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Investigation of the effect of Equivac® HeV Hendra virus vaccination on Thoroughbred racing performance

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Objective To evaluate the effect of Equivac® HeV Hendra virus vaccine on Thoroughbred racing performance.

Design Retrospective pre-post intervention study.

Methods Thoroughbreds with at least one start at one of six major south-eastern Queensland race tracks between 1 July 2012 and 31 December 2016 and with starts in the 3-month periods before and after Hendra virus vaccinations were identified. Piecewise linear mixed models compared the trends in 'Timeform rating' and 'margin to winner' before and after initial Hendra virus vaccination. Generalised linear mixed models similarly compared the odds of 'winning', 'placing' (1st–3rd) and 'winning any prize money'. Timeform rating trends were also compared before and after the second and subsequent vaccinations.

Results Analysis of data from 4208 race starts by 755 horses revealed no significant difference in performance in the 3 months before versus 3 months after initial Hendra vaccination for Timeform rating (P = 0.32), 'Margin to winner' (P = 0.45), prize money won (P = 0.25), wins (P = 0.64) or placings (P = 0.77). Further analysis for Timeform rating for 7844 race starts by 928 horses failed to identify any significant change in Timeform rating trends before versus after the second and subsequent vaccinations (P = 0.16) or any evidence of a cumulative effect for the number of vaccines received (P = 0.22).

Conclusion No evidence of an effect of Hendra virus vaccination on racing performance was found. The findings allow owners, trainers, industry regulators and animal health authorities to make informed decisions about vaccination.

Keywords Hendra virus; racing performance; Thoroughbreds; Timeform rating; vaccination

Abbreviations APVMA, Australian Pesticides and Veterinary Medicines Authority; HeV, Hendra virus

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Hendra virus (HeV: genus *Henipavirus*) is a zoonotic paramyxovirus of major veterinary and public health significance that first emerged in Queensland in 1994. Sporadic spill-over events from its natural host, the flying fox/fruit bat (genus *Pteropus*), to horses and from horses to humans, have occurred in Queensland and northern New South Wales.[1–3] The most likely mode of transmission is contact with infected flying fox urine on equine oronasal and/or conjunctival mucosa, either directly or via urine-contaminated pasture or surfaces.[2] Infected horses are further able to transmit HeV to other horses, dogs and humans, acting as an amplification host for the virus.[3,4]

Clinical signs of HeV infection in horses are highly variable and non-specific, and infection has been confirmed in some cases in the absence of or with only minimal clinical signs, including, in some instances, a lack of pyrexia.[5] Acutely infected horses usually display depression and a range of general, respiratory and/or neurological signs, mediated by an endothelial vasculitis, such as fever, loss of appetite, abdominal pain, muscle fasciculations, terminal nasal discharge, ataxia and apparent loss of vision.[5] Horses may shed the virus prior to developing clinical signs.[6,7] Transmission from horses to humans requires exposure to infectious body fluids, blood or small droplets.[6] To date there have been 102 equine deaths caused by HeV infection or exposure, resulting in 19 human exposures. Seven human cases of infection have occurred, four of whom have died, giving a high human case fatality rate of 57%.[8] As yet there are no approved therapeutics available for human use;[9] however, monoclonal antibody therapy has been offered as post-exposure therapy on compassionate grounds to 13 people following natural exposure to infected horses and a further 2 people following exposures in a laboratory setting (P. Reid, pers. comm.).[10] For a localised HeV outbreak, the average response cost and the economic loss due to horse deaths are estimated to amount to A$30,660 per horse.[11]

Following the two most recent human fatalities in 2008 and 2009, and the unprecedented number of outbreaks in horses in 2011,[10] an equine HeV vaccine was developed and approved for use to reduce disease transmission and infection risk in November 2012.[12] The vaccine has been promoted as 'the single most effective way to reduce the risk of exposure to Hendra virus' in animal health authorities' communications to horse owners.[13–15] The subunit HeV vaccine is based on the HeV G glycoprotein, required by the virus for attachment to host cells and neutralising antibodies directed against this protein were found to prevent infection and disease.[12]

The vaccine release occurred under special permit from the Australian Pesticides and Veterinary Medicines Authority (APVMA). Conditions of this and a subsequent permit required all vaccinated horses to be microchipped. The vaccine was fully registered in August 2015. Initially, horses were required to be booster-vaccinated every 6 months following a primary course of two doses administered between 21 and 42 days apart. A vaccination schedule change from 6-monthly to 12-monthly boosters following the initial 6-month booster was approved in May 2016.

