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AN EXPERIMENTAL INFECTION WITH A CLASSICAL SWINE FEVER VIRUS ON WEANER PIGS. CAN THE SEROLOGICAL DATA BE USED TO ESTIMATE THE DAY OF VIRUS INTRODUCTION IN NATURAL OUTBREAKS ?*

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Une étude sur le virus de la peste porcine classique 5PPC), souche Lorraine, isolé à l'origine d'un élevage Belge touché par l'épidémie de 1993-1994, a été menée avec 3 boxes adjacents (15 porcs sevrés/cage). Après une inoculation expérimentale des porcs du box du milieu, la proportion des porcs séropositifs a été examinée, la régression logistique a été utilisée à partir du premier porc devenant virémique (dbf). Ce modèle a été utilisé pour tenter la modélisation de la séroprévalence au cours du temps afin de repérer la date d'introduction du virus dans l'élevage.

*Les paramètres du modèle de régression logistique n'étaient pas différents entre les 3 cages, de ce fait, les données ont été groupées. L'équation de régression de la séroprévalence au cours du temps, pour les trois boxes, était : p = 1/[1+exp(4.65-0.39*dbf)].*

On a conclu que l'équation de régression issue des données sérologiques lors d'une infection expérimentale, peut être utilisée pour estimer la date d'introduction du virus de la PPC dans l'élevage porcin.

INTRODUCTION

In Belgium, vaccination against Classical Swine Fever (CSF) has been prohibited since April 1988. Since, two epizootics, with considerable economic loss, have occurred (1990 and 1993-1994) (3).

During the Belgian CSF epizootic of 1993-1994, data were gathered of every outbreak with the intention to trace the origin of the infection. However, the origin of the infection could only be tracked for the first outbreak. Lack of knowledge of factors causing disease introduction considerably complicated the task of the Veterinary Services whose responsibility it was to control the CSF epizootic. Therefore, the estimation of the day the CSF virus introduction into a pig farm could be a helpful tool to direct the analysis of the data and to find the factors responsible for the spread of the disease.

In this paper, the applicability of the serological data of an experimental CSF infection on weaner pigs, to estimate the day of virus introduction into a pig farm, was tested on the serological field data of the Belgian epizootic of 1993-1994.

MATERIAL AND METHODS

Experimental design: Forty five conventional weaner pigs of 12 - 15 kg, negative for CSF virus and antibodies, were randomly allocated to one of the 3 adjacent pens in an isolation unit (15 pigs / pen). After a 10-day acclimatisation period, a randomly selected weaner pig from the middle pen was inoculated with the CSF-field isolate, originally isolated from the first Belgian CSF herd of the epizootic of 1993-1994, by deep intramuscular injection (2 ml) and by intranasal inoculation (2 ml). The post-inoculation period lasted 58 days.

Sample collection: Two days prior to inoculation, and every two days in the post-inoculation period, blood samples were taken from all live weaner pigs. Blood was collected from the jugular vein with needles for single use and evacuated sterile tubes (Becton Dickinson, Erembodegem, Belgium).

Sample analysis: Two serological tests were used. First, the CTB-ELISA, was used as a complete kit (4). Second, the seroneutrolisation test was used as described by Holm-Jensen (1).

Field outbreak data: Three outbreak herds of the Belgian epizootic of 1993-1994 were selected to test the applicability of the serological data of the experimental infection trial on the serological field data. Selected pig herds met the following requirements: (i) history data were available on the introduction of uninfected pigs of less than 50 kg into a pighouse where infected pigs were shown to be present or (ii) the CSF virus had been introduced into the pig herd by pigs of less than 50 kg and the date of this introduction was known and (iii) information was available on the seroprevalence of all pigs in each pen. The history and serological data of these 3 outbreaks herds are in Figures 1, 2, and 3.

Data analysis: The proportion of seropositive pigs in a pen was modelled as a function of time with logistic regression (Statistix 4.1, Analytical Software, P.O. Box 12185, Talahassee FL 32317-2185, USA) using the following model:

$$p = \frac{1}{1 + e^{-(b_0 + b_1 * pen2 + b_2 * pen3 + b_3 * dpf + b_4 * pen2 * dpf + b_5 * pen3 * dpf)}}$$

with

p	= the proportion of seropositive pigs at time dpf;
dpf	= days post first positive virological diagnosis in the pen;
weighting factor	= the number of live pigs at each time point.

