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Intra- and interspecies transmission of H7N7 highly pathogenic avian influenza virus during the avian influenza epidemic in the Netherlands in 2003

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Summary
The poultry epidemic of H7N7 highly pathogenic avian influenza (HPAI) virus in the Netherlands in 2003 was probably the result of the introduction of an H7N7 low pathogenic avian influenza (LPAI) virus (by interspecies transmission from wild birds) and the subsequent intraspecies transmission of this virus in poultry. The intraspecies transmission of the ensuing H7N7 HPAI virus was very successful both within and between flocks. Consequently, in the two poultry-dense areas that were affected, the epidemic could only be stopped by eliminating all poultry in the region. According to the spatial models these are the only areas where this was the case in the Netherlands. There was also interspecies transmission to mammals, i.e. to pigs and to humans. For pigs it was shown that possible subsequent intraspecies transmission was negligible ($R_0 < 1$). With hindsight the same was probably also true for humans.

Keywords
Epidemic – H7N7 – Influenza – Poultry – Reproduction ratio – Transmission.

Introduction

From February 2003 to May 2003 an epidemic of H7N7 highly pathogenic avian influenza (HPAI) virus occurred in the Netherlands (22). The epidemic started in one of the most poultry-dense areas of the country and later spread to another poultry-dense area. The cause of the epidemic was believed to be the introduction of a low pathogenic avian influenza (LPAI) H7N7 virus which evolved during transmission in poultry to become highly pathogenic (11, 20).

In the course of the epidemic, both of the poultry-dense areas were depopulated of poultry by the culling of all birds on infected premises, the pre-emptive culling of all birds on contiguous farms, and the culling of all off-farm poultry in the contiguous area.

The epidemic in the larger of the two areas consisted of 212 infected premises and the epidemic in the smaller area consisted of 43 infected premises. The number of farms on which birds were pre-emptively culled, not counting locations where poultry were kept for non-commercial purposes, was five times the number of infected farms.

One person handling infected poultry during the epidemic became fatally ill and was later diagnosed as having been infected with a similar H7N7 virus (16). In addition, several cases of conjunctivitis in humans occurred (16) and workers and some of their family members tested seropositive (18).

The authors give an overview of the investigations carried out into this epidemic. Data and samples were collected before and during the epidemic and both epidemiological

and virological techniques were employed. Investigations focused on the following:

– possible intraspecies transmission of similar H7N7 LPAI virus among wild fowl and interspecies transmission from wild fowl to poultry

– intraspecies transmission of H7N7 HPAI viruses in poultry

– further interspecies transmission of H7N7 HPAI virus to mammals, and the possibility of intraspecies transmission of this virus in these mammals.

Interspecies transmission of H7N7 low pathogenic avian influenza virus to poultry

Isolates of the LPAI precursor of the Dutch H7N7 HPAI virus have not been reported: not from wild birds prior to the epidemic or from poultry during the epidemic. Nevertheless, circumstantial evidence strongly suggests a wild bird origin, as follows:

– introductions of LPAI into poultry appeared to be sufficiently common in the Netherlands in 2003 (11)

– transitions from LPAI virus to HPAI virus have been documented (2, 8, 23)

– related H7 and N7 genes were found among the isolates obtained from two different mallard ducks in the Netherlands in 2000 (16).

The last point, that is, the finding of similar genes albeit in viruses from different animals, makes it reasonable to suppose that a similar LPAI H7N7 virus could have circulated among mallard ducks.

It is also reasonable to suppose that introduction of LPAI viruses does occur, as it was shown serologically that other LPAI viruses had been introduced into poultry farms in 2003 (11). A serological survey of all poultry farms found antibodies against avian influenza at three locations outside of the HPAI epidemic areas (11). One location consisted of a cluster of three poultry farms. From diseased turkeys on one of these three farms an LPAI H7N3 was isolated (30). The viruses introduced into the other two locations could not be subtyped, but were neither H5 nor H7 and thus, according to current knowledge, they could not have developed into epidemics of HPAI virus in poultry (1).

