Hendra exposure cases in Australia, and is currently in human clinical trials at the University Of Queensland. http://www.aibn.uq.edu.au/world-first-human-hendra-virus-clinical-trials-begin It has been shown to protect ferrets and AGMs in clinical trials from Hendra and Nipah viruses as a post exposure therapy. In one study the African Green Monkeys were infused with m102.4 at 1,3 and 5 days after Nipah virus exposure and were given a second infusion 2 days later and survived the infection. (Therapeutic treatment of Nipah virus Infection in nonhuman primates with a neutralizing human monoclonal antibody, Geisbert et al, 2014)

Apparently Dr Rodgers the veterinarian who died in 2009 was the first person to receive the monoclonal antibody treatment on a compassionate use basis but may have been used too late in the disease. http://statements.qld.gov.au/Statement/Id/70649 After this there were 2 people in 2010 that were considered to have had significant exposure, 1 in 2012, and a lab worker in USA in 2013. None of these people went on to have develop Hendra virus disease but whether this was due to the monoclonal antibody therapy could not be determined. (A treatment for and vaccine against the deadly Hendra and Nipah viruses. Broder et al, 2013)

As of 5/4/15, in the following link it mentioned, "To date, m102.4 has been successfully administered to 10 individuals (nine in Australia and one in the U.S., on a compassionate use basis." http://www.acrpnet.org/MainMenuCategory/Resources/News/ACRP-Wire/ClinicalTrialUpdates.aspx

This therapeutic treatment holds much promise but it would be important as in all post exposure immunoglobulin therapies to treat early after exposure if possible.

PPE, Awareness, Hygiene, Quarantines, Policy

I believe that Personal Protective Equipment (PPE) and hygiene changes have probably made the biggest contribution to the lack of transmission of the Hendra virus disease from horses to humans since 2009. This is backed up by the statistics. Prior to the last human death at the beginning of September 2009 there had been 42 cases of Hendra virus infection in horses. In this time period all 7 of the human infections occurred. (Note there was one further case of Hendra infection in a horse in 2009 after the last human case). After this date there were the remaining 51 horse infections and zero human infections. Between that last human infection in 2009 and the release of the vaccine there were a further 35 cases of Hendra virus disease, and zero infections. After the launch of the vaccine at the end of 2012

to the present there have been a further 16 cases of Hendra virus infection in horses. The uptake of the vaccine has been gradual. (I have used total cases that includes untested and unresolved cases as well).

Most of the protection from transmission from Hendra infected horses to humans to date, since the last human case, cannot be not statistically attributable to the vaccine. The PPE and hygiene improvements have been particularly important.

There have been 2 cases of Australian Bat Lyssavirus in horses in Queensland in 2013. Australian Bat Lyssavirus is also a bat borne zoonosis that also has a neurological presentation. One article said "*underdiagnosis is likely"* and *"Vets should also practice good personal biosecurity when attending sick horses."* Note there have been 3 human cases in Australia with 100% fatality. Australian Bat Lyssavirus was first identified in 2006 and is very rare emergent virus but fatal. www.abc.net.au/news/2013-05-19/no-bats-found-on-lyssavirus-horse-death-property/4698494 ; http://www.abc.net.au/news/2013-05-19/no-bats-found-on-lyssavirus-horse-death-property/4698494 There are good reasons to practice personal biosecurity, not just in unvaccinated Hendra horses. In the earliest human infections, Hendra Virus had not previously been identified and PPE wasn't practiced to the same extent as it is now, if at all, in those cases that became infected. The 2013 infections in horses with this Lyssavirus, as well as the more common infections that a veterinary health care worker can contract from an ill animal, highlights the importance of PPE and good hygiene.

I see it as a problem that vets are not protecting themselves to the same extent in a HeV vaccinated horse, with different policies as to PPE for example, as it is still possible to contract Hendra virus in the vaccinated horse. APVMA has emphasised the importance of taking the **same** steps to protect from disease transmission in the vaccinated and the unvaccinated horse. I have referenced this point and discussed this further in the section above in "Vaccination of Horses" under "2. Veterinary Surgeon Benefits", and in Terms of Reference 1.

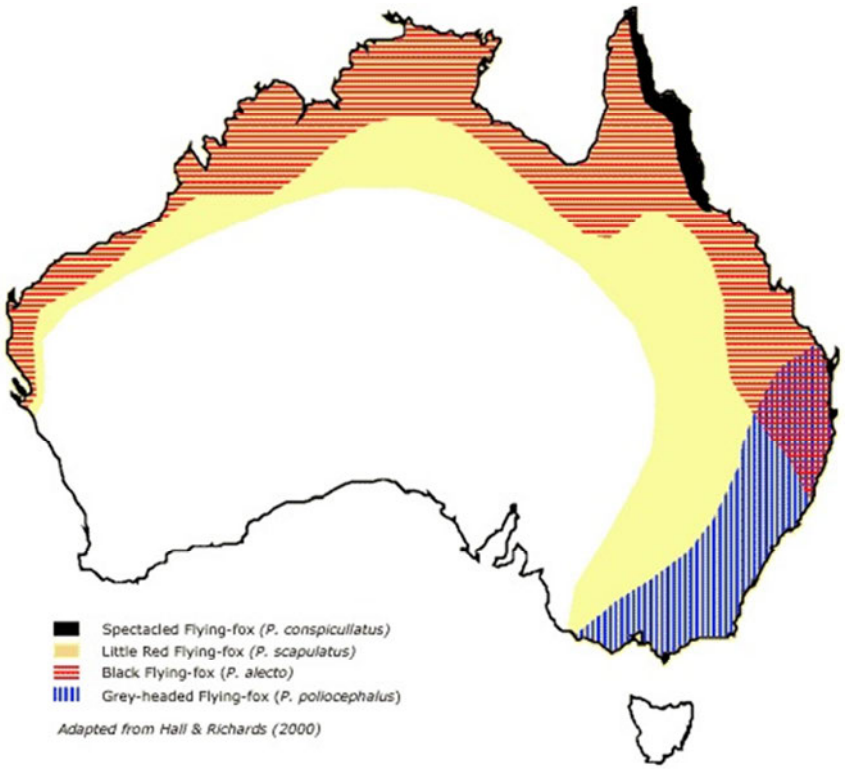
The down side of the PPE of course is risk to the vets and horses from a frightened horse seeing a human in a "moonsuit", and may become more dangerous as a result to those humans and cause themselves injury. Some may be difficult to work in and hot. I believe there must be a middle ground concerning PPE, perhaps a graded approach should be used that is sensible, and evidence based. Perhaps a working solution of more user friendly and lower cost PPE for lower risk situations may be worth exploring. Of course a higher degree of protection should be used during an autopsy of a horse, when the risk of transmission is considered to be at the highest level, and in nursing a terminally ill animal. Also an appropriate level of protection should be sought when performing high risk procedures on any animal regardless of vaccination status, that the level required for more common situations where the risk of exposure and transmission is much lower.

