RIVM report 703719012/2005

Quantitative Risk Assessment of Avian Influenza Virus Infection via Water

JF Schijven, PFM Teunis, AM de Roda Husman

Contact:
JF Schijven
Microbiological Laboratory for Health Protection
Jack.schijven@rivm.nl

This investigation has been performed by order and for the account of the Environmental Inspectorate, within the framework of project M/703719/05/BD, Advise on Drinking Water.

RIVM, P.O. Box 1, 3720 BA Bilthoven, telephone: 31 - 30 - 274 91 11; telefax: 31 - 30 - 274 29 71

Rapport in het kort

Kwantitatieve risicoschatting van vogelgriepvirus via water

Op grond van literatuurgegevens werden voor kippen en mensen dagelijkse infectierisico's door H5N1-vogelgriepvirus door consumptie van besmet drinkwater geschat voor Nederland. Een zeer infectieus virus en minder dan 4 $\log_{10}$ drinkwaterzuivering (redelijk inefficiënt) kunnen leiden tot een hoog infectierisico (meer dan 1%) voor pluimveebedrijven met meer dan tienduizend kippen. Goed gezuiverd drinkwater (8 $\log_{10}$) leidt tot een verwaarloosbaar infectierisico voor individuele kippen en mensen.

Aangenomen werd dat een enkele geïnfecteerde eend H5N1-virus uitscheidde in oppervlaktewater, dat werd ingenomen voor drinkwaterproductie en leidde tot consumptie van besmet drinkwater door een kip of mens.
Bij 8 $\log_{10}$ drinkwaterzuivering is het geschatte dagelijkse infectierisico voor een individuele kip laag, namelijk 10^{-15}–10^{-10}. Dit weerspiegelt de grote onzekerheden in virusuitscheiding en infectiviteit (10^{-5}–1). Desondanks, kunnen de 2000 pluimveebedrijven met meer dan tienduizend kippen (74% van alle Nederlandse pluimveebedrijven) een hoog risico (meer dan 1%) lopen indien het virus zeer infectieus en de drinkwaterzuivering minder dan 4 $\log_{10}$ is.

Uitgaande van een lage virusinfectiviteit (10^{-5}) werd het gemiddelde dagelijkse infectierisico voor de mens door drinkwaterconsumptie geschat op 2×10^{-12}, wat zeer laag is, en door oppervlaktewaterrecreatie op 10^{-8}.

Hoewel het H5N1-vogelgriepvirus voor mensen vermoedelijk minder infectieus is dan voor kippen, is efficiënte drinkwaterzuivering ook voor de mens van groot belang. Efficiënte en robuuste drinkwaterzuivering kan worden vastgesteld aan de hand van de in Nederland reeds wettelijk opgelegde risicoanalyse voor enterovirussen en in waterveiligheidsplannen.

Trefwoorden: vogelgriepvirus, risicoschatting, kippen, mensen, drinkwater

Abstract

Quantitative Risk Assessment of Avian Influenza Virus Infection via Water

Using literature data, daily infection risks of chickens and humans with H5N1 avian influenza virus (AIV) by drinking water consumption were estimated for the Netherlands. A highly infectious virus and less than 4 $\log_{10}$ drinking water treatment (reasonably inefficient) may lead to a high infection risk (more than 1%) of poultry farms with more than 10 000 chickens. Well treated drinking water (8 $\log_{10}$) leads to a negligible infection risk of individual chickens or humans.

It was assumed that a single infected duck was shedding H5N1 AIV in surface water used for drinking water production, after treatment resulting in consumption of contaminated water by chickens or humans.
At 8 $\log_{10}$ treatment, the estimated daily infection risk of an individual chicken is low, 10^{-15}–10^{-10}, reflecting the large uncertainties in viruses shedding and infectivity (10^{-5}–1). Nevertheless, the 2000 farms with more than 10000 chickens (74% of all Dutch farms), may run a high risk (more than 1%) if the virus is highly infectious and treatment is less than 4 $\log_{10}$.

Assuming a low virus infectivity (10^{-5}), the average daily infection risk for humans by consumption of contaminated drinking water was estimated as low as 2×10^{-12}, and by surface water recreation as low as 10^{-8}.

Although H5N1 AIV is presumably less infectious for humans than chickens, efficient drinking water treatment is also of utmost importance for humans and may be determined by risk analysis for enteroviruses already required by Dutch law and warranted by water safety plans.