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Uptake of the vaccine has been moderate, with over 550,000 doses having been administered to approximately 140,000 horses Australia-wide (up to November 2017; R. L'Estrange, pers. comm.). The total number of horses in Australia is unknown; however, domestic horse numbers were estimated at just under 1 million in 2007,[16] suggesting a vaccination uptake of approximately 14%. The Australian Veterinary Association recommends that all vaccination events and horse details are recorded on a vaccination registry to allow all vaccinated horses to be monitored and traced.[15]

Reported adverse reactions to the vaccine include injection site reactions (swelling), fever, muscle stiffness and general depression. The rate of probable and possible adverse reactions, as calculated by the APVMA, based on over 513,890 doses of vaccine administered from the launch of the vaccine to 31 March 2017, was approximately 0.23%, with the majority being injection site reactions (0.18%).[17] Concerns regarding adverse reactions and reduced performance are frequently given as reasons for non-adoption of vaccination by some horse owners.[18,19] As part of a Queensland Parliamentary Inquiry into the HeV vaccine and its use by veterinarians in Queensland, the APVMA, the vaccine manufacturer Zoetis and the Australian Veterinary Association argued that the levels of adverse reactions to the HeV vaccine are low and well within the normal acceptable range for other types of vaccines.[20]

Other individuals and performance horse association representatives lodged their concerns regarding vaccine safety at the Inquiry. Representatives of the Queensland Thoroughbred Breeders Association made public claims of anecdotal evidence relating to alleged poor performance in some horses following HeV vaccination.[20,21] Other prominent trainers and veterinarians have disputed these claims.[22] The Queensland Parliamentary Inquiry noted that the fear of adverse reactions is a major factor in some owners' decisions not to vaccinate their horses.[20]

In their submission to the Inquiry, the Queensland Endurance Rider Association Incorporated outlined the substantial veterinary involvement in their sport and the fact that many veterinarians were withdrawing their services to this industry because of the absence of a mandatory HeV vaccination policy. This was based on the veterinarians' concerns about the possibility of an investigation by Workplace Health and Safety Queensland into any potential HeV case occurring at an event. Three investigations have occurred to date, leading to the prosecution of the veterinarians involved. All three cases arose as part of routine equine consultations, highlighting the significant responsibilities for animal and human health held by veterinarians in such scenarios.[20]

Another horse performance industry body, Equestrian Queensland (representing the Olympic equestrian disciplines), indicated that the majority of their members oppose mandatory vaccination, based on their perception of encroachment on civil liberties entailed in the removal of free choice on whether to vaccinate with a product subject to under-reporting of known cases of adverse reactions.

Meanwhile, the Brookfield Horse and Pony Club Incorporated Management Committee indicated that vaccination had been compulsory for their members since 2013 and that there have been no serious side effects or membership withdrawals because of fear of adverse effects from vaccination.[20] The Queensland Horse Council also fully supported the vaccine.[20] The issues of adverse reactions and potential performance effects caused by HeV vaccination have clearly polarised opinion within the Australian horse industry. This jeopardises the important relationship between horse owners and veterinarians that is fundamental to maintaining satisfactory horse welfare standards and optimising emergency disease investigation efforts, which has great public health significance.

To date, no objective evidence exists regarding the potential effects of Hendra vaccination on horse performance. Indeed, to the authors' knowledge, there is no other comparable study that has been conducted anywhere in the world examining the effects of any other vaccinations on horse performance. Therefore, this study aimed to evaluate the potential effect of Hendra vaccination on Thoroughbred racing performance. The specific objectives of the study were: (i) to compare the trend in Timeform rating and 'margin to winner' in the 3 months before versus after administration of initial Hendra vaccination; (ii) to compare the trend in Timeform rating in the 3 months before versus after administration of the second and subsequent Hendra vaccinations to test for a potential cumulative effect of vaccination on performance; and (iii) to compare the odds of winning any prize money, winning and placing (1st–3rd) in the 3 months before versus after administration of initial Hendra vaccination.

Materials and methods

The study was conducted as a retrospective pre-post intervention study by comparing horse performance data for vaccinated horses for the 3-month periods before and after Hendra vaccinations. The target population for the study was Australian Thoroughbreds and the study population was horses racing at least once at one of the six major south-eastern Queensland race tracks (Eagle Farm, Doomben, Ipswich, Toowoomba, Gold Coast, Sunshine Coast) between 1 July 2012 and 31 December 2016. These inclusion criteria were set to be representative of horses racing in competitive races in areas considered to be high-risk for HeV infection.

Data sources

Data for this study were obtained from four different sources. Racing data for horses racing in Australia between 1 July 2012 and 31 December 2016 were acquired from Racing Australia Limited. Track status (metropolitan, provincial or country) and dates for group and listed races were obtained separately from the Racing Australia Limited website (http://racingaustralia.horse/FAQ/Track-Information.aspx). For the dates on which metropolitan status race days were held at provincial tracks (including group or listed race days), the track status was adjusted from provincial to metropolitan status to reflect the larger amounts of prizemoney available to attract participation by higher performing horses. These data sets were linked to the performance data by track name.

Timeform rating performance data for each race start were obtained from Racing and Sports Proprietary Limited (https://www.racingandsports.com.au/en/), matched by horse name. Vaccination data for selected horses were obtained from Zoetis Incorporated (https://www.zoetis.com.au/). The performance and vaccination data

were linked by microchip number and the vaccination status for each race start was determined. Horses were considered vaccinated for the purpose of initial selection and primary analysis if they had received their first Hendra vaccination during the study period and had race starts before and after this vaccination event. Following this initial selection, we identified horses that had race starts in the 3-month periods before and after initial vaccination for the primary analysis presented here. For further analysis, the second dose or any subsequent Hendra vaccination received by a horse was also considered. The distribution of race starts by vaccination event was described. The 3-month periods before and after vaccination were chosen to minimise the potential for known time-varying confounding whereby performance changes with horse age, but also to allow for several race starts before and after vaccination to assess trends over time.