Flying Foxes - Relocation of Roosts

The single largest study for Hendra virus surveillance in individually wild caught flying foxes, so far conducted, attempted to identify major routes of HeV excretion in 3 of 4 Australian flying fox species in QLD and NSW and species prevalence of Hendra virus excretion. (Routes of Hendra Virus Excretion in Naturally-Infected Flying-Foxes: Implications for Viral Transmission and Spillover Risk, D. Edson et al, 2015). They found that the level of Hendra viral RNA was highest in urine samples. The *only species* in this study that was found to have Hendra viral RNA on PCR was the P. alecto which is the Black flying fox. HeV RNA was detectable in 42 of the 1410 animals sampled. They found 78 positive samples in 4436 in P. alecto (they took multiple samples from each animal), and found 1.76% of all samples had positive HeV RNA. There were zero instances of positive HeV RNA in P. scapulatus, which is the Little red flying fox (N=262 animals and n= 985 samples), and zero instances also in P. poliocephalus, which is the Grey Headed flying fox. (n= 1168

animals and n=2958 samples). A comment was made *"The complete lack of detections in P. poliocephalus and P. scapulatus adds to a growing body of evidence suggesting that P. alecto and P. conspicillatus are the main reservoir host species for HeV",* and in discussing the higher risk animals for shedding virus which are the pregnant females, "*The complete absence of HeV detections in this higher infection risk cohort strengthens our contention that P. scapulatus is not a primary reservoir host."* The discussion about P. poliocephalus in this article also reinforces the concept that this species also has a low HeV infection rate. However, P. conspicillatus (Spectacled flying fox) occurs in far North East Queensland and is thought to be a primary HeV reservoir host species, like P. alecto. Note the maps of the distribution of the different flying fox species.

Map of flying fox prevalence



Flying foxes serve essential roles ecologically, especially in the pollination of hardwood trees. http://www.batsqld.org.au/aboutbats.html . Strategies of risk mitigation through exposure reduction to HeV from bats by relocation of Flying fox roosts should take these issues and studies into account. If flying fox roost relocation is considered from high horse and people population density areas, maybe the roosts with a preponderance of the P. alecto species mostly needs to be targeted, particularly those that may have had higher prevalence of positive HeV RNA in sampling. Many people have concerns with roosts of the Little red flying fox (P. Scapulatus) and the Grey headed flying fox (P. conspicillatus) in urban areas but densities of these 2 species are epidemiologically associated with "*weak negative or negligible correlations with equine cases*". (Flying –Fox Species Density – A Spatial Risk Factor for Hendra Virus Infection in Horses in Eastern Australia, C. Smith et al, 2014). Another study, "*Flying-Fox Roost Disturbance and Hendra Virus Spillover Risk"* (Edson, 2015), found no statistical association between flying-fox roost disturbance and Hendra virus urinary excretion. It maintained this indicated *"that roost disturbance does not precipitate increased HeV infection and excretion in dispersing flying-foxes*". This article emphasised the importance of a best practice approach, however, to limit distress to flying foxes in roost dispersal and modification. It also mentioned that there had been recent legislative changes devolving urban flying fox management from state to local authorities.

Terms of Reference 4: Whether the guidelines/procedures required for veterinarians attending horses that are not vaccinated against HeV are proportionate to the consequences.

In discussing this point I would need to identify what the guidelines and procedures are and what the consequences are.

The **guidelines and procedures:** I would expect to include use of PPE in a sick horse, getting testing done early if Hendra virus would be part of a differential diagnosis (almost every systemically sick horse), notifying authorities re possible Hendra cases, counselling horse owners as to risks to themselves and advice re containment and so on.

The **consequences** are: vets, assistants or owners getting Hendra virus infection; Horses infecting other companion horses, dogs or cats; Prosecution of vets, fines and threat of loss of registration to practice for not following the Workplace Health and Safety Guidelines perfectly.

The **assumption** is that these consequences won't or are extremely unlikely to happen in a vaccinated horse, so the guidelines and procedures can be relaxed in a vaccinated horse.

How the Guidelines differ between vaccinated and unvaccinated horses:

"*Guidelines for veterinarians handling potential Hendra virus infection in horses"2013, QLD Govt,* or in the "*Hendra Virus-Information for Veterinarians",* 2015, Work Place Health and Safety Queensland

There is no mention of different requirements that I could find reading the guidelines above for veterinarians in treatment of HeV vaccinated horses as opposed to unvaccinated. The government guidelines pertain to the management for a horse suspected of having Hendra Virus disease. Whether to treat an unvaccinated horse or not, or delay treatment while waiting for a HeV result is at the discretion of the vet.

In assessing a sick horse in which Hendra could be part of a large range of possibilities in the differential diagnosis, many vets are managing in 2 different ways. Vaccinated or unvaccinated. What about the possibility of a vaccinated horse having the virus? Ignoring this possibility and reducing level of PPE and caution goes against the advice of the regulatory body that has assessed the vaccine, the APVMA.