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For pen 2, the data of the experimentally inoculated weaner pig were not included in the analysis. The first pen mate of the experimentally inoculated weaner pig became viraemic at day 12 of the post-inoculation period (2). Therefore, for pen 2, day 12 of the post-inoculation period was equal to 0 dpf. For both pen 1 and pen 3, the first weaner pig became viraemic at day 18 of the post inoculation period. For pens 1 and 3, day 18 was equal to 0 dpf.

The applicability of this regression equation, to estimate the day the infection was introduced into a pig farm, was tested on the serological field data of the Belgian epizootic of 1993-1994 from 3 outbreak herds.

From pens, populated with originally uninfected or infected pigs introduced in a pighouse previously already infected or uninfected, respectively, the pen with the highest seroprevalence, was selected. The day the CSF virus was introduced in this pen was estimated using the proportion seropositive pigs in that pen and the regression equation of the experimental infection. The estimated day of introduction of the CSF virus was compared with the day the uninfected pigs were introduced in the infected pighouse (herd A and herd B) or with the day the infected pigs were introduced into the pig herd (herd C).

Figure 1. History and serological data of herd A - Pighouse II

Pen identification	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Number of pigs	7	0	11	1	7	12	8	8	0	13	10	11	12	5	10	9	13	12
Seroprevalence	43	....	0	0	71	27	12	62	....	0	10	0	8	60	25	0	0	0
Weight	120	....	45	50	115	60	70	50	....	55	85	55	60	120	120	85	45	60
Weight	90	115	115	120	105	....	90	55	85	125	125	50	125	60	125	50	80	90
Seroprevalence	0	0	50	0	0	....	0	0	0	67	20	0	78	0	38	0	10	30
Number of pigs	10	7	4	12	11	0	3	12	9	7	6	11	9	9	8	6	11	12
Pen identification	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1

▨: Pigs were already housed in pighouse II before February 19, 1994 (Overall seroprevalence: 28 %)

□: Pigs were housed in pighouse II at February 19, 1994 (Overall seroprevalence: 6 %)

History

February 19, 1994: Re-population of the empty pens of pighouse II with fattening pigs of pighouse I
February 27, 1994: First clinical symptoms in pighouse II (cuffing and increased mortality)
March 5, 1994: Suspicion of CSF
March 7, 1994: Confirmation of CSF
March 8, 1994: Blood sampling for serological examination and destruction of the pigs on the farm

Figure 2. History and serological data of Herd B - Part of Pighouse V

24[1] 12[2] 50[3] 135[4]	25 5 80 135	72 2 50 90	73 10 100 115
23 12 20 100	26 11 46 115	71 7 75 115	74 8 67 105
22 11 54 100	27 11 70 100	70 10 60 90	75 1 100 100 17 0 35
21 0	28 12 36 120	69 0	76 8 50 135

[1] Pen identification; [2] number of pigs; [3] seroprevalence; [4] weight

History

April 11, 1994: Housing of 17 pigs in pen 75 of pighouse V together with the pig already housed in pen 75
April 13, 1994: Blood sampling of 10 fattening pigs: virologically negative and 1 sample serologically positive
April 14, 1994: Increased mortality in pighouse V
April 18, 1994: Suspicion of CSF
April 19, 1994: Confirmation of CSF
April 20, 1994: Blood sampling for serological examination and destruction of the pigs on the farm

Figure 3. History and serological data of Herd C - Part of pighouse V

Pen identification	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	
Number of pigs	15	14	8	16	16	16	15	16	14	16	16	15	15	16	17	
Seroprevalence	0	36	38	19	12	12	7	0	0	0	6	0	0	0	0	
Weight	38	35	35	40	45	45	48	48	50	50	50	50	55	55	55	
Weight	40	40	35	38	45	48	40	45	50	42	48	27	48	50	50	
Seroprevalence	0	7	45	14	0	0	0	0	0	0	6	0	0	0	6	
Number of pigs	16	14	11	14	16	15	16	16	16	14	16	16	16	16	17	
Pen identification	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	

History

September 24, 1993: Re-population of pighouse V with pigs originating from Germany
October 8, 1993: First clinical symptoms in pighouse V
October 12, 1993: Suspicion of CSF
October 13, 1993: Confirmation of CSF
October 13, 1993: Blood sampling for serological examination and destruction of the pigs on the farm