The findings of de Wit *et al.* (11) on the number of introductions of LPAI have led to the following recommendations:

– to better monitor the situation with regard to the introduction of LPAI by serological sampling

– to better monitor the possible emergence of a new HPAI by applying polymerase chain reaction (PCR) methods to detect avian influenza in poultry after excessive mortality.

Based on the data from the infected farms during the 2003 epidemic it was found that excessive mortality is indeed the best indication of HPAI virus circulation in poultry (13). Later, Elbers *et al.* (12, 14) refined this finding to establish notification thresholds for poultry. In poultry flocks with animals less than 11 weeks old it is, for example, compulsory to notify the veterinary authorities when the mortality exceeds 0.5% on two consecutive days; the set threshold mortality level is higher for turkeys and lower for caged layer birds.

Furthermore, the conclusion that LPAI is probably introduced into poultry from wild birds has led to recommendations to reduce the opportunities for contact between poultry and wild birds. To that end, it is now compulsory in the Netherlands to keep poultry indoors during an epidemic and during periods and in areas where the possibility of introduction is thought to be high.

For a virus to be able to have continued transmission it is necessary that on average each infected host infects more than one other host. The average number of hosts infected by one typical infected host is called the reproduction ratio (R_0) (R_h indicates the average number of secondary infected herds/flocks caused by one infectious herd/flock). Thus $R_0 > 1$ implies that there can be prolonged transmission. When an LPAI virus is newly introduced into poultry it cannot always transmit sufficiently, which is not surprising, because, as with any introduction into a new bird species, the virus has not yet adapted. It is important to note that apart from ducks, poultry species are not closely related to the wild bird species that carry the LPAI viruses (15). Even introductions that did lead to transmission, i.e. infection of more than one bird in the poultry flock, were often self-limiting and did not spread beyond the flock.

Thus, introductions of LPAI viruses do probably occur regularly, but many of these introductions into poultry remain a minor outbreak, in terms of either the number of birds or the number of farms. In other words, the reproduction ratio of newly introduced avian influenza viruses is often below one, either at the between-bird or at the between-farm level. This was reflected in the monitoring of LPAI in the Netherlands in 2003 (11). Of the four known introductions with subsequent transmission in that year, two stopped within the farm, the one with H7N3 virus stopped between farms (30), and only the H7N7 virus caused an epidemic after mutating to high pathogenicity.

In the epidemic of 2003, the circulation phase of H7N7 LPAI virus was probably limited. On one of the first detected farms there was also one flock with H7 seropositive poultry which may be an indication of the transmission of H7N7 LPAI virus into that flock. If that were true, it could be that that farm was the index farm.

In the literature there are different accounts with respect to the duration of LPAI virus circulation before HPAI virus occurs. Sometimes short periods are mentioned, for example in Chile with H7N3 (23), and sometimes longer periods are recounted, for example, in Pennsylvania with H5N2 (2) and in Italy with H7N1 (8).

The supposed origin of HPAI viruses implies that during an early period both LPAI and HPAI viruses occur together and, thus, that there will already be cross-immunity against HPAI virus in some birds, in which the HPAI virus will continue to replicate, or attempt to replicate, after transmission. It has been shown experimentally that immunity against the related LPAI virus can prevent the infection and transmission of the descendant HPAI virus (26, 28). Such cross immunity may explain why the flock of H7 seropositive hens mentioned above did not experience high mortality despite being next to a flock in which high mortality was occurring as a result of HPAI H7N7 virus infection. With cross immunity and initial occurrence in the same animal population the HPAI virus can only take over when the transmission of the HPAI virus is faster than the transmission of the LPAI virus. For the HPAI virus to give rise to an epidemic, the resulting reproduction ratio both within herds and between herds has to be higher than one.

Intraspecies transmission of H7N7 highly pathogenic avian influenza virus in poultry

The H7N7 HPAI virus that occurred in the Netherlands in 2003 transmitted very successfully both within chicken layer flocks and between these layer flocks. The within-flock transmission was fast. Depending on assumptions, it was estimated as follows:

– 4.5 $days^{-1}$ (95% confidence interval 2.7 to 7.6) assuming no latent period

– 19.9 $days^{-1}$ (95% confidence interval 11.7 to 33.8) assuming a latent period of one to two days (6).

