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### Short communication:

## Attack risk on infected properties during the 2007 equine influenza outbreak in New South Wales, Australia

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### Abstract

The aim of this preliminary study was to estimate the proportions of seropositive horses on infected premises (IPs) in order to assess the attack risk of the disease. Logistic regression analyses were conducted to evaluate the differences in attack risks between enterprise sizes and predefined spatial clusters/regions. The average attack risk experienced during the outbreak was 96.88% (median 100%) but it differed according to the size of the enterprise and other geographical and demographic conditions. The highest attack risks were observed in the Dubbo cluster/region and the lowest in the Narrabri–Northern cluster. Properties with fewer horses were generally more likely to have higher attack risks than larger enterprises though this was not true for all cluster/region.

**Keywords** equine influenza, epidemic, seroprevalence

**Abbreviations** EI, equine influenza; IP, infected premises; bELISA, blocking enzyme-linked immunosorbent assay

Equine influenza (EI) is a highly infectious disease with a considerably higher attack risk (proportion of the animals-at-risk that become infected) in naïve horse populations<sup>1</sup> compared to vaccinated or previously exposed populations<sup>2,3</sup>. As expected, very high attack risks were observed during the first EI outbreak in Australia, in 2007, due to the generally naïve horse population. On most infected properties (IPs), all horses became infected within a short period of time, but on some IPs, a proportion of the horses were seronegative when retested a month or more after first being declared an IP. This study was conducted to estimate the attack risk of the disease and to compare the attack risk between enterprise sizes and predefined spatial clusters/regions.

## **Materials and methods**

The EI dataset maintained by NSW Department of Primary Industries was queried to extract the results of blocking ELISA (bELISA) undertaken on horses tested more than 25 days after the properties were first declared to be IPs. Properties on which none of the tested horses were seropositive were excluded from the analysis because they were unlikely to have been infected. Proportions of seropositive horses of the total number of horses tested were calculated for each property as an estimate of the attack risk. To make comparisons of attack risks between enterprise sizes, properties were categorised on the basis of number of horses sampled on each property into small (up to 5 horses), medium (6 to 20 horses) and large (>20 horses) enterprises. Note that in the absence of information about actual number of horses, the numbers of horses sampled were used as a surrogate measure of enterprise size.

Logistic regression analyses were conducted to evaluate the differences in attack risks between predefined clusters/regions and between enterprise sizes. Initially univariable logistic regression analyses were conducted to test the unconditional association of cluster/region and enterprise size with the outcome. This was followed by fitting multivariable models by including both the variables and their interaction term in the model (retained if significant).

Similar analyses were conducted to evaluate the association of cluster/region and enterprise size with the probability of detecting at least one seronegative horse. All statistical analyses were conducted using SAS statistical software (release 9.1, © 2002-03, SAS Institute Inc., Cary, NC, USA).

## **Results**

Of more than 6000 recorded IPs, a total of 1772 were identified as meeting the inclusion criteria and were included in this analysis. Attack risk - the proportions of seropositive horses on properties- ranged from 9.09% to 100.00% (mean 96.88%; median 100%). Significant differences in attack risk were observed between clusters/regions and enterprise sizes. Highest attack risks were observed in Dubbo cluster/region and lowest in Narrabri-Northern cluster (Table 1). Properties with fewer horses (up to 5 horses sampled) were more likely to have higher attack risk than larger enterprises (>20 horses sampled). The interaction between

cluster/region and enterprise size was significant in the multivariable logistic regression model, indicating that the attack risk was not consistently higher in smaller enterprises for all clusters/regions (see the adjusted odds ratios presented in Table 2).

