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Estimation Models for the Morbidity of the Horses Infected with Equine Influenza Virus

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Estimation formulas for the morbidity of horses infected with equine influenza virus by linear regression, logistic regression and probit transformation were developed, using data from the outbreak at the Sha Tin Racing Track in Hong Kong in 1992. Using these formulas, we estimated the equine influenza virus morbidity rates at training centers belonging to the Japan Racing Association (JRA) in October 1997 and in October 1998. In 1998 JRA started a new vaccination program, and every horse must now be vaccinated twice per year. At that time, the vaccine included two US lineage virus strains, the A/equine/Kentucky/81 strain and the A/equine/La Plata/93 (LP93) strain, against equine type-2 influenza viruses; it did not include any EU lineage virus strains, such as A/equine/Suffolk/89 (SF89). Comparing the geometric mean (GM) values of hemagglutination inhibition (HI) titers between the LP93 strain and the SF89 strain in 1997 and in 1998, they both rose significantly at every age ($p < 0.05$) by Wilcoxon test. Calculations by the simulation models show the morbidity rates for LP93 diminished from 0.439 (linear), 0.423 (logistic) and 0.431 (probit) to 0.276 (linear), 0.265 (logistic) and 0.271 (probit), respectively. On the other hand, the estimated morbidity rates for SF89 diminished only slightly from 0.954 (linear), 0.932 (logistic) and 0.944 (probit) to 0.946 (linear), 0.914 (logistic) and 0.927 (probit), respectively. Our simulation models could estimate the effect of the vaccine on each of the equine virus strains represented by the morbidity of infected horses. Thus, they are useful for vaccine evaluation.

Key words: equine-2 influenza virus, estimated morbidity, hemagglutinin inhibition test

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Equine influenza, caused by the equine-2 influenza A virus (H3N8), has become enzootic among the equine populations in American and European countries. Equine-2 influenza viruses classified as American (US) and European (EU) type [1, 4, 5] and antigenic variations also exist among the strains [6]. An equine-2 influenza virus outbreak involving A/equine/Hong Kong/92 (HK92) occurred among vaccinated Thoroughbred horses in Hong Kong during November and December in 1992. It resulted in the postponement of seven race meetings over a period of 32 days [3, 8]. This incident is the sole available report for estimating the morbidity in an outbreak of the equine influenza virus. Equine influenza caused by the equine-2 influenza virus is reported in Europe and

America throughout the year [2]. In Japan today, many horses are imported from Europe and America, so the risk of an equine influenza outbreak originating from imported horses has become higher. Therefore, in order to prevent an equine influenza outbreak, we must maintain high antibody titers against the equine-2 influenza viruses among horses all the year round, and also, the efficacy of the equine influenza vaccine must be carefully assessed on a routine basis [7, 13]. Although an outbreak of equine influenza occurred and one race was cancelled in 2007, despite the twice yearly vaccine policy, there is no way to estimate the risk of an outbreak of an equine influenza virus. Thus, we developed risk estimation model formulae to estimate the morbidity risk of the horses infected with an equine influenza virus.

The risk estimation of an outbreak of an equine influenza virus to the huge equine herd such as the

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Miho and Ritto training centers belonging to the Japan Racing Association (JRA) is very important. The epidemiological report on the Hong Kong outbreak by Powell *et al.* [8] is the one available precious source of information on the first equine influenza outbreak in vaccinated horses that hadn't previously been infected by the virus. Of the 215 horses at Sha Tin from which preliminary serum samples were taken, 161 (75 percent) showed evidence of HK92 virus infection irrespective of whether clinical signs were reported. These horses were vaccinated twice yearly and their morbidity rate was 19%. The inactivated equine influenza vaccine did not prevent the infection though it did prevent the showing of symptoms of the disease. We, therefore, developed estimation formulae for the morbidity of the horses infected with equine influenza virus, using the outbreak data of the Sha Tin Racing Course in Hong Kong in 1992 [8]. In this report, 149 infected horses, for which there were sera samples before and after the outbreak, were investigated. In the group of 1:20 HI titer against the HK92 before the outbreak, 20 horses showed clinical signs and 10 horses did not. In the group of 1:40 HI titer, 5 horses showed clinical signs and 8 horses did not. In the group of 1:80 HI titer, 3 horses showed infected and 28 horses did not. In the groups of horses with HI titers of 1:160 (31 horses), 1:320 (25 horses), 1:640 (12 horses) and 1:640 (7 horses), there were no horses with clinical signs. Using the data above, we estimated the morbidity of horses infected with equine influenza virus using a linear regression model, a logistic regression model and a probit transformation model.