However APVMA states "*The potential for a vaccinated horse to pass on the Hendra virus cannot be ruled out… Personal protective equipment should be worn whenever infection is suspected even in vaccinated horses."* It doesn't recommend reducing the level of PPE even in a vaccinated sick horse that has symptoms and signs indicating possible Hendra infection.

The **guidelines and procedures for vets should be based on the research, and evidence to date.** As I have described in Terms of Ref 1, the sample sizes for efficacy testing are very small, very few horse efficacy studies have been done, and so there has also been some reliance put on other research in other animal models and also in Nipah virus research.

Research Basis for Guidelines

I refer to the "*Guidelines for veterinarians handling potential Hendra virus infection in horses",* Department of Agriculture, Fisheries and Forestry QLD Govt, version 5.1, Dec 2013, which is the latest version as on their website. Sources of this research quoted in these guidelines include: "Hendra Virus Vaccine, a One Health Approach to Protecting Horse, Human and Environmental Health", D Middleton et al, 2014; and "Experimental Infection of horses with Hendra Virus/ Australia/ Horse/ 2008/ Redlands", G Marsh et al, 2011.

Following in italic are 2 excerpts from this Guideline for vets document, discussing the research which helps form a basis for their guidelines:

"The AAHL research has also shown that viral ribonucleic acid (RNA) could be detected continually in nasal swabs from as early as two days post exposure, which was three to five days before the onset of clinical signs indicating that systemic spread of the virus may be preceded by local viral replication in the nasal cavity or oropharynx. The data indicates that nasal secretions of asymptomatic horses may pose a transmission risk during the early phase of disease that precedes viraemia, fever, or other discernable clinical signs of HeV infection. However, the increasing gene copy number recovered over time also suggests that the risk provided by these animals is relatively low compared with animals in the immediate pre-symptomatic and symptomatic stages of infection."

I do note however the inoculation of the horses experimentally was oronasal, so that could contribute to the nasal swabs being positive earlier for the HeV on PCR testing.

These guidelines also commented about the 6 month trial in vaccinated horses. (note in following Guidelines for Vets excerpt, inoculation was oronasal not intranasal, refer D. Middleton et al, 2014 in sources above)

"*At approximately six months post-vaccination, when horses were challenged with HeV via the intranasal route, all were protected from clinical signs of HeV disease. In addition, virus was not reisolated from any clinical samples (collected pre-and post-mortem) from any of the horses, and there was no evidence of virus spreading beyond the site of administration (i.e. the upper respiratory tract). In non-immunised (surrogate control) ferrets, viral infection was detected and all succumbed to acute HeV infection*."

A different way of putting it from reading the results from the research into the 6 month trial is that of the total of 3 vaccinated test horses, **one Hendra vaccinated horse was found to have viral genome in 4 days of nasal swabs.** The horse was euthanized on day 8, the day after a positive reading on day 7 and even before another test horse that did not have evidence of viral replication. Day 8 is still in the middle of the possible incubation period in the horse. There were comments discussing this evidence in scientific manuscript mentioned in the sources above, of Hendra viral replication in the vaccinated horse, that it was thought to be transient and low level. It was definitely transient as the horse was destroyed before one could see if the likely viral replication settled or increased and would go on to cause a viraemia and systemic disease. I wouldn't expect to see multi-organ disease until the virus spread systemically after local replication, and after the horse became febrile and unwell. This horse would be unlikely to have reached that stage by the time of euthanasia without other clinical signs. What may have happened if horse could have lived longer would only be conjecture as the horse was euthanized before these questions could be answered. The article also mentioned viral genome was low level, actually this vaccinated horse had a level of viral genome roughly the same on day 3 as the only control horse for the whole series on day 6 prior to this unvaccinated control horse becoming febrile and sick and was euthanized the next day. (in figure 2, from Middleton et al, 2014 above) (Refer- 6 month trial – in my terms of ref 1). **There was mention that there was no virus isolated, in the vaccinated horse that had positive viral genome, but this is not remarkable. It also didn't mention that virus was not isolated before post mortem in any of the experimentally infected 4 unvaccinated horses' samples** including historical experimental horse infection- 3 horses, and the one control associated with an earlier efficacy trial. These included late incubation, also clinically sick horses' samples, those that had positive viral genome on PCR in many samples. **A problem is that viral isolation as a technique is not as sensitive as PCR, a negative viral isolation doesn't mean the virus isn't there.** This has been stated by other researchers in other papers. I have discussed this in Terms of Reference 1.

I wonder how sweeping generalizations for all horses can be reliably made from the very small study findings in horses, especially with potential red flag results that were not repeated or questioned.

"Always take appropriate precautions based on any suspicion of HeV; do not wait for confirmation. Even with vaccinated horses it is important to take appropriate precautions as no vaccine is completely effective in 100 per cent of animals."(From the "Guidelines for veterinarians handling potential Hendra virus infection in horses", Department of Agriculture, Fisheries and Forestry QLD Govt, version 5.1, Dec 2013)

It is good that the guidelines have made this statement as the only 6 month efficacy trial (in horses), did show prevention of HeV viral infection in 2 of the 3 horses in the trial (66.6%). However, one horse (33.3%) did have evidence of HeV infection and nasal viral replication over 4 days. This horse did not develop clinical disease or evidence of any systemic spread in the 8 days post exposure to HeV at euthanasia. Unfortunately it is unknown if this may have progressed or settled completely as the incubation period in horses is up to 16 days. Nasal viral replication is said to precede systemic spread and onset of clinical illness.

There are other examples as I have reviewed in Terms of Reference 1 demonstrating possible limitations of the vaccine, in other species vaccinated with HeVsG vaccine then challenged with Hendra and Nipah viruses. The vaccination does appears to reduce the likelihood of clinical disease and infection when challenged with Hendra virus, but remember that most of the trials have been performed at the time of likely the most robust antibody protective level at around 21 days post booster. Also in some smaller animal species trials much higher comparative antigen levels in the vaccine (ie dose of vaccine) are used that may stimulate a bigger and more prolonged response in protective antibody levels. In some HeVsG vaccine challenge studies (ferret 12 month post booster NiV, ferret 21 day HeV and cat 21 day NiV) several animals even with much higher antibody levels than the horse 6 month trial did demonstrate infection (not clinically unwell though). Furthermore, in real world conditions not all horses will have very good antibody levels. Also infection is still possible and has been demonstrated in animal studies, in animals with good anti-HeV antibody levels but less often perhaps. I just thought I would address those issues briefly first to provide a balance as to some vaccine limitations which seems to me to be generally glossed over if at all ever acknowledged, or even known about in most instances.