**Table 1. Descriptive and univariable logistic regression analyses to evaluate the differences in attack risks between clusters/regions and enterprise sizes during the 2007 equine influenza outbreak. The results are based on properties tested >25 days after being declared as infected properties (IPs).**

Variable	Categories	Total horses	Number (%) seropositive	Univariable odds ratios (95% CI)	P
Cluster/region					<0.001
	Dubbo	747	734 (98.26%)	8.49 (4.72, 16.40)	
	Hunter valley	2159	1922 (89.02%)	1.22 (0.88, 1.66)	
	Maitland-Central coast	2844	2742 (96.41%)	4.04 (2.84, 5.68)	
	Parkes	586	566 (96.59%)	4.25 (2.55, 7.38)	
	Sydney south	5570	5410 (97.13%)	5.08 (3.65, 6.98)	
	Tamworth	1151	1028 (89.31%)	1.26 (0.89, 1.76)	
	Other	353	336 (95.18%)	2.97 (1.73, 5.37)	
	Narrabri-Northern <sup>a</sup>	421	366 (86.94%)	1.00	
Enterprise size <sup>b</sup>					<0.001
	Small ( $\leq 5$ horses)	3166	3073 (97.06%)	2.23 (1.78, 2.82)	
	Medium (6-20 horses)	3782	3583 (94.74%)	1.22 (1.02, 1.445)	
	Large (>20 horses) <sup>a</sup>	6883	6448 (93.68%)	1.00	

<sup>a</sup> Reference category for logistic regression analyses

<sup>b</sup> Number of horses sampled was used as a surrogate indicator for enterprise size: small enterprise ( $\leq 5$  horses sampled); medium enterprise (6–20 horses sampled); large enterprise (>20 horses sampled).

CI, confidence interval.

**Table 2. Association of cluster/region and enterprise size<sup>a</sup> with attack risk on infected NSW properties during the 2007 equine influenza outbreak in Australia. Adjusted odds ratios are based on the multivariable logistic regression model of attack risk on region/cluster, enterprise size and their interaction.**

Clusters/regions	Total number of horses tested			Number (%) of horses positive			Adjusted odds ratios		
	Small	Medium	Large	Small	Medium	Large	Small	Medium	Large <sup>b</sup>
Dubbo	193	304	250	192 (99.48%)	294 (96.71%)	248 (99.20%)	2.32	0.36	1.00
Hunter valley	171	324	1664	156 (91.23%)	285 (87.96%)	1481 (89.00%)	1.93	1.35	1.00
Maitland-Central coast	622	662	1560	599 (96.30%)	647(97.73%)	1496 (95.90%)	1.67	2.77	1.00
Parkes	159	216	211	153 (96.23%)	206 (95.37%)	207 (98.10%)	0.74	0.60	1.00
Sydney south	1618	1419	2533	1582 (97.78%)	1382 (97.39%)	2446 (96.57%)	2.35	1.99	1.00
Tamworth	280	490	381	272 (97.14%)	447 (91.22%)	309 (81.10%)	11.89	3.63	1.00
Other	66	136	151	65 (98.48%)	128 (94.12%)	143 (94.70%)	5.46	1.34	1.00
Narrabri-Northern	57	231	133	54 (94.74%)	194 (83.98%)	118 (88.72%)	2.29	0.67	1.00

<sup>a</sup> Number of horses sampled was used as a surrogate indicator for enterprise size: small enterprise ( $\leq 5$  horses sampled); medium enterprise (6-20 horses sampled); and large enterprise ( $>20$  horses sampled).

<sup>b</sup> Reference category for logistic regression analyse

## Discussion

All of the tested horses were seropositive on 1547 of the properties (87.30%) but at least one horse was seronegative on the remaining 225 properties (12.70%). The proportions of properties with at least one seronegative horse were significantly different in different clusters/regions and enterprise sizes (Tables 3). For example, properties in Dubbo cluster/region had 80% less chances of having at least one seronegative horse than properties in Narrabri-Northern cluster. Similarly, smaller enterprises had 94% less chances of having at least one seronegative horse compared to larger enterprises (Table 3).