Key words: avian influenza virus, risk assessment, chickens, humans, drinking water

Contents

Summary

In the present study, risks of infection of chickens and humans were estimated due to consumption of drinking water that was contaminated with H5N1 AIV. It was assumed that an infected duck was shedding this virus in surface water and that a fraction of this virus was able to pass drinking water treatment and reach a chicken or human. This risk assessment was conducted in order to evaluate the potential transmission of AIV to poultry and humans via drinking water. Despite the lack of data and the large uncertainties involved, this quick microbiological risk assessment allowed us to draw conclusions about the risks for chickens and humans associated with the possible exposure to H5N1 AIV via water.

It was found that the mean daily risk of infecting an individual chicken by consumption of contaminated drinking water is low, namely $8.9 \times 10^{-10} – 2.3 \times 10^{-7}$, depending on the assumed virus infectivity from $10^{-5} – 1$. The wide range reflects the large uncertainties in the estimates of number of viruses shed in the surface water and the infectivity of the virus.
The risk of infection of at least one chicken in a farm is more relevant, because once one chicken in a farm has been infected, the whole farm must be considered lost. This infection risk of a farm was found to be four orders in magnitude higher than the risk of infection of an sinlge chicken. Especially large farms, and even more in the case of a highly infectious virus, may run a high risk. A ten times larger farm is at a ten times higher risk. A ten times lower risk level requires drinking water treatment to be ten times more efficient.

This risk assessment demonstrated that especially the numbers of shed viruses and the infectivity of the virus determine the risk of infection. This emphasizes the need to put effort into quantifying numbers of viruses that are excreted by an infected organism and, if possible, determine a dose-response relationship for a wide range of doses in order to reduce the large uncertainties encountered in evaluating the importance of transmission of such agents as H5N1 AIV via water.
Nevertheless, the current data for AIV allowed concluding that highly efficient drinking water treatment is required in the case high numbers of highly infective virus are shed into surface water, but the actual estimated risk is very uncertain.
On the basis of this evaluation it is recommended to remain alert on the spread of AIVs by water fowl in neighboring European countries.

Assuming a low infectivity of 10^{-5} of AIV for humans, the daily risk of infection of humans was estimated to be on average 1.8×10^{-12}, which is very low. Thus, it was concluded that H5N1 infection of humans in the Netherlands from properly treated drinking water is negligible.

The risk of infection by ingestion of contaminated surface water by bathers, divers, surfers and kayakers, was estimated to in the order op 10^{-8}.

1. Introduction

Wild waterfowl are the natural reservoir of all avian influenza viruses (AIVs), and these viruses are usually nonpathogenic in birds. Influenza viruses are pleomorphic, enveloped RNA viruses belonging to the family of Orthomyxoviridae. Protruding from the lipid envelope are two distinct glycoproteins, the hemagglutinin (HA) and neuraminidase (NA) (De Jong and Hien, 2005). Virus representatives of all 16 hemagglutinin and all 9 neuraminidase subtypes have been isolated from waterfowl. From this reservoir, AIVs occasionally be transmitted to other avian and mammalian hosts, including humans, and can cause outbreaks of severe disease (Sturm-Ramirez *et al.*, 2005). Most AIVs isolated from birds are avirulent. The H5 and H7 subtypes are highly pathogenic and are capable of causing outbreaks of severe disease in chickens or turkeys. Although all HA and NA subtypes are found in aquatic birds, the number of subtypes that have crossed the species barrier and established lineages in mammals is limited (De Jong and Hien, 2005). However, since late 2002, H5N1 outbreaks in Asia have resulted in mortality among waterfowl in recreational parks, domestic flocks, and wild migratory birds (Sturm-Ramirez *et al.* (2005). Li *et al.* (2004) indicated that domestic ducks in southern China had a central role in the generation and maintenance of H5 N1 AIV and that wild birds may have contributed to increasingly wide spread of it.
Frequent long-term cloacal shedding of H5N1 viruses by ducks increases the likelihood of transmission to the environment, to other ducks, and potentially, to other species. Water in which ducks swim, drink and eat present a high exposure risk to humans and domestic chickens (Hulse-Post *et al.*, 2005). Because birds are the natural hosts of these viruses, birds obviously are at a higher risk of infection and disease than humans.

Since 2004 outbreaks caused by an H5N1 AIV occurred in several Asian countries, and in December 2005 also among chickens in Turkey, Romania and Croatia. In Vietnam, Cambodja, Indonesia and Thailand few human cases of bird flu by H5N1 were confirmed.

In 2003 in the Netherlands, avian influenza virus H7N7 caused an outbreak that resulted in destruction of 25 million chickens (Galama, 2003), but no signs of spread of H5N1 to the Netherlands at present have been observed.