These guidelines mention the importance of adopting the "precautionary principle" which is prudent.

However precautionary principle should also indicate including vaccinated horses as well as unvaccinated horses for consideration for HeV in the differential diagnosis of a sick horse, and testing for HeV PCR accordingly.

The other very important consideration would be the use of PPE in sick horses both vaccinated and unvaccinated, rather than changing the approach due to vaccination status. This point of considering HeV in the vaccinated sick horse is more explicit in advice from the regulatory body that considers the data and issued the registration, the **APVMA**, as follows:

"The potential for a vaccinated horse to pass on the Hendra virus cannot be ruled out. As a precaution, it is recommended people take the same steps to protect vaccinated horses from exposure to infection—and to prevent humans being infected by horses—as are recommended for unvaccinated horses. Personal protective equipment should be worn whenever infection is suspected even in vaccinated horses."

Most vets just don't seem to consider that Hendra Virus infection could be a possibility once vaccination commences, then don't test for it and relax PPE standards, but remember the consequences are high in getting it wrong, and PPE also protects from other disease, that may also be infectious. Vets are potentially exposing themselves by adopting 2 different approaches to managing a sick horse based on vaccination status that may

not necessarily ensure protection from infection with Hendra virus. This divergent approach based on vaccination status is not reflected in the above recommendations given by the APVMA in 2015.

The current evidence indicates that **human HeV infection has occurred in those performing high risk procedures in a horse, having close contact with secretions and doing invasive procedures in a terminally ill horse, and performing autopsies on a dead horse**. It is therefore logical to use suitable PPE in these situations obviously in any horse, again regardless of vaccination status, or even a HeV PCR virus result. (There have been examples of a negative HeV PCR on samples before death that have had HeV infection. In the first human fatality with HeV encephalitis, there was negative PCR on samples before death and HeV was found in the man's brain post- mortem. Also in an experimental ferret study with 12 month duration of immunity, all pre-euthanasia samples were negative, but a bronchial lymph node was found to be PCR positive for Nipah virus post mortem.)

PPE is important but needs to be graded, low cost, comfortable and easy to use, so it becomes habit. To date no one has contracted Hendra virus infection from an asymptomatic horse without doing unprotected high risk procedures. **I think it is not practical to enforce a certain level of PPE for every situation**, vets have done very well in the last almost 7 years with no record of human infections, once there was greater awareness of the Hendra issue, they are professionals capable of a making risk assessments of infection and clinical judgement as to the level of PPE required. Guidelines however are useful.

From the "*Guidelines for veterinarians handling potential Hendra virus infection in horses"2013, QLD Govt,* or in the "*Hendra Virus-Information for Veterinarians",* 2015, Work Place Health and Safety Queensland, I understand that policy doesn't appear to delineate different treatment recommendations or policies in the management of the sick horse, between vaccinated and unvaccinated horses. What they do is recommend if a potential Hendra virus case requires ongoing treatment before the Hendra virus test results are received to conduct a risk assessment to ensure health and safety of the person administering the treatment, and appropriate risk communication with clients. Non-veterinary staff should not be requested to administer invasive treatment.

The decision whether to delay treatment awaiting a test result appears to be at the discretion of the veterinarian. There is nothing written I have come across requiring non vaccinated horses to have different guidelines or treatment to a vaccinated horse.

The treatment guidelines are for a horse suspected of suffering with Hendra Virus infection. The problem is that Hendra virus infection especially early in the symptoms and signs can be similar to many common illnesses. The different management of vaccinated and unvaccinated horses by individual vets is based on their assumption that a vaccinated horse won't get Hendra virus infection. A management plan based on this assumption allowing a reduction of protection standards for example even in a vaccinated horse is possibly exposing the veterinary health workers to HeV infection or other infectious disease. The APVMA advice state that "***potential for a vaccinated horse to pass on the Hendra virus cannot be ruled out".*** The experimental research also has examples of infection in vaccinated animals with Hendra and Nipah viruses.

Not all people will vaccinate all horses due to concerns of past history of adverse reactions, concerns adverse reaction risk may outweigh risk of Hendra infection in their horse, cost of ongoing immunization particularly where owners have multiple horses, or a stud situation, the fact that the immunizations are due every 6 months for the rest of the horses life, I am sure there are many reasons. Some vet clinics are recommending yearly immunizations instead as an option, but APVMA advice was clear, a ***"****booster dose of the vaccine is required every 6 months. This is because a duration of immunity has not been demonstrated for more than 6 months."* Some of these vaccinated horses with an off label duration of immunization interval may have very low or non-immune protective antibody levels, and Hendra should be considered. I also think a blanket

approach to all horses in a practice to order then wait for results prior to treatment for every instance of colic in an unvaccinated or under-vaccinated horse, will inevitably result in horse deaths and illness. Human safety is also paramount, but to date there have only been evidence of transmission from horse to human during high risk procedures, care of the moribund horse with exposure to body fluids, and autopsies all without adequate or any PPE. The disease is both rare and serious. The shadow of Work Place Health and Safety reviewing positive Hendra case histories and management and imposing fines also creates stress for the vets. I respect that it is up to the vet to run their own practice in whatever way they choose, and I recognise the difficulties.

Terms of Reference 5: Impacts on the equine industry and the economy arising from veterinarians applying a policy not to treat unvaccinated horses.