Outcome variables

The primary outcome variable was the Timeform rating, expressed in pounds of weight, for each horse's performance in a race https://www.racingandsports.com.au/en/. Since 1948, Timeform ratings have been an internationally recognised standard for the global measurement of Thoroughbred racetrack performance. The Timeform rating was selected over the principal racing authority rating in use by each state, because it allows direct comparison of horse racing merit across all jurisdictions and age groups. Factors considered in compiling a final Timeform rating for each performance are pre-race horse ratings (Timeform ratings compiled from each runner's previous race starts), sex, weight carried and the beaten margin for each runner, the race time and sectional times (if available), as well as the race class, type of racing surface and track condition. Timeform rating also offers the advantage of ease of interpretability, being expressed directly in pounds carried by a horse in a race. To this end, 3.3 Timeform rating points are equal to 3.3 pounds, which equates roughly to one horse length at 1200 m. The pounds-per-length conversion decreases with increasing race distance (Gary Crispe, pers. comm.).

Additional secondary performance outcome variables considered for analyses were: (i) 'margin to winner' (in horse length to one decimal point whereby 1 length equals 2.4 m), (ii) total prize money (A$), (iii) placing (1st–3rd; yes/no) and (iv) win (yes/no).

Explanatory variables

Covariates and potential confounders of performance considered for analysis were age in years (to 1 decimal point), sex (entire male, gelding, female), distance raced (sprint: < 1400 m, middle-distance: 1400–2200 m, staying distance: > 2200 m), track status (metropolitan, provincial, country), track type (turf, dirt, sand, synthetic) and track condition (firm, good, soft, heavy). Additionally, the variable 'runs from spell' (the number of race starts since a break of more than 61 days from training and racing) was calculated for each race start. The 'runs from spell' data were categorised into 1st-up, 2nd-up, 3rd-up, 4th–10th run and ≥ 11 runs. This categorisation was based on published performance trends of Australian Thoroughbreds showing improvement in race performance up until the 4th start in a campaign and decline after the 10th start.[23]

Descriptive analyses

The quantitative and qualitative outcome measures were described individually and by vaccination status using summary statistics and tabulation of frequencies and relative frequencies, respectively. The primary outcome measure, Timeform rating in the 3-month periods before and after vaccination, was also plotted in so-called 'spaghetti plots' that illustrate an individual's performance over time. These plots were stratified by age and sex for ease of interpretability.

The demographic factors for race starts were described by tabulating frequencies (counts) and relative frequencies (percentages). All explanatory variables were described individually and cross-tabulated with vaccination status.

Linear mixed modelling

The two quantitative outcome variables, Timeform rating and 'margin to winner', were analysed using linear mixed modelling. A manual forward stepwise approach was used for model building to identify variables that had a significant association ($P < 0.05$) with the outcome variable. Covariates considered for adjustment were age (on a continuous scale), as well as sex, distance, track type, track status, track condition and runs from spell (all categorical). Collinearity among pairs of covariates was tested using Pearson's correlation coefficients or Pearson chi-square tests. If collinearity existed ($|r| > 0.70$ or Pearson chi-square $P < 0.05$), only the variable of each pair that was more strongly associated with the outcome was retained for further analyses. A random effect term was included for horse to account for clustering of starts by each horse. To be able to compare performance trends before and after vaccination, a two-piecewise random coefficient model was fitted.[24] The effect of vaccination on performance trends was tested using a contrast that compared the slopes of the two lines before and after vaccination, respectively. To achieve this, the intercept, time before vaccination (in months) and time after vaccination (in months) were also included as random effects, so that each horse as a cluster had the intercept and the slopes over the two periods. The covariance structure of the data was unstructured to allow for estimation of unique correlations of residuals for each pair of race starts by the same horse. Model fit was assessed by plotting the modelled trend lines for each period against the observed data using model-based marginal means calculated using the 'lsmeans' package in R statistical software[25,26] and the 95% confidence intervals of the marginal means. Additionally, the distribution of standardised residuals was assessed for normality.

Generalised linear mixed modelling

Because of model assumption difficulties, prize money won was categorised into a dichotomous categorical variable (prize money won – yes/no) and analysed using a generalised linear mixed model alongside two other categorical outcome variables: win (yes/no) and placing (1st–3rd; yes/no). The approach to model building was similar to that for the linear mixed models, using a manual forward stepwise approach, testing the same covariates, including a random effect term for horse, an unstructured covariance structure and a significance level $\alpha = 0.05$. A two-piecewise random coefficient model was fitted and the effect of vaccination on the odds of a positive outcome was tested using a contrast to compare the odds before versus

after vaccination. Model fit was assessed using the Hosmer-Lemeshow goodness-of-fit test.

Additional analyses

An additional linear mixed model analysis was conducted to test for a potential cumulative effect for additional vaccines administered. Initial vaccinations were not included in this analysis because of the overlap of the before and after periods for the first and second doses of vaccine, which are to be administered 3–6 weeks apart, and the stipulated requirement not to race for 7 days following vaccination.[27] A fixed effect term for the number of vaccination was included to test for a potential cumulative effect of multiple vaccination events, but otherwise the same model form as for the Timeform analysis was specified.