In response to the spread of pathogenic Avian Influenza Virus (AIV) of type H5N1, the Dutch Ministry of Housing, Physical Planning and the Environment questioned whether this virus could contaminate drinking water in the Netherlands and to which health impact.

The present study aimed to estimate risks of infection of poultry and humans in the case that one infected duck has been shedding AIV of type H5N1 in surface water that is being abstracted for drinking water production. This risk assessment was based on data from literature. By means of Monte Carlo simulations, uncertainties in these data were included in the risk assessment. In addition, this risk assessment aimed to give insight into the potential threat from pathogenic AIV contaminating drinking water.

2. Methods

2.1 Outline of Quantitative Risk Assessment

It is assumed that one infected duck is shedding H5N1 AIV in surface water, that is being used for drinking water production. The shed virus mixes with the water and gradually inactivates in the water. During treatment for drinking water production the virus particles that enter the treatment plant are removed for most part. The virus particles that are able to pass the treatment may reach poultry farms. Chickens drink unboiled drinking water and thus may be exposed to these viruses. Chickens that become infected this way may rapidly spread the virus through the farm by direct bird-to-bird transmission. Also humans consume unboiled drinking water and may therefore be exposed to these viruses.
The following equations describe the exposure (ingested dose) to AIV, the exponential dose response model that was applied for calculating the individual risk of infection and the risk of infection of at least one chicken in a farm (Teunis and Havelaar, 2000):

$$D = \frac{N}{W} 10^{-R} V \qquad (1)$$

where, D is the ingested daily dose [number of viruses/day], N is the number of shed viruses/day in surface water, W is the volume of surface water [liter], R is the $\log_{10}$ removal of virus by drinking water treatment and V is the daily ingested volume of unboiled drinking water (by chickens or humans) [liter].

$$p_i = 1 - e^{-rD} \qquad (2)$$

where, p_i is the daily invidual risk of infection of a chicken or human and r is the infectivity of the virus. It is assumed that each virus particle has the same infectivity.

$$p_F = 1 - \left(1 - \left(1 - e^{-rD}\right)\right)^F \qquad (3)$$

where, p_F is the risk of infection of at least one chicken in a farm with F chickens. Note that in this case the whole farm is considered to be infected, because of the high transmission rate from chicken to chicken.

Accordingly, the following data were collected from literature for this risk assessment:

1. Numbers of AIVs shed by ducks (N)
2. Volume of contaminated surface water (W)
3. Inactivation rate of AIV in surface water (μ)
4. Decimal removal by drinking water treatment (R)
5. Size of poultry farms (F)
6. Ingested volume of water by poultry or humans (V)
7. Infectivity of AIV to poultry or humans (r)

For each of these model parameters, distributions were constructed by applying Monte Carlo drawings in Mathematica 5.1 (Wolfram Inc.)

2.2 Shedding of AIV, *N*

Since duck/Memphis influenza virus is shed in high concentration in the feces, the most likely method of spread of the virus in ducks in their natural habitat would be through the water (Webster *et al.*, 1978).
Much higher numbers of AIVs have been isolated from cloacal samples than from the respiratory tract. A/Duck/Memphis/546/74 influenza virus can pass through the digestive tract, despite the low pH in the gizzard, and replicate in the lower intestinal tract of mallard ducks, without producing any signs of disease. This transition through the digestive tract of the duck and replication in the lower intestinal tract was possible without prior replication in the lungs (Webster *et al.*, 1978).

Webster *et al.* (1978) also reported that the mammal virus strains WSN/33 (H0N1), Memphis/110/76 (H3N2), Texas/1/77 (H3N2) and swine/Tn/1/75 (Hsw1N1) each replicated in the upper respiratory tract of ducks, but the viruses were not detected in cloacal samples. The human A/Japan/305/57 strain (H2N2) did not multiply in ducks. The relatively high titers of virus obtained from tracheal swab samples (1000 – 50 000 particles per swab) together with the high antibody titers 14 days after infection, leave no doubt that the above mentioned mammalian influenza A viruses can replicate in ducks.

Lu *et al.* (2003) infected chickens with H7N2 AIV in four trials ($5 \times 10^4 - 5 \times 10^5$ EID_{50} per bird). H7N2 was reisolated from cloacal swabs two days after infection. The first week was the most active period of shedding through the respiratory and intestinal tracts. In trial 1, 10% of cloacal swabs were positive at 24 and 31 days after infection. Cage swabs and manure samples were also positive for AIV. In trial 4, cage swabs and manure samples were detectable for at least three weeks.