I believe if veterinarians choose not to treat horses that are not vaccinated with the Hendra virus vaccine that is really their choice. I see that many vets are under an enormous amount of pressure due to the Hendra virus issue. They may have fears of themselves or their employees catching the virus, they are at odds often with owners about the vaccine, they may have issues of insurance for veterinary hospitals they may run on the Hendra issue, and there is the threat of Work Place Health and Safety investigating positive Hendra case and imposing massive fines and prosecution. I think for some it just is easier to be vaccinated only veterinarians. However vets being generally compassionate people that care for animals, in a way a higher calling in life I believe, many are finding the situation stressful. Their practice or they may have a policy that calls for a delay in treatment of sick unvaccinated horses while waiting for a Hendra test, or deny treatment for all unvaccinated horses. To be that compassionate person, seeing the animal suffer, the owner's emotional distress during a delay without necessary treatment will cause stress to themselves, and possibly animals die or become much more ill prior to treatment. This does happen and it would be difficult. Some vets just take it all in their stride and choose to treat unvaccinated horses as per usual. The work place health and safety QLD fines and prosecutions are making the whole situation much more stressful. I feel vets are "the meat in the sandwich" in the current situation, they are in an unenviable position.

On the other side of the coin however, vets are making money from the vaccination itself and callouts for the vaccinations and this would potentially add several thousand to the income at the end of the year. Some see that vaccination of horses will provide virtually 100 % protection from transmission for Hendra virus to themselves from that horse, even if it doesn't it may engender a feeling of safety. So many vets would see the HeV vaccination as a win-win in many respects.

In any case, anyone who has animals and loves their animals really needs their vet, in some ways it is like having your own GP for people. This relationship is important for the health of the animal, in this case the horse, and also for the owner who feels that they can trust and rely on their chosen vet in times of need for their animal. I have great respect for vets in general. My vet has saved the life of my dog when she was seriously ill and my horse, from a confirmed brown snake envenomation when it was virtually unresponsive, shocked and at the point of death. He later saved the eyesight of the same horse when she had a serious corneal abscess. She was not expected to regain vision, but did. Without that intervention, she wouldn't be alive and not the pleasure she now is.

This brings me to my friend Lee C. I wrote about Lee and her horse "Little Girl" in the Terms of Ref 2 under Adverse Reactions. Little Girl had an adverse reaction to her 5th Equivac vaccine, that she considered as being serious. She believed that should she continue to vaccinate Little Girl the next vaccine reaction would kill her. She decided to cease the 6 monthly vaccination program due to concerns for the safety of her horse. At 12 months she was sent reminders from Zoetis stating if she didn't continue now she would have to start the whole program of prime then booster all over again. She declined to continue. One month past the 12 month reminder, Little Girl was found with a swollen, tightly closed eye, with copious purulent discharge in her eye and streaming down her cheek and in a lot of pain. This was not just the conjunctivitis that every horse owner sees from time to time. She contacted her vet who refused to see her horse, as her Hendra vaccination had lapsed.

The vet had seen Lee for spaying her 2 dogs and for issues with her horse many times in the previous 12 months. She respected him as a vet. He was aware she did not intend to continue the vaccination with Equivac HeV, but had not told her that he would not be her horse vet anymore. Little Girl presented with what I consider to have been an ophthalmic emergency. Lee said that her eye was very swollen and jammed shut, full of pus that also ran down its cheek and the horse was in obvious distress. The vet told Lee his wife didn't want him to see horses that weren't Hendra vaccinated anymore. She brought photos of the horse's eye into the vet practice and was given some medication to put in its eye. I received a phone call from my distressed friend asking if I could examine her horse's eye. I skipped a medical presentation that I had been scheduled to attend after work to see Little Girl. I am not a vet. I had no way to sedate Little Girl and am not confident to do a proper ophthalmic examination on a horse in any case. Little Girl's eye had a hazy, bluish tinge in her cornea, her eye looked a mess, I thought she had a serious corneal abscess. Lee did find another more sympathetic vet, who changed Little Girl's treatment program and the eye slowly settled, with intensive and appropriate eye care.

I was shocked at the absolute failure in duty of care by this first vet, in my opinion.

If that vet would told Lee that he would no longer look after her horse when she decided to stop vaccinating, she would have had time to find another vet for an emergency situation. This presentation, any way you look at it, is not characteristic of Hendra virus infection. At this time, being 13 months since the last vaccination, with a history of 5 vaccinations previously as per the 6 monthly protocol, if Hendra virus infection was seriously a risk in this case, then one would have to question the vaccine itself. Lee feels she has been failed by the current system that encourages vaccination, which she dutifully did. When her horse had a serious adverse reaction and the safety of continuing that program was in question, she was denied care without warning in an ophthalmic emergency situation. She is (uncharacteristically) tolerant towards the first vets' actions, now that the situation has settled. However, I see it an issue of importance that this sort of behaviour is now considered the norm and accepted by the vet profession (and the AVA?) as ok. **There have to be clear guidelines ahead of time on a will treat or not basis, so people can put in place their own contingency plans for veterinary care.** This case was obviously not a Hendra virus infection, but was a sight threatening situation for her horse that needed prompt attention. I see it as heartless for the vet to refuse to see her horse without warning at a critical time, when she had been a loyal client and had no other vet she knew to turn to.

Lee is a retired pathology nurse. She has worked for many years at infectious diseases wards at large public hospitals and also in the private domain. She collected samples of nasopharygeal swabs from various highly infectious respiratory patients. She didn't refuse to take blood samples from HIV and Hepatitis C patients due to valid concerns about possible needlestick injuries. This was part of the job and did entail more risk. An article looking at risks to health care workers from needlestick injuries, estimated that in the US there were 800,000 needlestick injuries per year in health care workers. Each instance incurs risk of transmission of infection. http://enhs.umn.edu/current/6120/needle/magnitude.html *"Of these 20 pathogens, HIV, HBV, and HCV are the three most common diseases transmitted via needlestick injuries (CDC, 2004). A single exposure to HIV, HBV, or HCV in the context of a needlestick*

injury places a worker at average risks of infection of 0.3%, 6% to 30%, and 1.8%, respectively (CDC, 2004; NIOSH, 2000)." Another US study estimated occupational death rates in health care workers and discussed transmission estimated that there were 10,000 Hepatitis B occupationally acquired transmission events (HBV annually from 1983 data), 50-150 HCV annual transmissions events, and 57 documented and 138 possible HIV transmission events due to occupational exposure http://wwwnc.cdc.gov/eid/article/11/7/04-1038_article. Many of these transmission events do eventually result in death. This study explored 4 types of infectious exposure there would be many others. In hers and my profession there is risk, as in any profession, some more than others, but as this last article maintained,

"The fundamental ethic of health care is that sick persons must receive care. This premise carries an unstated consequence: an occupational risk to healthcare workers who respond to the needs of contagious patients."