Using the data of 1:20, 1:40 and 1:80 groups, the morbidity of the horses infected with the equine influenza virus was estimated by the linear regression model with the REG procedure of the SAS system in which $M = \beta_2 + \beta_1 \log_2 T$ (f1) [9]. In this formula, M is the morbidity and T is the HI titer.

The logistic regression model was estimated by the NLIN procedure and the Gauss-Newton method with the logistic sigmoid curve formula expressed as

$$M = \frac{1}{1 + e^{\beta_1 (\beta_2 - \log_2 T)}} \quad (\text{f2}) [11].$$

The distribution of the numbers of each antigenic titer was revised by the weighting option. β_1 is the slope of the sigmoid curve and β_2 is the 50% protective HI titer of prevention presenting symptoms of equine influenza.

For the estimation of the probit transformation model, the Probit procedure of SAS system and the Newton method were used: $\text{probit}(M) = \beta_2 + \beta_1 \log_2 T$

(f3) [11]. The distribution of the numbers of each antigenic titer was revised by the weighting option.

The results of the estimated formulae were as follows; The linear regression model:

$$R = 1.899 - 0.285 \log_2 T \quad (0 \leq R \leq 1) \quad (\text{f4})$$

The logistic regression model:

$$R = \frac{1}{1 + e^{-1.468 (4.795 - \log_2 T)}} \quad (\text{f5})$$

The probit transformation model:

$$\text{probit}(M) = 4.282 - 0.882 \log_2 T \quad (\text{f6})$$

For the intercept (β_2) of the linear regression model (f1, f4), the standard error was calculated as 0.090 and 95% confidence limits were calculated as being from 1.785 to 2.013. For the x (β_1), the standard error was calculated as 0.0167 and 95% confidence limits were calculated as being from -0.306 to -0.264.

For β_1 of the logistic regression model (f2, f5), the approximation of the standard error was calculated as 0.0347 and the approximations of 95% confidence limits were calculated as being from -1.909 to -1.027. For the β_2 , the approximation of the standard error was calculated as 0.0169 and the approximations of 95% confidence limits were as being calculated from 4.580 to 5.009.

For the intercept (β_2) of the probit transformation model (f3, f6), the standard error was calculated as 0.913, 95% confidence limits were calculated as being from 2.492 to 6.071, and χ^2 was calculated as 22.00. For x (β_1), the standard error was calculated as 0.173, 95% confidence limits were calculated as being from -1.222 to -0.542, and χ^2 was calculated as 25.87. The log likelihood of the probit procedure model was calculated as -37.746.

The morbidity estimates using the linear regression model (f4), the logistic regression model (f5) and the probit transformation model (f6) are shown graphically in Fig.1. The logistic regression model estimates and the probit regression model estimates fall on the almost the same curve. The linear regression model estimate shows a higher morbidity rate than other models below the 1:20 HI titers and it shows a lower morbidity rate than other models above the 1:80 HI titers.

Using the HI data against equine influenza viruses

for the racing horses belonging to the JRA Miho and Ritto training centers in 1997 and 1998, we estimated the morbidity of the infected horses. In 1998, the vaccine program for racing horses belonging to JRA was changed from once per year to twice per year. HI titers against A/equine/La Plata/93 (LP93) and A/equine/Suffolk/89 (SF89) at the Miho and Ritto training centers from 1997 to 1998 increased significantly (P value <0.05) in each group as estimated by the Wilcoxon test (Table 1) [10], though HI titers

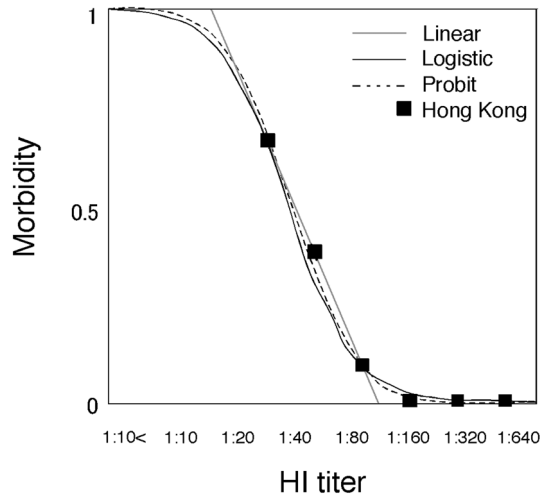


Fig. 1. The relationship between HI titers and morbidity of the horses infected with an equine influenza virus by the estimation formula. The gray solid line is the linear regression model, the black solid line is the logistic model and the black dot line is the probit conversion model.

against the SF89 in 1998 were not high (see GM values in Table 1). At that time, the vaccines included A/equine/Kentucky/81 (KY81; old extinct strain) and LP93 (the US lineage virus strain) but did not include the SF89 (EU) strain.