The latter estimate is in accordance with experimental transmission of this H7N7 HPAI virus in groups of chickens (27).

Rapid mortality accompanying infection limited the level of the reproduction ratio. However, from the estimates of Bos *et al.* (6) it can be seen that even with a high mortality rate, and hence a short survival period, the reproduction ratio within herds is well above one. Massive mortality within the infected flocks occurs within one to two weeks after introduction of the virus (7, 22).

For the purposes of epidemic control it is also important to quantify the transmission between flocks. Stegeman *et al.* (22) provided an estimate of the transmission between flocks without using the spatial location of the cases in the analysis. Before the control measures were introduced a flock was infected for 13.8 days and its reproduction ratio was estimated as R_h = 6.5 (95% confidence interval 3.1 to 9.9) (22).

The control measures implemented included enhanced biosecurity measures such as transport limitations in the surveillance zone, and cleaning and disinfection requirements for those contacts that were permitted. All infected premises were depopulated and there was extra effort to find and diagnose infected premises. In addition, all neighbouring farms and dangerous contact farms were also depopulated. This resulted in a reduction in the infectious period of infected farms to 7.3 days in the first affected area. As a consequence (22), the reproduction ratio (the number of new cases per infected farm) was reduced from the previously mentioned 6.5 to 1.2 (95% confidence interval 0.6 to 1.9).

An attempt was made to identify risk factors for introduction of HPAI into poultry farms (24). The only risk factor found was a higher risk for poultry farms having layer hens versus farms having other poultry. The difference is attributed to the fact that layer hen farms have more contacts than other poultry farms. Alternatively, layer hen farms may have more contacts with other layer hen farms than with other poultry farms. In either case, further analysis is needed to confirm this hypothesis.

Even after implementing all control measures the reproduction ratio between flocks was above one, which implied that the epidemic was not terminated by the control measures. The epidemic only seemed to have stopped because the boundaries of the poultry-dense area were reached. The same epidemic pattern was observed after the introduction in the other poultry-dense area in the Netherlands. In this area the reproduction ratio between farms was initially 3.1 and after implementation of all the control measures it dropped to 1.2. Again, the epidemic seemed to have stopped only because the boundaries of the area were reached (22).

Given this conclusion, the spatial analysis of the transmission between farms was important; the question being, to what extent transmission depended on the

density of the poultry farms in an area during this epidemic. The analysis of Boender *et al.* (5) showed that the density of poultry farms in the two epidemic areas could indeed explain the observed patterns. The two infected areas were the only areas in which the reproduction ratio between farms, even with control measures, was above one. In contrast, in all other areas in the Netherlands the reproduction ratio was below one, even without control measures, again according to the model (5).

In the poultry-dense areas, control was thus not possible without culling almost all poultry in the area. Gassing the whole hen house with carbon dioxide proved to be the most efficient and humane manner of killing poultry on farms with large numbers of chickens (17).

Inter- and intraspecies transmission of H7N7 highly pathogenic avian influenza virus in mammals

Infection of mammals is of concern, particularly when transmission among these mammalian hosts occurs. Moreover, transmission among mammals other than humans heightens the concern that humans may also become infected and diseased. Before the Dutch H7N7 virus epidemic in poultry, isolation of H7 viruses from humans, seals and horses had been reported (21). In humans, infections with H7 viruses have caused conjunctivitis, but also influenza-like illnesses such as mild respiratory disorders.

To find infections with the H7N7 virus in mammals, virus isolation and serology can be employed. Whereas virus detection by isolation or PCR provides evidence of at least exposure of the mammalian host to the virus, interpretation of serology is difficult in the absence of other research validating the serological test, i.e. verifying the correlation between infection and serology.

