TRANSMISSION OF A HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS TO SWINE IN THE NETHERLANDS

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Introduction

At the end of February 2003 a highly pathogenic avian influenza H7N7 was introduced in the Dutch poultry population. In the early stages of the epidemic it became apparent that humans could also be infected with this virus. Although this mainly resulted in mild symptoms of conjunctivitis, also one fatal case was recorded[2]. In early April a serological surveillance program was started to determine whether the virus was also infecting pigs. Infections of pigs may have consequences for both animal and human health.

Material and Methods

Field surveillance:

Three categories of swine herds inside the protection zones were sampled:

Category 1: Mixed herds with swine and poultry; poultry infected.

Category 2: Mixed herds with swine and poultry; poultry pre-emptively culled (no infection established).

Category 3: Swine herds with no poultry present.

The first part of the surveillance consisted of 13 herds from category 1 where a possible infection had the highest chance of detection. The poultry was culled 3-5 weeks before the pigs were sampled, so there had been enough time for any serological responses, had the virus infected the pigs. Eight of the herds were sampled according to a representative sampling schedule, allowing for the detection of even small outbreaks (60-118 samples per herd). The five smallest herds were sampled completely, to allow detecting even very small outbreaks (62-376 samples per herd.

After the first results from these herds, five herds were sampled again. Eleven days after the first sampling, all individual animals above 4 weeks of age were sampled and tested (173-713 per herd, total 2373 samples).

After that, all category 1 herds were tested serologically after at least 3 weeks had passed since the culling of the infected poultry. They were sampled according to the same representative sampling schedule used during the first part of the surveillance in pigs (60-217 samples per herd). Including the first 13 herds, in the end a total of 46 herds in this category were identified and tested during the avian influenza outbreak.

During the outbreak the surveillance was soon expanded to swine in mixed herds where poultry was preventively culled (category 2), and to swine in swine herds with no poultry at all (category 3). All these herds were located in the protection zones, within a 3 kilometer radius of an infected premises. From category 2 a total of 21 herds were sampled and from category 3 a total of 23 herds. In each herd 60 samples were randomly taken, resulting in a total of 2640 blood samples.

Pilot experimental infection:

An experimental infection of pigs with influenza subtype H7N7 was carried out to determine the serological response of an infection and to get a first indication of transmission. Four 10-week-old SPF piglets were inoculated intranasally (I.N., 2ml per animal, 10^6 EID_{50} per ml). Two piglets were inoculated intramuscularly (I.M.) with the same doses and two piglets were added 24 hours later as non-infected contact pigs. Blood samples were taken at days 0, 7, 11, 14, 18, 22, 28 and 32 for antibody detection in an Np-ELISA and a haemagglutination inhibition (HI) test. Oropharyngeal swabs were taken daily at days 0 to 9, and furthermore at days 11, 14 and 18 for virus isolation.

Transmission experiment:

To quantify transmission between pigs, a transmission experiment was carried out with 40 pigs. Half of these pigs were inoculated intranasally (2ml per animal, 10^6 EID_{50} per ml). The other half was added 24 hours later as non-infected contact pigs. Blood samples were taken at days 0, 4, 7, 11, 14, 18, 21, 28 and 35 for antibody detection in an Np-ELISA and a haemagglutination inhibition (HI) test. Oropharyngeal swabs were taken daily at days 0 to 9, and furthermore at days 11 and 14 for virus isolation.

With these numbers of pigs, a contact infection of up to 4 pigs results in an R_0 (reproduction ratio as a measure for transmission) below 1.

Testing:

Samples were tested in a haemagglutination inhibition (HI) test, using the avian H7N7 as antigen. Sera were pre-treated with cholera toxin and chicken erythrocytes. 969 blood samples from swine herds in the Northern parts of the Netherlands, from before the introduction of H7N7 in the Netherlands, were tested to determine the test specificity. From these samples the specificity of the HI-test, based on the titre 40 threshold level, was determined at 97.4%. To determine whether a herd was infected , the seroprevalence was corrected for this specificity and an estimated sensitivity of 80%. Around this estimated true seroprevalence a 95% confidence interval (CI) was calculated[1].

While decisions during the outbreak had to be made mainly based on HI-results, on selected field samples also a virus neutralisation test (VNT) was carried out to confirm the HI-results.

For the experimental infections an Np-ELISA was used that detects antibodies against all influenza subtypes. Virus isolation was carried out on embryonated chicken eggs.

Results

Field surveillance:

The results of the first part of the surveillance (13 category 1 herds) showed that in five of these herds the seroprevalence was significantly above 0, indicating that the H7N7 virus was introduced into the swine population. Seroprevalences in these herds (counting titers of 40 and higher) were 5.1%, 5.6%, 8.3%, 15% and 27% respectively.