I don't know how this compares to a vet's feeling about their own profession and this current situation. They have to balance the need to care for themselves and their employees against the care needs and risk presenting from their animal patients.

A consequence of actions of some vets to delay treatment while waiting for a HeV test result or refusal to treat, will inevitably and has already resulted in horse illness and deaths. Hendra virus infection can share symptoms with other acute presentations of an ill horse such as colic. Colic is common, often serious illness in horses. It is the most common cause of horse death apart from old age. As I wrote earlier in this submission, there were found to be 4.2 colic events per 100 horses per year and 11 % case fatality from those colic episodes in a very large US study into the incidence of colic in US horses. (Incidence of Colic in U. S. Horses, Info Sheet Veterinary Services, United Department of Agriculture, Oct 2001). I extrapolated that to Australia with our domestic horse population of 1 million. This calculates to 42 000 cases of colic per year in Australian domestic horses. Compare this to the average rate of Hendra virus infection in horses per year which averages at 4.4 for all of Australia. That 4.4 average is horse infections including suspected, but untested and unresolved cases. The number of HeV infections which only includes confirmed cases of HeV is 73cases/21 years or 3.5 cases per year on average. https://www.business.qld.gov.au/industry/service-industries/veterinary-surgeons/guidelines-hendra/incident-summary . Colic is a serious condition, but the 11% case fatality quoted included cases with veterinary care - without care, that case fatality rate will climb. It is an example of an important condition in which veterinary intervention can make all the difference.

One friend recently had a horse die from colic. Trying to find a vet who would see her unvaccinated horse delayed veterinary care. I am not sure if it would have survived had treatment been more timely, but it may have had more of a chance. Another friend was looking after a friend's unvaccinated horse that developed colic, but treatment was delayed while the horse suffered awaiting a negative Hendra result. Fortunately, this horse lived. This policy of delaying treatment while waiting for a Hendra virus test in unvaccinated horses or denying them treatment altogether is at the discretion of the vet, as I have said in the last Term of Reference. Each vet needs to make their own risk assessment, and that is their prerogative, but the consequence of applying this policy will be increased horse suffering and more, otherwise preventable, horse deaths.

Term Of Reference 6: The Impact of Workplace Health and Safety actions on the decision by veterinarians not to attend unvaccinated horses and results of previous Workplace Health and Safety HeV investigations where there have been human infections.

The investigations by Work Place Health and Safety Queensland (WHSQ) into Veterinary surgeons that had treated HeV positive horse cases have resulted in subsequent aggressive prosecutions and created increased stress on veterinary surgeons in a situation that was already difficult. In some of these prosecutions, the vets may be facing fines from $100,000 for individuals and $600,000 fines for veterinary practices. http://www.abc.net.au/news/2015-09-25/vets-prosecuted-over-hendra-cases/6801422, http://www.horsetalk.co.nz/2016/01/25/horses-welfare-issues-aussie-hendra-stand-state-mp/#axzz47UMaqbHe

What have these vets done that have resulted in such onerous fines and malicious court actions? There were no human infections as a result of their management of the cases, nor to my knowledge any new horse cases either. They were doing their jobs as best they could, often in difficult situations, and may not have followed the fine print of the almost 50 page government "Guidelines for veterinarians handling potential Hendra virus infection in horses", to the letter. (the latest version is 5.1 from Dec 2013 https://www.daf.qld.gov.au/__data/assets/pdf_file/0005/126770/2913_-Guidelines-for-veterinarians-handling-potential-Hendra-virus-infection-in-horses-V5.1.pdf)

It may well be that these cruel actions in prosecutions of vets by government are driven by an agenda to effectively enforce vaccination by intimidation, which is pushing vets to reduce or deny treatment of unvaccinated horses. Comments in the media such as by Dr Nathan Anthony the Hendra spokesman for Equine Veterinarians Australia reinforces this sentiment, *"At the end of the day if it's a vaccinated horse you're not going to end up in a work health and safety inquiry".* http://www.abc.net.au/news/2015-09-25/vets-prosecuted-over-hendra-cases/6801422 These Work Place Health and Safety actions could be seen as an implicit government threat, which provides an option to have an easier life if one does not see unvaccinated horses at all, which would in effect engender compliance with HeV vaccination. This policy of no treatment for unvaccinated horses or no or reduced treatment during the delay while awaiting a HeV results is at the discretion of the vet after making appropriate risk assessment. This has taken 3-4 days in some areas to come back, which makes treatment of an acutely ill animal somewhat academic - they get better or they die. This also serves a secondary purpose, owners are finding it increasingly difficult to get satisfactory vet care, if at all, for unvaccinated animals, again indirectly enforcing vaccination.