Using the linear regression model, the logistic regression model and the probit regression model to calculate, the morbidity of the horses infected with SF89 in 1997 were 0.954 (linear), 0.932 (logistic) and 0.944 (probit) and those in 1998 were 0.946, 0.914 and 0.927. On the other hand, the morbidity of the horses infected with LP93 in 1997 were 0.439, 0.423 and 0.431 and those in 1998 were 0.276, 0.265 and 0.271. It seems that the twice yearly vaccination program, started in 1998, is effective against the LP93 strain though not against the SF89 strain, because the new vaccine did not include the EU strain, SF89.

Generally speaking, inactivated influenza vaccine cannot protect horses from influenza virus infection effectively because it lacks the activation ability of IgA. When an outbreak of equine influenza occurred in 1992, more than 70 % of horses were infected in spite of vaccination [8]. When an outbreak occurred in Japan in 2007, more than 50% of horses were infected with the equine influenza virus (personal communication) in spite of the twice yearly vaccination policy. Also, the rate of infection is difficult to estimate, because it is affected by humidity, temperature, the organization of the epidemic prevention facilities, the system of quarantine etc., and these are difficult to compare between Japan and Hong Kong. Thus, our models can calculate the morbidity in infected horses

Table 1. Calculation results of HI data analysis against the LP93 strain

Antigen	A/equine/Suffolk/89 (SF89)					A/equine/La Plata/93 (LP93)				
	Linear ^a	Nlin	Probit	GM ^b	W.test ^c	Linear	Nlin	Probit	GM	W.test
1997										
2 yr	0.937	0.913	0.925	5.9	–	0.437	0.425	0.431	39.4	–
3 yr	0.991	0.972	0.982	4.4	–	0.482	0.458	0.468	30.9	–
over 4 yr	0.980	0.959	0.970	4.6	–	0.394	0.375	0.384	42.6	–
All ^d	0.954	0.932	0.944			0.439	0.423	0.431		
1998										
2 yr	0.941	0.907	0.920	6.9	0.017	0.280	0.271	0.276	71.0	0.017
3 yr	0.963	0.937	0.949	5.6	0.001	0.317	0.302	0.310	56.7	0.002
over 4 yr	0.947	0.916	0.928	6.4	<0.001	0.215	0.202	0.208	78.1	0.001
All ^e	0.946	0.914	0.927			0.276	0.265	0.271		

a) Linear: linear regression model; Nlin: logistic regression model; Probit: probit transformation model. b) Geometric mean values. c) P values of HI titers in 1997 compared to the titers in 1998 by the Wilcoxon test [10]. d) These values were calculated with the following weightings: 2 yr horses, 64.1%; 3 yr horses, 20.2%; over 4 yr horses, 15.8%. e) These values were calculated with the following weightings: 2 yr horses, 64.4%; 3 yr horses, 18.6 %; over 4 yr horses, 17.0%.

but not among all horses. Although the morbidity of the infected horses would be affected by the antibody titers arising from the inactivated equine influenza vaccine, by virus strain, health condition etc., the morbidity of horses infected with the influenza virus would be mainly affected by the antibody titers. Generally it is believed that the symptoms do not appear titers of 1:80 or above in a human influenza virus. Our models are also capable of making estimates below 10% at 1:80 (linear; 0.098, logistic; 0.096 and probit 0.098). In 2007, an outbreak of equine influenza occurred at the Miho and Ritto training centers and 23.2% of infected horses showed clinical signs (personal communications). Our models estimated 0.276 (linear), 0.265 (logistic) and 0.271 (probit) for LP93 in 1998 (Table 1). The antigenicity of A/equine/Ibaraki/2007 is almost the same as LP93 and the distribution of antibody titers against the LP93 was almost same as in 1998 (data not shown). The difference in the vaccine program between in 1998 and in 2007 was only the vaccine strains; that is, the vaccine in 2007 includes A/Avesta/93 for the EU strain instead of KY81 (old extinct strain). Thus our model formulas can be used to evaluate the morbidity of horses infected with equine influenza virus.

The difficulty in estimating morbidity rates of infected horses is that the available data for calculating the morbidity rates of vaccinated horses that have not been infected by the equine influenza virus before, is only from the outbreak in the Sha Tin racing horses in 1992. Thus, we could not conclude which model would be more suitable for estimating the morbidity in infected horses. The real data originated from the virus strain and humidity, temperature etc. is uneven, however, the estimation results of the 3 models were almost the same. The linear model would be easy to calculate using an electronic calculator, and the logistic model and probit model could be calculated using Microsoft Excel or similar software package. Thus, all of the models would be of practical use.

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