During the epidemic in the Netherlands, pigs were reported to be seropositive for H7 antibodies (19). These pigs were found only on mixed farms (poultry and pigs), where the poultry was infected. No virus was isolated and no clinical signs were seen and there were no indications that there were infected pigs in other herds. It was concluded that all the pigs must have been directly exposed to the virus from the poultry, but that no further transmission occurred.

Experimentally, the virus did not transmit to contact pigs in a setting where 20 inoculated pigs were placed together with 20 contact animals (19). Based on the data reported in Loeffen *et al.* (19) the estimated reproduction ratio for this H7N7 virus in pigs was $R_0 = 0.0$ (95% confidence interval $R_0 \leq 0.32$).

Cases of conjunctivitis in humans also occurred during the epidemic. In several instances the H7N7 HPAI virus was also isolated from these human conjunctivitis cases (16). Serological positive results in people other than those presenting with symptoms also occurred, indicating that more workers may have been exposed or possibly infected (18). Thus, interspecies transmission to humans had occurred (16, 18). Moreover, in some humans, clinical disease was observed and one person became fatally ill (16). Based on further analysis of the serological data (18) it was concluded that intraspecific transmission from these infected humans to other humans could also have occurred (18, 25).

Virus isolates both from a human conjunctivitis case (A/NL/230/03) and from the human fatal case (A/NL/219/03) were studied using animal models. First it was shown that in Balb/c mice the virus from the fatal case (NL219) was highly virulent (10). Similarly, Belser *et al.* (4) showed that NL219 was highly virulent for both mice and ferrets (4). In addition, Belser *et al.* (4) found that the other Dutch virus isolate (NL230), the one isolated from a conjunctivitis case, was much less virulent for mice and ferrets.

The same two H7N7 virus isolates from human cases were also tested for their ability to transmit in mammals (3). Belser *et al.* (3) used the ferret transmission model as a model to study potential differences in the transmission of these viruses among humans. Having found a difference in transmission among ferrets they looked at underlying genotype and phenotype differences in the viruses to see if they could be responsible for that difference in transmission. Belser *et al.* (3) observed that the NL219 transmitted less well in ferrets (0 out of 3 contact animals infected) than did the NL230 isolate (2 out of 3 contact animals infected). The experiment with NL230 was repeated and gave the same result.

Looking at their data (3) from the perspective of quantifying the transmission of the viruses (9), one can extract estimates of the underlying transmission parameters for the two isolates in ferrets. For NL219 the reproduction ratio estimate is $R_0 = 0$ (95% confidence interval $R_0 < 3.4$) and for isolate NL230, based on two replicate experiments, $R_0 = 1.5$ (95% confidence interval 0.36 to 9.2). According to statistical inference based on the models (9) these two isolates show in this experimental set-up no significant difference in transmission parameters for ferrets. This remains true when the experiment with the NL219 isolate is repeated and gives the same result as before (Table I).

Table I
Significance levels for some transmission experiments
To test whether two experimental set-ups (for example two different host species, two different virus isolates or two different vaccines) yield different results with respect to transmission, the difference in the number of contact infections is documented. The probability (p) that the observed difference or an even more extreme difference can occur is then calculated *assuming* the two set-ups are identical ('null hypothesis'). These p values are given in the Table for different experimental designs each with two experimental set-ups and different observed differences. The observed difference is the difference in the number of animals which become infected in the two set-ups. It is thus assumed that the observed number of infected contact animals is identical for both groups using the same experimental design as given in the first column. The design is a group of animals with S susceptible animals and I infectious animals repeated n times coded in the Table as n × (S,I) (the p values were calculated using methods described in de Wit *et al.* [10] and van Boven *et al.* [25]). For example, when each parameter is tested with one experimental group of 3 susceptible animals and 3 infectious animals the maximum difference in outcome is 3 (in one set-up all infected and in the other none). In that case the Table provides us with a p-value of 0.060, which means not significant

Experimental design	Difference observed						
	0	1	2	3	4	5	6
1 × (3,3)	n.s.	0.375	0.182	0.060			
2 × (3,3)	n.s.	0.413	0.254	0.133	0.056	0.018	0.0036
1 × (5,5)	n.s.	0.411	0.251	0.130	0.053	0.013	

Concluding remarks

Methods

In this paper the authors looked at the epidemic of H7N7 in the Netherlands in 2003 using a combination of observational data, experimental data and quantitative methods (9).