Hulse-Post *et al.* (2005) compared the pathogenicity and transmissibility in ducks of the H5N1 influenza viruses isolated from poultry and humans in 2003 and 2004 in various regions of Asia (two isolates from Hong Kong, one from mainland China, six from Vietnam, one from Thailand and one from Indonesia in mallards). Tree additional viruses isolated in previous years were tested for comparison to the new 2003/2004 viruses. All of the isolates tested, including the four human isolates, replicated in the inoculated ducks (inoculated with 10^6 EID_{50} of virus; 0.5 ml was applied to the cloaca, 0.2 ml to the trachea and 0.1 ml each to the throat, nares and eyes) and were transmitted efficiently to contact ducks, which shed virus at high titers. Hulse-Post *et al.* (2005) categorized low pathogenicity by the absence of deaths and high pathogenicity by the death of at least one duck out of four (two inoculated and two contact ducks). Within the high-pathogenicity category there was a wide range of disease signs and mortality. Viruses that caused the death of at least one duck could cause very mild symptoms in another animal, such as cloudy eyes with no neurological signs or could cause severe clinical signs, such as weight loss, cloudy eyes and severe neurological dysfunction. A/Vietnam/1203/04 was the only human isolate tested that caused the death of ducks. Several ducks infected with the human Vietnamese virus isolate A/Vietnam/3046/04 had cloudy eyes upon close examination but showed no other disease signs. The human virus isolate from Thailand and the chicken H5N1 isolates from mainland China and Indonesia were non-pathogenic in ducks. H5N1 viruses isolated from healthy ducks (*i.e.* not pathogenic to ducks) remain pathogenic to chickens and to mammals (mice). Therefore, ducks may represent a reservoir of H5N1 viruses, transmitting them to other bird species and potentially to mammals.

Hulse-Post *et al.* (2005) summarize from older studies that nonpathogenic AIVs were shed by ducks for as long as 20 days, but highly pathogenic H5N1 viruses for only 2 to 5 days with one exception of 10 days. Hulse-Post *et al.* (2005) showed that all tested 2003- and 2004 - isolates of H5N1 were shed by inoculated and contact ducks for at least 11 days after infection and all the isolates they tested were shed for 7 to 17 days. All of the viruses tested showed similar results: Virus titers were highest on day 3 and were consistently higher in the tracheal than in the cloacal swabs at that time. The titers then decreased progressively until day 11, after which the titers were consistently close to 10 EID_{50} per ml or were undetectable. Hulse-Post *et al.* (2005) did not report virus titers. Hulse-Post *et al.* (2005) also demonstrated that virus can be isolated for a longer period from ducks infected with the newer (2003-2004) H5N1 isolates than from those infected with the tested viruses from 1997 and 2001. The longer period of virus-shedding appears to be another characteristic of the H5N1 viruses currently circulating in ducks.

Sturm-Ramirez *et al.* (2005) inoculated juvenile mallards with 23 different H5N1 influenza viruses isolated in Asia between 2003 and 2004. All viruses replicated efficiently in inoculated ducks and 22 were transmitted to susceptible contacts. Viruses replicated to higher levels in the trachea than in the cloaca of both inoculated and contact birds, suggesting that the digestive tract is not the main site of H5N1 influenza virus replication in ducks and that the fecal-oral route may no longer be the main transmission path. The pathogenicities of the virus isolates varied from completely nonpathogenic to highly lethal and pathogenicity was positively correlated with tracheal virus titers. Nevertheless, the eight virus isolates that were nonpathogenic in ducks replicated and transmitted efficiently to naïve contacts, suggesting that the highly pathogenic H5N1 viruses causing minimal signs of disease in ducks can propagate silently and efficiently among domestic and wild ducks in Asia and that they represent a serious threat to veterinary and human public health.

Each of the viruses tested by Webster *et al.* (1978) (Hav1Nav2, Hav4Nav1, Hav5N2 and an unknown H?N?) replicated in the upper respiratory tract of the duck, were shed in high concentrations in fecal material ($30 – 6 \times 10^5$ per cloacal swab for a week), and caused no signs of disease. These data show that a number of avian influenza A viruses possessing different hemagglutinin and neuraminidase antigens can replicate in the intestinal tract of ducks (Webster, *et al.* 1978). A/Duck/Memphis/546/74 influenza virus was detected in all segments of the intestines posterior to the duodenum. The high titers of virus obtained from the mucosal cells of the intestines ($6 \times 10^3 – 6 \times 10^7$ per ml) suggested that the virus was replicating in these cells (Webster *et al.*, 1978).
The virus titers of these cloacal swabs from these four AIVs taken in a period of a week are given in ***Table 1*** and appear fairly uniformly distributed, encompassing a range of 1.4 to 5.8 $\log_{10}$ EID_{50}/swab.