**Table 3. Descriptive, univariable and multivariable logistic regression analyses to evaluate the association of cluster/region and enterprise size with the probability of at least one horse remaining seronegative on infected properties (IPs) during the 2007 equine influenza outbreak. *P*-values are presented only for the multivariable model but were similar for the univariable models.**

Variable	Categories	Total number of IPs	IPs with at least one seronegative horse (%)	Univariable odds ratios (95% CI)	Adjusted odds ratios (95% CI)	<i>P</i>
Cluster/region						<0.001
	Dubbo	106	7 (6.60%)	0.17 (0.06, 0.45)	0.20 (0.07, 0.56)	
	Hunter valley	125	49 (39.0%)	1.52 (0.74, 3.13)	1.73 (0.79, 3.92)	
	Maitland-Central coast	325	38 (11.69%)	0.31 (0.15, 0.64)	0.45 (0.21, 1.00)	
	Parkes	95	8 (8.42%)	0.22 (0.08, 0.56)	0.27 (0.09, 0.73)	
	Sydney south	850	73 (8.59%)	0.22 (0.11, 0.43)	0.33 (0.16, 0.71)	
	Tamworth	178	30 (16.85%)	0.48 (0.23, 1.00)	0.62 (0.28, 1.40)	
	Other	46	6 (13.04%)	0.35 (0.12, 1.02)	0.41 (0.12, 1.24)	
	Narrabri-Northern <sup>a</sup>	47	14 (29.79%)	1.00	1.00	
Enterprise size <sup>b</sup>						<0.001
	Small (≤5 horses)	1262	74 (5.86%)	0.05 (0.03, 0.08)	0.06 (0.04, 0.09)	
	Medium (6-20 horses)	387	84 (21.71%)	0.23 (0.15, 0.36)	0.24 (0.15, 0.37)	
	Large (>20 horses) <sup>a</sup>	123	67 (54.47%)	1.00	1.00	

<sup>a</sup> Reference category for logistic regression analyses

<sup>b</sup> Number of horses sampled was used as a surrogate indicator for enterprise size

High attack risks observed in the current outbreak are characteristic of EI outbreaks in naïve populations, whereas the attack risks are usually lower in vaccinated horses or in previously exposed populations. Morbidity rate was only 19.4% in an EI outbreak involving regularly vaccinated horses in Japan <sup>2</sup> in August 2007, probably the source of the virus that caused the outbreak in Australia.

Interestingly, a small number of properties had quite low attack risks, as low as 10%. The reasons for this are unclear, but could include false-positive reactions on properties that were never infected (specificity of the bELISA used in the response was estimated at about 97%<sup>4</sup>) or false negative reactions in horses that were exposed to the virus but had not seroconverted by the time of sampling. Alternatively, properties with low attack risk could have been due to infected premises where the infection diminished before it had a chance to infect all horses.

The differences in attack risks in different clusters/regions could be due to differences in geographic, social or demographic factors, but needs further evaluation. For example, many of the infected properties in the Narrabri/Northern cluster (the cluster with the lowest seroprevalence) were larger, rural properties compared to smaller peri-urban properties in some clusters/regions (Dubbo, Sydney-South, Parkes, Maitland-Central Coast). The larger property size provided opportunity for less dense horse populations and presumably slower spread, so that some horses might have avoided exposure before the infection diminished on the property.

Infection of a greater proportion of horses on properties with a fewer number of horses could be due to comparatively smaller property area facilitating spread of virus in close contact horses. Note that we used sample size as a surrogate measure for the number of horses on a property because an accurate estimate of number of horses was not available at the time of analyses. We acknowledge that this could have introduced bias in our estimates.

To sum up, this preliminary investigation indicated that not all of the horses on infected properties became infected during the EI outbreak and the proportions of horses that became infected were not similar for different enterprise sizes or for different geographical and demographic conditions. Further analysis of the data is recommended to provide insights into the epidemiology of EI, particularly the spread of infection within naïve populations under Australian conditions.

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