Additionally, two sensitivity analyses were conducted to test the model assumptions. A random intercept for each horse was assumed for the primary analysis, as descriptive spaghetti plots suggested no marked changes in slope at time of vaccination but large within- and between-horse variation. To test this assumption the final model was re-run excluding the random effect term to create a single population intercept only.

Further, as the 3-month periods before and after vaccination were chosen arbitrarily, a sensitivity analysis using 1-month before and after the vaccination periods was also conducted to further reduce the potential for time-varying confounding.

All statistical analyses were conducted using SAS Enterprise Guide statistical software (v6.1, SAS Institute Inc., 2013) unless otherwise indicated. The University of Sydney Animal and Human Ethics Offices confirmed in writing that no ethical approval was required for this retrospective data linkage project that used existing data only.

Results

There were a total of 40,505 race starts for 1764 horses that received their initial HeV vaccination during the study period. Of those, 12,066 race starts by 1154 horses were eligible for analysis (Table 1). For the primary analysis of race starts within the 3 months before and after vaccination, 4208 race starts by 755 horses were available. The demographic factors of these race starts by vaccination status are presented in Table 2.

The primary outcome variable, Timeform rating, was normally distributed, ranging from 0 to 117 with a mean of 60.9 and a standard deviation of 19.1. There were 11 observations missing for this outcome variable. Large variation in Timeform rating between starts by the same horse and between starts of different horses were evident in the age- and sex-stratified 'spaghetti plots'; however, overall a linear, banded trend was discernible in all plots (Supplementary Figures 1–12). The two quantitative secondary outcomes, total prize money earned and 'margin to winner', were positively skewed with medians of A$425 (interquartile range: A$0–2,235) and 3.5 lengths (interquartile range: 1.3–6.5 lengths), respectively. There were 535 wins (12.7%) and 1513 placings (1st–3rd; 36.0%) recorded for the 4208 race starts.

Comparison of trends in Timeform rating and 'margin to winner' before and after vaccination

A total of 4208 race starts by 755 horses in the 3 months before and after initial Hendra vaccination were considered for analysis. The rate of performance change during the 'before vaccination period' as measured by the Timeform rating was slightly but not significantly decreasing (estimate = −0.54 ± 0.29 point per month; P = 0.066) and the rate of performance change in the period following vaccination was also not significantly different from 0 (estimate = −0.04 ± 0.25; P = 0.86; Table 3; Figure 1). The difference in rates was not significant (Difference = 0.49 ± 0.49; P = 0.32). Sex, track type, track status, track condition and distance raced were other factors that were associated with the Timeform rating. The results have been adjusted for these variables.

The variable 'margin to winner' was square-root transformed for the analysis to meet model assumptions. Similarly to the results for initial Hendra vaccination and Timeform rating, no significant difference in trend before versus after vaccination was observed for

Table 1. Distribution of vaccination events and eligible race starts to pre-post vaccination analysis

Vaccination event	All horses[a]	%	Eligible horses[b]	%	Race starts by eligible horses (n)	%
1	755	32.2	755	36.6	4208	34.9
2	693	29.5	680	32.9	3885	32.2
3	404	17.2	347	16.8	2178	18.1
4	225	9.6	155	7.5	949	7.9
5	137	5.8	85	4.1	578	4.8
6	84	3.6	30	1.5	191	1.6
7	38	1.6	8	0.4	51	0.4
8	9	0.4	4	0.2	26	0.2
9	2	0.1	0	0	0	0.0
Total	1764		1154		12,066	100.0

[a]All horses that raced at least once at one of six major Queensland race tracks and received their initial Hendra virus vaccination between 1 July 2012 and 31 December 2016.
[b]Eligible horses with race starts in the 3 months before and the 3 months after the vaccination event.
The same horse is counted for multiple vaccination events.

Table 2. Demographic information of 4208 race starts by vaccination status used for comparison of racing performance in the 3 months before versus 3 months after initial Hendra virus vaccination in a study of 755 Australian Thoroughbred horses

Variable	Category	Not vaccinated (n = 2210)		Vaccinated (n = 1998)		Total (n = 4208)	
		n	Row %	n	Row %	n	Total %
Sex							
	Entire male	77	57.9	56	42.1	133	3.2
	Gelding	1216	52.3	1109	47.7	2325	55.2
	Female	917	52.4	833	47.6	1750	41.6
Age[a] (years)							
	< 3	179	69.9	101	36.1	280	6.7
	3	665	58.3	476	41.7	1141	27.1
	4	662	50.1	658	49.9	1320	31.4
	5	398	49.1	413	50.9	811	19.3
	6	203	47.5	224	52.5	427	10.1
	≥ 7	103	45.0	126	55.0	229	5.4
Distance (m)							
	Sprinter (≤ 1400 m)	1664	52.5	1505	47.5	3169	75.3
	Middle (1401–2200 m)	478	51.5	450	48.5	928	22.1
	Stayer (> 2200 m)	68	61.3	43	38.7	111	2.6
Track status							
	Metropolitan	666	54.8	549	45.2	1215	28.9
	Provincial	885	53.0	784	47.0	1669	39.7
	Country	659	49.8	665	50.2	1324	31.4
Track type							
	Turf	2135	52.3	1950	47.7	4085	97.1
	Dirt	18	48.7	19	51.3	37	0.9
	Sand	27	51.9	25	48.1	52	1.2
	Synthetic	30	88.2	4	11.8	34	0.8
Track condition							
	Firm	45	36.0	80	64.0	125	3.0
	Good	1513	50.0	1514	50.0	3027	71.9
	Soft	454	60.0	303	40.0	757	18.0
	Heavy	198	66.2	101	33.8	299	7.1
State of race							
	QLD	1689	50.7	1643	49.3	3332	79.2
	NSW	452	56.8	344	43.2	796	18.9
	Other (VIC/NT/SA/WA)	69	86.3	11	13.7	80	1.9
Runs from spell[23]							
	1st-up	429	55.2	348	44.8	777	18.5
	2nd-up	377	56.9	286	43.1	663	15.8
	3rd-up	327	57.7	240	42.3	567	13.5
	4th–10th run	956	49.3	983	50.7	1939	46.1
	≥ 11 runs	121	46.2	141	53.8	262	6.2