This policy of treating unvaccinated horses differently, however, ignores several salient points about the Hendra virus vaccine. It has had scant testing in horses and there has been evidence for early infection in a vaccinated horse and other vaccinated test animals in efficacy studies with NiV and HeV, that I would think merit further investigation. While it appears to protect from clinical disease in animals, and in many cases infection, most efficacy studies have occurred in situations where there has been a greater likelihood of success with challenge occurring 21 days after booster for example, and in different protocols that use comparatively higher doses of antigen to that given to a horse. This situation is different to what vets may see in the real world. The serology levels provided by Zoetis in a 2013 news release showed that only half of the group 1 horses before their 6 month booster and over half of the horses in a second group with that proposed 12 month protocol had just detectable or not detectable titres of HeV antibody (≤ 8), and Zoetis commented ***"It is unknown whether horses with undetectable titres can withstand challenge with live Hendra virus".*** Of course after the 6 month booster the titres were higher, but whether they would have been protective prior to the booster would be questionable. The particular 12 month group with low antibody status was not

challenged with live virus. Zoetis said that it would first trial the protocol that included a 6 month booster prior to the proposed 12 month duration of immunity challenge, as it would have a better chance of success. In any case, what would happen if immunized just every 12 months, many more horses may possibly have very low titres some of the time, some vets are already advocating this. Horses with such very low titres to my knowledge have never been tested for efficacy in viral challenge studies, yet judging from these serology levels may well be common. Remember the 12 month duration of immunity application by Zoetis was rejected by the APVMA. http://www.abc.net.au/news/2015-08-05/apvma-aprroves-hendra-vaccine/6673542 The APVMA put on their website at the time of registration, *"The approved instructions of the product indicate that a booster dose is required every 6 months. This is because a duration of immunity has not been demonstrated for more than 6 months".* In the real world there will be a big variation in the immunogenic response in individual horses too.

Sorry to reiterate these points yet again, but think it is important, as the vaccine is usually promoted as being virtually infallible with a "no vaccine is 100%" disclaimer, this is strongly promoted by both AVA and WHSQ. These issues underlie the divergent approaches to treating a vaccinated versus unvaccinated horse. Many vets will test before treatment for HeV in the unvaccinated horse and not test for the same condition in the vaccinated horse. How can they know that HeV is not possible in the vaccinated horse? This will completely skew cases of positive HeV as it is mostly only being tested for in vaccinated horses. Vaccination may result in a milder illness in a low titre vaccinated horse and transmission events may still be possible. It is possible that there would be cases of fulminant disease perhaps in an undetectable or barely detectable titre horse.

This belief in the virtual infallibility of the vaccine also underlies a divergent approach to PPE by vets - a comment by Dr Nathan Anthony, *"Because if you vaccinate your horse against Hendra virus you will protect your horse against this disease and when veterinarians see a sick horse that's fully vaccinated they will not need to wear these high levels of PPE."* http://www.abc.net.au/news/2015-09-25/vets-prosecuted-over-hendra-cases/6801422 This is in contrast with the APVMA that analysed data and granted registration of the vaccine. *"The potential for a vaccinated horse to pass on the Hendra virus cannot be ruled out. As a precaution, it is recommended people take the* ***same steps*** *to protect vaccinated horses from exposure to infection—and to prevent humans being infected by horses—as are recommended for unvaccinated horses. Personal protective equipment should be worn whenever infection is suspected even in vaccinated horses."* This advice was added in 2015 by the APVMA when the registration was approved for Equivac HeV. Wording in the Work Place Health and Safety Guidelines and the AVA should more clearly reflect this above advice from the registering body. There should be consistency between APVMA, AVA and WHSQ. Applying the precautionary principle, one should extend caution to vaccinated horses, the same as unvaccinated, to avoid potential risk of exposure in this group.

What is the motivation for the Work Place Health and Safety Queensland investigations and prosecutions - to reduce risk and prevent human infections? There have been 7 human infections in over 21 +years resulting in 4 deaths. 2 deaths occurred as a result of infection with Hendra virus contracted before it was even identified in 1994, there were a further 2 deaths in 2008 and 2009. Since 2009, awareness has grown about HeV, the use of PPE has become more standard and there have been no further human cases of infection despite a spike in horse cases in 2011. Vets have obviously broken the "chain of transmission from bats to horses to humans" themselves through their own changed practices. So for each year after 2009, there were zero infections and zero human deaths as a result of Hendra virus infection, now it is 2016. I compare this to workplace deaths from other occupations and find, according to a US study, fishermen and construction workers each had over 1000 deaths per million workers per year. http://wwwnc.cdc.gov/eid/article/11/7/04-1038t3 These are US statistics, but surely there are other occupations in Australia that need the attention of WHSQ where there have been injuries and fatalities more than the veterinarians, with zero infections and zero fatalities for 7 years due to Hendra virus infection. A young construction worker died last year falling off a roof down the road, and this sad fatality hardly made it into the news. If it had been a Hendra virus fatality there would have been national coverage for an extended period of time.

Work Place Health and Safety investigation should be an excellent tool to ascertain deficiencies that could lead to better standards and reduction in work place injury or infection. The way it is being used at the moment for aggressive prosecution of vets is malicious and unfathomable to me. If the agenda was to promote safer veterinarian and client health care and reduction in HeV transmission risk, a more positive and cooperative approach would be preferable.

Some possibilities of a positive approach to possible deficiencies identified in patient/client management in the WHSQ investigation:

A WHSQ case that I have heard of could be typical of a common scenario these days. The vet ordered a test on an unvaccinated, unwell horse to exclude Hendra virus, but thought Hendra was unlikely. It took 4 days for the HeV PCR result to come back, by which time the horse had already been euthanized and buried. There may have been human exposures, which would be less of an issue if more rapid diagnostic testing was available. Obviously there is a problem with turnaround time on the testing. PCR HeV testing is done once a day, only on weekdays, at 2 pm at Biosecurity Queensland's laboratory at Coopers Plains. Results are usually reported to the submitter within one to two working days of receiving the samples. (In private human medicine urgent pathology testing is usually same day, depending on what it is.) There are possibilities for urgent testing, however, if there has been significant human exposure to body fluids of a suspect horse. (from the guidelines for vets, version 5.1). Even using a courier service, it can take time as many people live in rural Queensland, and weekends are unavoidable. The knock on effects of this sometimes tardy process is that people may not be as likely to be careful, including owners even if they are told and they already know that Hendra is a possibility, than if they do know it is Hendra. Also if high risk exposure is suspected in anyone, monoclonal antibody treatment for compassionate use is available and most effective close to the time of exposure. Prompt confirmation is in everyone's best interest, (except maybe a recovered horse that may have to be euthanized). I have read about an on the spot HeV PCR that may become available. If introduced, it would be useful and could precede a more formal lab analysis if equivocal. Why doesn't WHSQ work at ways of rectifying this delay in testing and reporting, rather than focus on the possible issues that could have emanated from this delay.

