Maternal Antibody Acquisition and Decline to Arboviruses and After Hendra Virus Vaccination

Dr Allison J. Stewart[1]

[1]The University of Queensland, School of Veterinary Science, allison.stewart@uq.edu.au

Keywords: Horse, Foal, Immunology, Virus.

1.1 Maternal Antibody

Foals are born immunologically naive at birth. In horses, the 6-layer epitheliochorial placentation prevents in-utero transfer of maternal circulating immunoglobulins to the foetal foal. Mares concentrate antibodies in their colostrum over the last 2-3 weeks of gestation, such that immunoglobulin G (IgG) concentrations are often higher in colostrum verses serum. Mare factors can affect maternal antibody concentrations: age, parity, health status, previous exposure to pathogens and vaccinations.

Foals ingest colostrum in the first 12-24 hours after birth. Intestinal absorption of immunoglobulins occurs in the small intestine by specialized enterocytes by pinocytosis. Colostral absorption is maximal during the first 6 hours of life but declines to zero by 24 hrs of age as the specialized pinocytotic enterocytes are replaced with mature enterocytes that are incapable of immunoglobulin uptake.

Colostrally derived maternal antibodies provide passive immunity for foals over the initial few months of life, but they can also interfere with the foal's endogenous production of antibodies in response to disease exposure or vaccination. The duration of interference by maternal antibody varies between pathogens, and is affected by the maternal colostral antibody concentration and the amount of colostrum ingested by the foal prior to loss of pinocytotic enterocytes. The influence of maternal antibody on a foal's immunologic response to vaccination has been studied after vaccination for tetanus, equine influenza and equine herpes virus types 1 and 4. It has been recommended to delay vaccination of foals that are born to vaccinated mares relative to foals born to unvaccinated mares.

Maternal antibody can also interfere with serological testing of foals. Foals will test positive to pathogens if they have acquired colostral antibodies from the previously exposed dam. This is important for endemic diseases. False positive diagnosis may be made if a serological diagnosis is made from a single sample. A rise in antibody titre (usually ≥ 2 fold serial dilution) after a lag of 14-21 days is required to confirm infection and rule out transfer of passive immunity and previous exposure in the mare. Testing of the mare or asymptomatic herd mates is also advisable.

We have studied the effects of maternal antibody acquisition and decline in a group of foals who's dams were vaccinated against Hendra virus and many of who's mares were naturally infected with orthoflaviviruses.

2.1 Hendra virus

Hendra virus first emerged in Australia in 1994 and is classified as a biosafety level (BSL) 4 organism, which is the highest level of biocontainment. Hendra virus (HeV) infection is a notifiable disease in all states and territories of Australia. Horses act as an amplifying host and are the only known mammalian species that has been infected directly from bats. Direct (ocular/nasal or oral mucous membrane contact when horses are resting or browsing under trees in which flying-foxes roost) or indirect contact (pasture or feed contaminated) with flying-fox urine is thought to be the main route of transmission of the disease from bats to horses. Other body fluids such as aborted fetuses or associated fetal fluid, blood, feces, nasal discharge, and saliva are less likely but potential transmission sources. Bats infected with HeV appear to be asymptomatic.

The incubation period of HeV in horses is between 4 and 16 days, and in experimentally infected horses shedding of virus occurred up to 5 days before development of clinical signs. There are no clinical signs pathognomonic for HeV infection in horses, and clinical signs are often non-specific and variable. HeV infection is often characterised by rapid deterioration in acutely affected horses. Horses infected with HeV predominately demonstrate clinical signs related to the respiratory and neurological systems, although non-specific early signs such as fever, depression, inappetence and restlessness are very common. Neurological manifestations of the disease include ataxia, disorientation, head tilt, facial nerve paralysis, circling, and seizures. Laboured breathing, tachypnoea, frothy or blood-tinged nasal or oral discharge are not uncommon terminal signs with respiratory involvement. Sudden death and colic have also been reported.

Infected horses can amplify the virus, and spread the disease via aerosol or fluid transmission to other horses and humans. So far, humans have only been infected directly from horses, and not from bats or other humans. Horse-to-horse and horse-to-human transmissions are likely via contact with infected bodily fluids, especially nasal or oral secretions, from an infected horse during all stages of disease from preclinical to post-mortem. Hendra virus (HeV) is a low incidence high consequence pathogen with high case fatality rates of 80% in horses and 57% humans.

Disease prevention in horses and humans is by vaccination of horses using the Equivac® HeV vaccine. The subunit vaccine contains recombinant HeV soluble G (sG) glycoprotein. This was the first vaccine commercialised against

a BSL-4 agent and currently is the only commercially licensed prophylactic treatment for HeV. There is no commercially available vaccine for humans, however, a human vaccine based on the same immunogen as Equivac® HeV using HeV-sG is now in phase 1 clinical trials.