[a]Age category calculated based on the difference between foal date and race date. NSW, New South Wales; NT, Northern Territory; QLD, Queensland; SA, South Australia; VIC, Victoria; WA, Western Australia.

square-root transformed 'margin to winner' (Difference = −0.03 ± 0.04; P = 0.45; Table 3). Track status, track type, track condition and distance raced were covariates significantly associated with 'margin to winner' and were adjusted for in the analysis.

Comparison of odds of winning, placing and winning any prize money

Analysis of the three dichotomous outcome variables (winning, placing and any prize money won) considered 4208 race starts by 755 horses

Table 3. Piecewise, multiple linear mixed model for Timeform rating and square-root transformed 'margin to winner' in the 3 months before versus the 3 months after initial Hendra virus vaccination for 4208 race starts by 755 Australian Thoroughbred horses

Parameter	Category	Timeform rating (points/lb)[a]		Margin to winner (lengths)	
		Estimate (± SE)	P value[b]	Estimate (± SE)	P value[b]
Intercept		63.6 (± 0.97)	< 0.001	1.66 (± 0.05)	< 0.001
Time before vaccination (months)		−0.54 (± 0.29)	0.066	0.04 (± 0.02)	0.09
Time after vaccination (months)		−0.04 (± 0.25)	0.86	0.01 (± 0.02)	0.71
Track status					
	Metropolitan	0	0.008	0	< 0.001
	Provincial	−0.94 (± 0.54)		−0.11 (± 0.04)	
	Country	−2.01 (± 0.68)		−0.24 (± 0.05)	
Track type					
	Turf	0	< 0.001	0	0.002
	Dirt	−6.26 (± 2.04)		0.27 (± 0.17)	
	Sand	−6.34 (± 2.24)		0.24 (± 0.19)	
	Synthetic	−8.40 (± 2.22)		0.66 (± 0.19)	
Track condition					
	Good	0	< 0.001	0	< 0.001
	Firm	1.08 (± 1.14)		−0.12 (± 0.10)	
	Soft	−1.44 (± 0.50)		0.14 (± 0.04)	
	Heavy	−3.18 (± 0.77)		0.23 (± 0.07)	
Distance raced					
	Sprinter	0	< 0.001	0	0.011
	Middle-distance	2.42 (± 0.57)		0.13 (± 0.05)	
	Stayer	6.15 (± 1.37)		0.20 (± 0.11)	
Sex					
	Gelding	0	< 0.001		–
	Entire male	−2.91 (± 2.42)			
	Female	−5.33 (± 1.17)			

[a]Model for Timeform rating includes data for 4197 race starts by 755 horses because of 11 missing values for this outcome variable;
[b]P value for type 3 tests of the fixed effect for the entire variable. SE, standard error.

in the 3-month periods before and after initial Hendra vaccination. None of the three final models found a significant difference in odds before versus after vaccination (Table 4). The estimated difference in the odds of winning any prize money before versus after vaccination was 0.094 ± 0.082 (P = 0.25). Similarly, the differences in odds for winning and placing (first to third) were −0.050 ± 0.106 (P = 0.64) and 0.022 ± 0.077 (P = 0.77), respectively.

Additional analyses

Further analysis for the second and subsequent Hendra vaccinations based on data for 7844 starts by 928 horses, which started in the 3-month periods before and after their second and/or subsequent vaccinations revealed no significant difference in the trend in performance before versus after vaccination events (estimate = −0.53 ± 0.38; P = 0.16; Table 5). There was no cumulative effect for the number of vaccinations received (P = 0.22).

A sensitivity analysis excluding a random effect for intercept for each horse in the model for Timeform rating found no demonstrable variation in horses. Compared with the model including random intercepts, the model estimates were very similar and there was no significant difference in the rate of performance change before versus after vaccination (Difference = 1.24 ± 0.74; P = 0.09).

Another sensitivity analysis restricted the time period to only 1 month before and after initial Hendra vaccination. Modelling based on 1115 starts by 352 horses revealed no significant difference in the rate of performance change before versus after vaccination based on the 1-month periods before and after vaccination periods (Difference = −2.72 ± 3.86; P = 0.48).