Other ideas that may help with possible identified issues with the WHSQ investigation are deficiencies in client and possible contacts advice. Some useful suggestions from WHSQ could be for example well prepared print out sheets to give owners for undiagnosed sick (vaccinated or not) horses, in which Hendra is part of the list of possibilities, that are not coercion or blame based, but informative to protect people and companion horses, so they can read over later, when emotions settle, that is standard. When people are stressed little of what they told is taken in, understood and remembered. At the time of euthanizing a horse, give owners another handout re safe carcass disposal, even in non-Hendra diagnosed cases for owners to pass onto to the machinery operator. A lot of information is already available on the AVA site, it would be good to have it streamlined.

Vets are educated to be animal health practitioners, they can't be responsible for owner's behaviour, appropriateness and monitoring of containment facilities, and so on, but they can educate by advice and giving out a standard brochure for example. Luckily, WHSQ changed a recommendation in the 5.0 version of "Guidelines for veterinarians handling potential Hendra virus", stating that vets needed to "Advise neighbours that a horse is being investigated for HeV infection" to being an owner undertaking. It would be understandably frustrating for vets to be on the phone or visiting all the bordering properties (especially if there are many) for every case of colic with Hendra way down the list of possibilities. The guidelines are bordering on ridiculous with clauses such as this. Those 50 pages of guidelines that the vets are being prosecuted over, should be reviewed and made simpler and more realistic. If Workplace Health and Safety, and Biosecurity Queensland keep putting more of the onus on vets and divest some of what should be

government responsibilities, they need to pay vets for their time. Government employees get paid for this work, checking on containment and whether advice is followed through or not, but the vets don't get reimbursement for this.

It does mention in the *"Guidelines for veterinarians handling potential Hendra virus infection in horses"* referenced by WHSQ and from the QLD government that "comments on its content are encouraged", so points to consider follow.

One further area I feel that could be improved for clarity would be in the pathology testing for Hev. Those vets not up on all of the nuances of pathology testing could possibly become confused in reading this section on laboratory testing. I refer to the Laboratory testing - 9.3.1 "Hendra testing with vaccinated horses" in the Guidelines above. It doesn't even mention that the primary test in an acute case, that would help determine a Hendra virus infection is the PCR test for HeV, irrespective of vaccination status. If a vet were to just look up "Hendra virus testing with vaccinated horses" which just discusses serology, and the person may not know much about the tests available, would come away from reading that section with the idea that it was difficult to test for Hendra virus infection and not bother in the vaccinated horse. It just needs to be clearer I think. (PCR testing is discussed 9.3 but it doesn't mention vaccinated horses - I just thought it confusing how it is set out).

Serology tests are useful, but horses may not have detectable titres until 10-14 days after infection. (Hendra Virus Infection, The Centre for Food Security and Public Health, ISU, Dec 2015)

Lastly, I note in the section under Disinfectants 8.5 it says "specific testing of disinfectant compounds against HeV has not been conducted." I came across an article, *"Antiviral activity of gliotoxin, gentian violet and brilliant green against Nipah and Hendra virus in vitro*", Aljofan et al, 2009, which discussed screening of 8,000 small molecules for antiviral activity and demonstrated potent antiviral activity of three commercially available compounds. It mentioned that they may be too toxic for systemic use, but may be useful as topicals and antiseptics in field situations. I don't know if they are used here in Australia at all, but they seem to be available in the USA.

If Work Place Health and Safety QLD, instead of vindictively prosecuting vets and creating a future, possibly irrevocable, breakdown of relations and trust between WHSQ and vets, wouldn't it be more proactive to examine what they consider to be the deficiencies in the handling of potential Hendra positive cases and work out positive approaches that will improve the system. Surely a better way forward is to instil a spirit of co-operation and approachability to government bodies leading towards safer, better management and care, rather than this fear-based persecution.

In Conclusion

Some horses have had what owners consider **severe side effects** from vaccination and choose not to continue vaccinating with Equivac HeV. Some other owners elect not to vaccinate at all as they are more **concerned about vaccination adverse reactions** in an otherwise healthy horse, which have been undeniably documented. Others are **happier vaccinating,** as they feel more comfortable to help prevent the real but relatively rare possibility of Hendra virus infection, or for reasons such as still being able to access veterinary care, or to compete in vaccinated only horse events. There have been **sweeping generalizations** as to the almost infallible efficacy of the vaccine. I believe this has been based on very little reliable data from efficacy and longer term serology studies in horses in real world conditions. This belief that the vaccine is virtually 100% effective reduces the vet's and owner's alertness to the possibility of Hendra in a vaccinated horse, which presents other risks to health concerns. **All these concerns are valid.** There should be objectivity and transparency, rather than emphasizing only one perspective, for example to pursue a pro-vaccination agenda and downplaying, ignoring or not releasing any data that could tarnish the 100% effective image. The converse of course would be true for those that only focus simply on adverse reactions to the vaccine. There has to be a middle ground that can consider all these real concerns rather than one blanket black or white approach that serves only one agenda as to whether to vaccinate or not. I believe pointless confrontation on these various issues is not constructive but the way forward is possible in a spirit of objective and respectful co-operation.

About Me: I am a horse owner and a general practitioner (doctor), with some science background. I have been following the research on occasion from before the Hendra vaccine was released with interest. Like most people involved in the horse world, I feel the situation emanating from issues about the Hendra virus and its vaccine have become untenable, which has brought about this inquiry. I thought, as there was to be a Parliamentary inquiry, that I should prepare a submission, and hope it is a useful contribution. Sorry it has some redundancy in each of the different terms of reference, but find there was a degree of overlap in answering each item.