The results of re-sampling these 5 herds showed no significant increase in seroprevalence (now 2.5%, 7.7%, 6.9%, 14% and 29% respectively). In the first three herds the seropositives were found scattered across the farms, rarely more than one seropositive pig in one pen. In the herd with the highest seroprevalence, these seropositives were correlated with feeding of broken eggs from infected poultry in two compartments. Paired blood samples from over 200 individual animals showed 5 conversions from positive to negative and 3 conversions from negative to positive. All other results showed to be reproducible. In all cases the titres of these conversions were around the threshold value of 40 and test variation and aspecific responses were most likely to be the cause of these 'conversions'.

Further testing of all category 1 herds, up to a total of 46, revealed an additional 8 herds with an estimated true seroprevalence of more than 0. This brought the total number of likely infected herds at 13, with estimated true seroprevalences ranging from 3-42% (table 1).

From 5 of the 13 infected herds, samples were tested in a VNT to confirm the HI-results. All 5 herds were positive in the VNT as well. From all herds with very few (presumably false-)positives, positive samples in the HI were retested in the VNT and turned out to be negative.

The test results for the category 2 and 3 herds showed an overall seroprevalence of 1.1%, with no significant difference between both categories. Most of the titres found were at the threshold level of 40 and could well be explained by non-specific reactions. An additional 457 blood samples were taken on 5 herds from category 2 and 3 with one or two titres >40. Two percent of these samples were positive, again with mainly low titres and within what could be expected, based on the specificity of the test. Confirmation of all HI-positive samples in a VNT showed that they were indeed false-positives. In the end, no antibodies were detected in category 2 and 3 herds.

Table 1 : Results on 13 herds with an estimated true seroprevalence significantly above 0% (Prev=measured seroprevalence, True prev=estimated true prevalence).

Herd	Samples	Positive	Prev.	True Prev.	95%CI
1	60	5	8.3%	7.4%	3.4-11
2	60	9	15%	16%	11-21
3	61	16	27%	32%	25-38
4	118	6	5.1%	3.2%	1.0-5.5
5	72	4	5.6%	3.9%	0.9-6.9
6	60	17	28%	33%	26-39
7	60	21	35%	42%	35-49
8	60	18	30%	35%	29-42
9	60	9	15%	16%	11-21
10	116	5	4.3%	2.2%	0.1-4.3
11	60	5	8.3%	7.4%	3.4-11.3
12	60	4	6.7%	5.3%	1.7-8.9
13	60	6	10%	9.6%	5.3-14

Pilot experimental infection:

After inoculation of the pigs, either I.M. or I.N., no clinical signs related to influenza were ever noticed. After 7 days, all inoculated pigs were seropositive in the Np-ELISA. In the HI-test, pigs showed higher titres after I.N. inoculation than after I.M. inoculation (table 2). Virus could be isolated from oropharyngeal swabs at 1 to 4 different days between D1 and D5 from all four I.N. inoculated pigs.

Table 2 : Serological results of the pilot experimental infection. HI-titers are given (< means <10) and positive Np-ELISA results are shown in shades (Con=contact pig, I.M.=intramuscular, I.N.=intranasal)

DPI:	0	7	11	14	18	22	28	32
Con	<	<	<	<	<	<	<	<
Con	<	<	<	<	<	<	<	<
I.M.	<	<	40	40	40	40	40	20
I.M.	<	<	<	<	<	<	<	<
I.N.	<	<	160	320	160	160	80	80
I.N.	<	40	80	80	40	20	20	20
I.N.	<	<	160	160	80	80	80	40
I.N.	<	<	40	40	40	40	20	20

Transmission experiment:

All 20 inoculated pigs became seropositive in both the Np-ELISA and the HI-test. None of the 20 contact pigs seroconverted. No clinical signs were seen in any of the infected pigs.

Discussion

During the Dutch outbreak of avian influenza, subtype H7N7, it was found for the first time that pigs also could get infected with this particular influenza strain. The results from the field surveillance already indicated that a high exposure to the virus was necessary to infect the pigs. Only pigs in mixed herds with infected poultry were at a significant risk for introduction of the avian influenza strain. No evidence for (ongoing) transmission between pigs was found in the field. No evidence was found that the virus was able to remain for long in the swine herds after the source of infection, the infected poultry, was removed.

The experimental infections confirmed the results from the field and no transmission at all was noticed during both experimental infections. This means that without further adaptation of the virus, and even without additional measures, the infection will always come to an end and the virus will disappear from the pig population.

Conclusion

Pigs were infected during the Dutch outbreak of avian influenza in 2003. Transmission between pigs was however very low to negligible. Therefore, without further adaptation of the virus, there was no chance that it could have become endemic in the pig population

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References

1] Cameron AR, Baldock FC. A new probability formula for surveys to substantiate freedom from disease. Prev Vet Med. 1998 Feb 6;34(1):1-17.

2] Fouchier RA, Schneeberger PM, Rozendaal FW, et al. Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. Proc Natl Acad Sci U S A. 2004 Feb 3;101(5):1356-61.