Discussion

To the authors' knowledge, this is the first scientific study providing objective evidence to assess the potential adverse effects of HeV vaccination on horse performance. The findings of this study indicated there was no difference in performance before versus after initial HeV vaccination for the variables examined. Immune-related adverse reactions to vaccinations are more likely to occur with doses

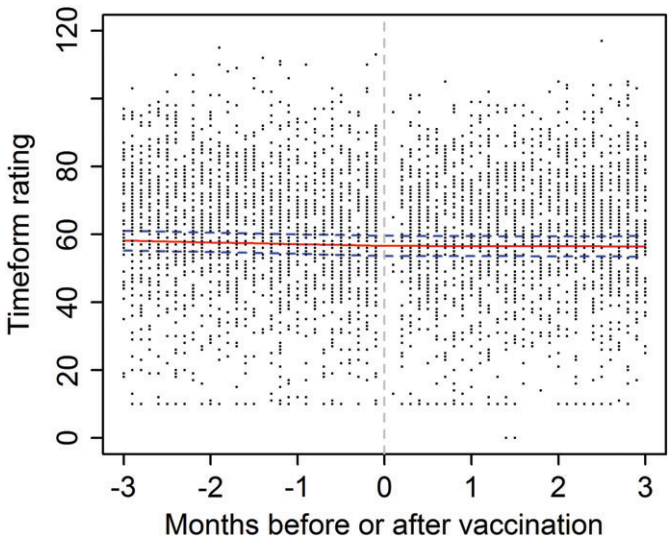

Figure 1. Fitted line plot of two slopes for mean modelled Timeform rating in the 3 months before versus after initial Hendra virus vaccination with observed Timeform rating data for 4197 race starts by 755 horses overlaid. The blue dashed lines contain the 95% confidence interval for the modelled mean.

subsequent to the primary dose.[28] By comparing the performance before and after two or more vaccinations in the same horse, this study also found no evidence of a cumulative effect on racing performance of multiple vaccinations.

These findings have important implications for horse owners, trainers, the Australian horse industry and its regulators, equine veterinarians and government animal health authorities. The most frequently given reasons for non-adoption of HeV vaccination by some horse owners and horse associations have been concerns about the potential for adverse reactions and effects on performance caused by vaccination.[18–20] The findings of this research provide evidence to diminish concerns regarding the HeV vaccine's safety and specifically its potential effect on horse performance. Equine veterinarians may use these results to provide evidence-based recommendations regarding preventive horse healthcare and allow horse owners to make informed decisions regarding HeV vaccination. Similarly, horse racing regulators and those of other performance horse industries, as well as government animal health authorities, may use this information to guide policy decision-making on HeV vaccination. Previous research has suggested that diversification of HeV biosecurity policies and an intervention ladder, ranging from monitoring the current situation and providing information to legislation restricting or even eliminating choice, might provide a useful approach to catering for all subpopulations within the horse industry in increasing biosecurity uptake and managing HeV risk.[29] Likewise, such an approach may be useful for HeV vaccination policy.

Equine HeV vaccination has proved controversial for a variety of reasons, including the way the vaccine was brought to market, workplace health and safety considerations for veterinarians, delays in HeV exclusion testing and implications for equine export and insurance.[18–20,30] Unlike most other vaccines in Australia, the HeV vaccine was initially launched under a Minor Use Permit, which was

Table 4. Generalised linear mixed model estimates for any prize money won, winning, placing (1st–3rd) in the 3 months before versus the 3 months after initial Hendra virus vaccination for 4208 race starts by 755 Australian Thoroughbred horses

Parameter	Category	Winning prize money[a]		Winning[b]		Placing (1st–3rd)[c]	
		Estimate (± SE)	P[d]	Estimate (± SE)	P value[d]	Estimate (± SE)	P value[d]
Intercept		0.59 (± 0.11)	< 0.001	1.36 (± 0.20)	< 0.001	-0.01 (± 0.16)	0.96
Time before vaccination (months)		−0.14 (± 0.05)	0.004	0.028 (± 0.06)	0.64	−0.06 (± 0.05)	0.33
Time after vaccination (months)		0.04 (± 0.04)	0.36	−0.02 (± 0.06)	0.72	0.01 (± 0.05)	0.66
Track status							
	Metropolitan	0 (–)	< 0.001		–	0 (–)	0.004
	Provincial	0.07 (± 0.08)				0.18 (± 0.08)	
	Country	0.74 (± 0.09)				0.30 (± 0.09)	
Track condition							
	Good	0 (–)	0.048		–		–
	Firm	0.20 (± 0.20)					
	Soft	−0.05 (± 0.09)					
	Heavy	0.34 (± 0.14)					
Distance raced							
	Sprinter	0 (–)	0.014		–	0 (–)	0.025
	Middle-distance	0.19 (± 0.09)				−0.05 (± 0.08)	
	Stayer	0.50 (± 0.22)				0.54 (± 0.21)	
Age (years)			–	0.13 (± 0.04)	< 0.001	−0.10 (± 0.03)	< 0.001

[a]Winning prize money Hosmer-Lemeshow P = 0.58; [b]winning Hosmer-Lemeshow P = 0.36; [c]placing (1st–3rd) Hosmer-Lemeshow P = 0.96; [d]P value for type 3 tests of the fixed effect for the entire variable. SE, standard error.