The key element in this approach is the transmission parameters that summarise the transmission characteristics of the different virus isolates in different host species.

Quantitative knowledge with respect to transmission parameters provides a basis for controlling epidemics and for testing control measures (27, 29).

Transmission parameters can be estimated both from experiments and from observed chains of infection. Comparison of these estimates can give insight into the dynamics of the virus, e.g. the differences in transmission in pigs as observed in the field and in experiments.

It is therefore advised that, in addition to good field observations, properly designed experiments are carried out and are statistically analysed.

Findings

An outbreak with the H7N7 HPAI virus was impossible to control with biosecurity and culling of infected poultry in those areas where the density of poultry farms was too high (5).

The transition from LPAI virus to HPAI virus has most probably taken place in poultry. Thus, circulation of H7 LPAI viruses in poultry must be seen as a risk for HPAI virus outbreaks.

Infection in humans with an H7 virus is possible and can lead to lethal disease.

Intraspecies transmission in mammals did not occur during this epidemic ($R_0 < 1$).

La transmission intra et inter-espèces du virus H7N7 de l'influenza aviaire hautement pathogène lors de l'épizootie d'influenza aviaire survenue en 2003 aux Pays-Bas

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Résumé
L'épizootie d'influenza aviaire hautement pathogène (IAHP) due au virus H7N7 survenue aux Pays-Bas en 2003 est probablement le résultat de la transmission inter-espèces d'un virus de l'influenza aviaire faiblement pathogène (IAFP) à partir d'oiseaux sauvages vers les populations de volailles domestiques, suivie de la transmission intra-espèce du virus chez ces dernières. La transmission intra-espèce du virus H7N7 (devenu hautement pathogène) a abouti facilement, aussi bien entre troupeaux qu'à l'intérieur d'un même troupeau. De ce fait, dans les deux zones affectées qui comportaient une forte densité avicole, l'élimination de toutes les volailles était la seule solution permettant d'endiguer l'épizootie. D'après les modèles spaciaux, cette solution n'a été pratiquée que dans ces deux zones aux Pays-Bas. Il y a eu également des cas de transmission inter-espèces au porc et à l'homme. Il a été établi que la transmission ultérieure intra-espèce a été négligeable chez le porc ($R_0 < 1$). Rétrospectivement, on peut considérer qu'il en a été de même chez l'homme.

Mots-clés
Épidémie – H7N7 – Influenza – Ratio de reproduction – Transmission – Volaille.

Transmisión intra e interespecífica de virus H7N7 de la influenza aviar altamente patógena durante la epidemia que asoló los Países Bajos en 2003

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Resumen
La epidemia de virus H7N7 de la influenza aviar altamente patógena (IAAP) que en 2003 sufrieron los Países Bajos fue probablemente causada por la introducción de una cepa H7N7 de influenza aviar levemente patógena (IALP) (por transmisión interespecífica a partir de aves salvajes) y la subsiguiente transmisión intraespecífica de ese virus en las aves de corral. El virus H7N7 de la IAAP resultante adquirió después gran eficacia en la transmisión intraespecífica, tanto en una misma bandada como entre bandadas. A consecuencia de ello, en las dos zonas de alta densidad avícola que resultaron afectadas la única solución para atajar la epidemia fue eliminar a todas las aves domésticas de la región. A juzgar por los modelos espaciales, ésas fueron las dos únicas zonas de los Países Bajos donde ocurrió tal cosa. También hubo

transmisión interespecífica a mamíferos, concretamente a porcinos y humanos. Se demostró que, en el caso del cerdo, el nivel de la subsiguiente transmisión intraespecífica era insignificante ($R_0 < 1$). Visto retrospectivamente, es probable que otro tanto ocurriera con las personas.

Palabras clave
Ave de corral – Coeficiente de reproducción – Epidemia – H7N7 – Influenza – Transmisión.

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