It is recommended that horses older than four months of age commence the vaccination schedule, with first two doses given three to six weeks apart and a third dose six months after the second, followed by annual boosters. Pregnant mares should not be vaccinated during the first 45 days of gestation or two weeks prior to parturition. Vaccination of foals born to vaccinated mares should have their primary series of vaccines delayed and the current recommendation was to commence the vaccination schedule at six months of age.

Virus neutralisation test can be used to detect antibodies after vaccination. The test can only be conducted at the Australian Centre of Diesase Preparedness (ACDP) and is expensive. It requires use of live virus and a BSL-4 laboratory. A high throughput Luminex ELISA has recently been validated and may become commenrcially available allowing for cheaper titre testing.

In an experimental study, horses that had a HeV titre ≥ 1:16 did not develop clinical signs and survived while unvaccinated horses succumbed to infection. Antibody titres ≥1:32 are considered protective. Foals may represent an overlooked source of infection for in-contact humans. The duration of persistence of maternal antibody or the best time to vaccinate foals with the HeV vaccine to ensure vaccine efficacy but decrease the time foals have unprotective titres (<1:32) was previously unknown.

Our group measured the serum neutralising antibody titres (SNT) on 37 foals that had periodic blood sampling between 0 and 57 weeks-of-age. Foals were vaccinated against Hendra virus at 24, 27 and 54 weeks-of-age. All foals had detectable maternal Hendra antibody titres at 24 weeks (Geometric mean ± SD: 50.8 ± 4.4) with 16/32 foals having SNT ≥1:32. Three weeks after the first HeV vaccine 28/29 had detectable titres and 19/29 had SNT ≥1:32. Three weeks after the second HeV vaccine 28/30 had detectable titres and 23/30 had SNT ≥1:32. Prior to the 3rd vaccine 10/15 had detectable titres and 5/15 had SNT ≥1:32. Three weeks after the third HeV vaccine 9/9 had detectable titres and 7/9 had SNT ≥1:32. Foals that had maternal antibody titres <1:32 had a greater response to their first and second HeV vaccines than foals with titres ≥1:32 (p = 0.017 and 0.0025, respectively).

Ideally titre testing of foals at 6 months of age should guide whether HeV vaccination should be delayed due to persistence of maternal antibody. Vaccination of foals with maternal antibody has poor efficacy.

3. Ortho-flaviviruses

Pathogenic *Orthoflaviviridae* affecting Australian horses include Murray Valley encephalitis, Japanese encephalitis and Kunjin strain of West Nile viruses. Our group aimed to determine the changes in maternally-derived orthoflavivirus antibodies in foals and natural infection to orthoflaviviruses.

For our 34 mare/foal pairs, 90% of mares were serologically positive to an orthopan-flavivirus blocking ELISA with a cut off of 40% inhibition considered positive. All foals testing negative on pre-suckle samples, but 65% tested positive at 24 hours of age after ingestion of colostrum. By 5 months of age only 1 foal tested positive indicating decline in the maternally derived antibody. By 2 years of age 57% of foals tested positive, indicating natural infection with at least one orthoflavivirus. In 2022 Japanese encephalitis virus became endemic in Australia and many of the horses on our campus were asymptomatically infected. Only 1 foal showed any neurological signs. Although Japanese Encephalitis virus can cause severe neurological signs in humans and horses, most cases are asymptomatic. Even if serological tests are positive, a rising titre should be used to help determine if the neurological signs are due to current infection. Other differentials such as HeV, MVEV, EHV1, Australian Bat Lyssavirus should always be considered in addition to non-viral causes of neurological disfunction.

4. Further reading:

Wang X, Wise J, Stewart AJ. *Hendra Virus: An update on diagnosis, vaccination and biosecurity protocols for horses*. Vet Clin Nth Am: Equine DOI: 10.1016/j.cveq.2022.11.009

Yuen KY, Fraser NS, Henning J, Halpin K, Gibson JS, Betzien L, Stewart AJ. *Hendra virus: epidemiology dynamics in relation to climate change, diagnostic tests and control measures.* One Health https://doi.org/10.1016/j.onehlt2020.100207 2021:12:100207 2021-06, Vol.12, p.100207

Stewart AJ, Habarugira G, Halpin K. *Maternal antibody and response to vaccination against Hendra virus in foals*. Australian Veterinary Association Conference Melbourne, May 2024 and Australian Veterinarians of Australian Bain Fallon Memorial Conference, Hunter Valley, July 2024.

Wang X, Meers J, Wise J. *Flavivirus maternal antibody positivity and natural infection in foals*. American College of Veterinary Internal Medicine Forum. Minneapolis, MN, USA, June 2024 and Australian Veterinarians of Australian Bain Fallon Memorial Conference, Hunter Valley, July 2024.