Table 5. Piecewise, multiple linear mixed model of Timeform rating for 7844 race starts by 928 Australian Thoroughbred horses in the 3 months before versus the 3 months after the second and subsequent Hendra virus vaccinations

Parameter	Category	Estimate (± SE)	P value[a]
Intercept		61.9 (± 1.62)	< 0.001
Time before vaccination (month)		0.10 (± 0.21)	0.64
Time after vaccination (month)		−0.43 (± 0.21)	0.04
Sex			
	Gelding	0	< 0.001
	Entire male	1.03 (± 1.54)	
	Female	−4.79 (± 1.07)	
Track status			
	Metropolitan	0	< 0.001
	Provincial	−0.88 (± 0.38)	
	Country	−2.40 (± 0.50)	
Track type			
	Turf	0 (–)	< 0.001
	Sand	−4.81 (± 1.36)	
	Dirt	−4.90 (± 1.26)	
	Synthetic	−9.13 (± 2.17)	
Track condition			
	Good	0 (–)	< 0.001
	Firm	−0.36 (± 0.72)	
	Soft	−1.16 (± 0.36)	
	Heavy	−3.89 (± 0.56)	
Distance raced			
	Sprinter	0 (–)	0.002
	Middle-distance	0.99 (± 0.43)	
	Stayer	3.33 (± 0.99)	
Runs from spell			
	4th–10th run	0 (–)	< 0.001
	1st-up	−2.61 (± 0.42)	
	2nd-up	−0.75 (± 0.42)	
	3rd-up	−0.05 (± 0.41)	
	≥ 11 runs	−0.53 (± 0.64)	
Age (years)		0.83 (± 0.28)	0.003

[a]P value for type 3 tests of the fixed effect for the entire variable. SE, standard error.

issued by the APVMA in 2012 following the exceptionally high number of outbreaks (18) in Queensland and New South Wales during the previous year. Unlike other large animal vaccines in Australia, the HeV vaccine was released for use by registered veterinarians only. In order to be able to administer the vaccine, veterinarians had to undertake accreditation with the vaccine manufacturer. Conditions of use included mandatory reporting of adverse effects and vaccine use exclusively in microchipped horses, with all vaccination events to be recorded in a central animal register maintained by the manufacturer, which was also made available to the Chief Veterinary Officers of each state. The vaccine was fully registered in August 2015 and is the first vaccine in the world to be registered against a biosafety level-4 pathogen.[9]

Despite the sound reasoning behind the vaccine's unconventional release, early views held by some horse owners were that the vaccine was unsafe and insufficiently tested.[18,19] The initial requirement for boosters to be administered at 6-monthly intervals, and the delay in APVMA approval for use in pregnant mares until 2016, were also likely reasons for some to conclude that there may have been early safety and efficacy concerns with the vaccine and that veterinarians' recommendations to vaccinate were largely motivated by financial gain.[18,19,31]

All facets of the safety, efficacy and adverse reaction reporting process were reviewed by the Queensland Parliamentary Inquiry in 2016, with the vaccine being verified to be safe and effective.[20,32]

Because of the very significant human health, horse health and welfare, biosecurity and workplace health and safety implications of HeV disease, the Australian Veterinary Association recommends that the vaccine should only be available to, and transported, stored and administered by registered veterinarians in order to fulfil the current label conditions.[33] The practice of restricting the administration of certain animal vaccines against significant diseases to veterinarians is commonplace internationally; for example, equine influenza vaccine in the UK and several other countries, and rabies vaccine in the USA. Similar restrictions also applied to equine influenza vaccine administration in Australia during the equine influenza outbreak of 2007–08.[34] During this outbreak, 0.04% of the 321,578 doses of equine influenza vaccine administered were officially reported to have caused adverse reactions; however, adverse reactions were likely under-reported, with vaccination teams providing anecdotal evidence of mild adverse reactions, such as transient local swelling at the injection site, being observed in 5–25% of horses vaccinated.[34] This compares with a reported adverse reaction rate of 0.23% for the HeV vaccine.[19]

The regulatory requirement for veterinary-only administration of the vaccine resulted in mistrust of private veterinarians, who were perceived by some in the horse industry to be motivated only by a vested financial interest.[19,31] Studies have also shown evidence of significant levels of distrust towards the vaccine manufacturer by some horse owners in regard to adverse vaccine reactions not being followed up or reported appropriately to the APVMA. Comments made by some horse owners suggest that veterinarians and the vaccine manufacturer were perceived to be in collusion.[19] This perception and resulting lack of trust have likely contributed to the controversy surrounding HeV vaccination. The importance of the veterinarian–client relationship extends beyond healthcare and welfare. It is of enormous industry security, human health and animal health and welfare importance to protect and repair the Australian veterinarian–client relationship. Encouragingly, a longitudinal study of horse owners found that the use and perceived usefulness of veterinarians as a HeV information source appeared to have increased from November 2012 to November 2014.[35]

In HeV-endemic areas, unvaccinated horses presenting with clinical signs referable to HeV are required to undergo HeV exclusion testing (performed at state government laboratories) prior to receiving invasive veterinary treatment or being admitted to a referral equine hospital. Despite significant efforts by the laboratories to deliver timely results, delays of up to 3 days are not uncommon, as a result of transport issues related to remoteness of sample collection site from testing laboratory or laboratory operating hours. A recent survey of Australian equine veterinarians in HeV-endemic areas indicated that such delays have led to compromised horse welfare outcomes, with disease progression ultimately resulting in death in some cases.[36]

Additionally, some equine veterinarians have chosen to decline to treat unvaccinated horses, change their clinic policy when dealing with potential HeV cases, move interstate or leave equine practice altogether to manage the risks associated with this disease.[37,38] Such responses have potential effects on horse welfare and the capacity for timely emergency animal and human disease recognition.