RESULTS AND DISCUSSION

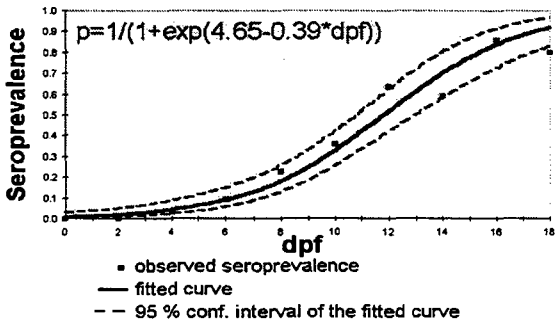
The parameters for the logistic regression model are in Table I. Since the seroprevalence did not differ significantly over time for the 3 pens, a single regression equation was fitted to the data for the 3 pens together up to 18 dpf. The regression curve, its 95% confidence interval (CI) and the regression equation is shown in Figure 4. The applicability of this regression equation was tested on the field data of the three outbreak herds.

Table I
The logistic regression analysis table of the seroprevalence over time for the 3 pens separately.

Predictor Variable	Coefficient	s.e.[1]	P
Constant	-4.92	0.84	< 0.001
Pen 2	1.36	1.10	0.22
Pen 3	0.82	1.23	0.51
dpf[2]	0.38	0.07	< 0.001
dpf*Pen 2	-0.13	0.09	0.16
dpf*Pen 3	0.04	0.12	0.72

[1] standard error
[2] days post first positive virological diagnosis in the pen

Figure 4
The pooled logistic regression model.



For herd A, the overall seroprevalence of the pigs that were already present in pighouse II on February 19, 1994 (Figure 1: grey pens) was superior to the seroprevalence of the pigs introduced in pighouse II on February 19, 1994, (Figure 1: white pens). Therefore, it was concluded that the former pigs (grey pens) were already infected before February 19, 1994.

Based on the regression equation, the first pig of pen 26, the pen with the highest seroprevalence (62.5 %), became viraemic on February 24, 1994 (13.1 dpf on March 8, 1994). As compared with the experimental inoculated weaner pig, that became viraemic 4 days post inoculation (dpi) (2), an extra 4 days was added to the 13.1 dpf to come to the day of infection. A total of 17.1 days (95% CI [12.5;27.3]) brings us back to February 20, 1994 (95% CI [February 10, 1994; February 25, 1994]).

In herd B, 17 pigs were introduced on April 11, 1994 in pighouse V and housed together with the 1 pig of pen 75. The 17 pigs had a seroprevalence of 0% on April 20, 1994 (Figure 2). Since it was not possible to calculate a CI for a seroprevalence of 0%. The estimation of the day of infection was calculated in a different way. Suppose that 1 out of 17 pigs was seropositive on April 20, 1994, then the seroprevalence would have been 5.9 % (95% CI: [0.0;16.2]), corresponding with 4.8 dpf (95% CI: [0.0;8.7]) on April 20, 1994. In the supposition that the 1 pig present in pen 75 before April 11, 1994, was excreting virus on April 11, 1994, the 17 pigs that were introduced on April 11, 1994, could get infected at the day of introduction. Therefore, an extra 4 days, the period from infection to viraemia (2), had to be added to the 4.8 dpf to come to the day of infection. The day of infection was thus estimated to be 8.8 days (95% CI: [4.0; 12.7]) prior to April 20, 1994, in the supposition that one of the pigs seroconverted. However, none of the pigs were seropositive, so the infection took place less then 8.8 days (95% CI: [4.0; 12.7]) prior to April 20, 1994, i.e. after April 11, 1994 (95% CI: [April 7, 1994; April 16, 1994]).

Herd C was the first outbreak of the epizootic of 1993-1994 (Figure 3). The infection originated from infected pigs that were imported from Germany. The seroprevalence (45.4 %) of pen 18, the pen with the highest seroprevalence, corresponded with an estimated 11.3 dpf (95% CI: [6.3;16.4]) on October 13, 1993. In the supposition that one infected pig was imported from Germany, an extra 12 days, the period from infection of the primary case to the first viraemia in his pen mates (2), had to be added, resulting in a total of 23.3 dpi (95% CI: [18.3;28.4]). The day of infection of the pig imported from Germany was estimated on September 21, 1993 (95% CI: [September 16, 1993; September 26, 1993]).

It was concluded that the application of the regression equation derived from the serological data of the experimental infection, to estimate the day the CSF virus was introduced into a pig farm and tested on the serological data of 3 pig farms of the Belgian epizootic of 1993-1994, was very promising.

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