The absence of scientific evidence regarding the effects of Hendra vaccination on horse performance may have contributed to inconsistent veterinary advice to horse owners and trainers, further contributing to the uncertainty regarding the safety of the vaccine. This study delivers critical, unbiased scientific information that will aid horse owners in making informed decisions regarding vaccination of their horses.

Study strengths and limitations

This study had a number of strengths: firstly, the analysis of performance of the same horses before and after vaccination over a short time period allowed for optimal control of confounding because each horse acted as its own control in the analysis. In addition, other confounding variables such as sex, track status, track type, distance, track condition, time since last start and number of runs from a spell were adjusted for during the analyses to reduce bias in estimating the effect of vaccination on horse performance.

However, because of the before-versus-after vaccination study design, only the data for horses with race starts in the 3-month periods before and after a vaccination event were included in the analysis.

To address this limitation, an analysis comparing the performance trajectories of horses that received multiple HeV vaccinations to that of unvaccinated horses could be performed. This would allow all vaccination events to be considered, irrespective of whether the horse started in a race during the 3-month periods before and after each vaccination. However, such a design would have its own limitations, including horse-level confounding variables not encountered in the present study, where each horse acted as its own control.

Another strong point of this study was the access to comprehensive racing performance and Timeform outcome data. For analyses, the study used race data from 755 and 928 horses. The large sample size resulted in very tight 95% confidence intervals, as illustrated in Figure 1, providing a high level of confidence that there is indeed no biologically meaningful change in slope. Furthermore, the use of a mixed model methodology allowed for each race start of each selected horse to be considered rather than calculating summary measures before and after vaccination, as in the case of a paired test. These analyses appropriately accounted for the multiple and variable numbers of starts for each horse, as well as the correlation of performance measures for a particular horse.

The sensitivity analysis for the 1-month periods before and after vaccination included only data for 352 horses, which may reflect the requirement in the Rules of Racing not to race horses for 7 days following vaccination[27] or a tendency for horses to be vaccinated prior to undergoing a short break from training and racing.

Conclusion

This study, the first to provide objective evidence regarding potential performance effects of HeV vaccination, concluded that there is no evidence of a measurable effect of vaccination on racing performance in Australian Thoroughbreds for the performance outcomes investigated. These results provide information that veterinarians can use to support evidence-based preventive healthcare recommendations and can assist horse owners in making informed decisions. Similarly, animal health authorities and regulators of performance horse sports may use this evidence to inform policy decisions regarding HeV vaccination.

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Conflicts of interest and sources of funding

Two of the six authors (EJA and PAR) are private practice equine veterinarians who provided advice on the study design and assessed the manuscript for publication. This study was commissioned and funded by the Queensland Racing Integrity Commission.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site: http://onlinelibrary.wiley.com/doi/10.1111/avj.12679/suppinfo.

Supplementary Figure 1. Spaghetti plot illustrating Timeform rating race performance of individual horses in the 3 months before and after Hendra virus vaccination for entire males of any age.
Supplementary Figure 2. Spaghetti plot illustrating Timeform rating race performance of individual horses in the 3 months before and after Hendra virus vaccination for less than 3-year old geldings.
Supplementary Figure 3. Spaghetti plot illustrating Timeform rating race performance of individual horses in the 3 months before and after Hendra virus vaccination for 3-year old geldings.
Supplementary Figure 4. Spaghetti plot illustrating Timeform rating race performance of individual horses in the 3 months before and after Hendra virus vaccination for 4-year old geldings.
Supplementary Figure 5. Spaghetti plot illustrating Timeform rating race performance of individual horses in the 3 months before and after Hendra virus vaccination for 5-year old geldings.
Supplementary Figure 6. Spaghetti plot illustrating Timeform rating race performance of individual horses in the 3 months before and after Hendra virus vaccination for 6-year old geldings.
Supplementary Figure 7. Spaghetti plot illustrating Timeform rating race performance of individual horses in the 3 months before and after Hendra virus vaccination for 7-year old or older geldings.
Supplementary Figure 8. Spaghetti plot illustrating Timeform rating race performance of individual horses in the 3 months before and after Hendra virus vaccination for less than 3-year old females.
Supplementary Figure 9. Spaghetti plot illustrating Timeform rating race performance of individual horses in the 3 months before and after Hendra virus vaccination for 3-year old females.
Supplementary Figure 10. Spaghetti plot illustrating Timeform rating race performance of individual horses in the 3 months before and after Hendra virus vaccination for 4-year old females.
Supplementary Figure 11. Spaghetti plot illustrating Timeform rating race performance of individual horses in the 3 months before and after Hendra virus vaccination for 5-year old females.
Supplementary Figure 12. Spaghetti plot illustrating Timeform rating race performance of individual horses in the 3 months before and after Hendra virus vaccination for 6-year old and older females.

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