Webster *et al.* (1978) also reported that experimentally infected Muscovy ducks (*Cairina moschata)* shed 6.4 g of fecal material per hour with an infectivity of 6.3×10^7 mean egg infective dose (EID_{50}). These birds shed an estimated 10^{10} EID_{50} of AIV (A/Duck/Memphis/546/74 Hav3Nav6) within a 24-hour period.

Sturm-Ramirez *et al.* (2004) inoculated mallard ducks via the cloaca, trachea, mouth, nares and eys with H5N1/02-03 AIVs (5.6×10^5- 3.2×10^8 EID_{50}/ml) and measured virusconcentrations in the cloacae of the inoculated and contact ducks (***Table 2***). Of those

ducks that shed a specific virus strain (above detection limit), these concentrations ranged from 2.2 – 4.4 $\log_{10}$ EID_{50}/ml ($1.6 \times 10^2 – 2.5 \times 10^4$ EID_{50}/ml).

Lee *et al.* (2005) reported virus titers in cloacal samples of A/chicken/TX/298313/04 H5N2 from parent and 14-day derivatives in chickens (***Table 3***).

Table 4 summarizes the virus titers found in cloacal samples (Sturm-Ramirez *et al.*, 2005).

To summarize, Webster et al. (1978) reported a range of 1.4 – 5.8 $\log_{10}EID_{50}$ per fecal swab for a number of different AIVs and the apparently high excretion of 7.8 $\log_{10}EID_{50}$/g feces of a H3N3 AIV. Sturm-Ramirez *et al*. (2004 and 2005) reported for cloacal samples a range of 2.2 – 4.4 $\log_{10}EID_{50}$/ml and 1 – 2.9 $\log_{10}EID_{50}$/ml of H5N1 AIVs. Lee *et al*. (2005) reported for cloacal samples a range 4.7 – 5.1 $\log_{10}EID_{50}$/ml H5N2 AIVs. These data were used in the risk assessment.

For risk assessment, virus excretion in $\log_{10}EID_{50}$/g feces was represented by a normal distribution, with parameters μ=3 and σ=1 to encompass this range of 1 – 5 $\log_{10}$ within the 95%-interval and with a maximum near 7.8. To calculate numbers of viruses excreted in a day 6.4 g feces per hour (Webster *et al.*, 1978) was assumed.

Table 1Virus titer (log_{10} EID_{50}/swab) in cloacal swabs over 7 days (Webster et al., 1978)

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Hav1Nav2	3.3	5.8	5.8	5.3	4.5	2.6	2.3
Hav4Nav1	3.5	4.8	3.5	2.3	2.0	1.5	
Hav5N2	1.5	2.3	3.4	1.6	1.4		
H?N?	4.0	5.8	5.8	5.3	5.8	4.5	3.5

Table 2 Virus titers (log_{10} EID_{50}/ml) of H5N1/02-03 AIVs in cloaca of mallard ducks (Sturm-Ramirez et al., 2004)

AIV	3 days post infection		5 days post infection	
	Inoculated ducks	Contact ducks	Inoculated ducks	Contact ducks
HK/156/97	2.4	0	0	<1
Ck/HK/220/97	<1	0	0	3.5
Ck/HK/YU562/01	3.5	0	0	<1
Ck/HK/FY150/01	3.0	0	2.2	2.5
Ph/HK/FY155/01	4.4	2.3	<1	3.5
Ck/HK/822.2/01	<1	0	0	0
A/Ck/HK/873.3/01	<1	2.5	2.8	2.2
Ck/HK/86.3/02	2.5		0	
Teal/HK/2978.1/02	<1	2.2	0	2.3
RB poch/HK/821/02	0	<1	0	2.3
Gs/HK/739.2/02	2.2	2.6	0	0
HK/213/03	<1	<1	2.5	<1

Table 3 Titer of A/chicken/TX/298313/04 H5N2 in cloacal samples of chicken (Lee et al., 2005).

Virus	Inoculation	Virus titer in cloacal samples (log_{10} EID_{50}/ml)
Parent	Intranasal	<0.9
	Intravenous	4.7 ± 0.3
14-day derivative 2	Intravenous	4.8 ± 0.4
14-day derivative 10	Intravenous	5.1 ± 0.3

Table 4 Virus titers (log_{10} EID_{50}/ml) of H5N1/03-04 AIVs in cloaca of mallard ducks (Sturm-Ramirez et al., 2005)

AIV	3 days post infection		5 days post infection	
	Inoculated ducks	Contact ducks	Inoculated ducks	Contact ducks
Low pathogenicity group				
A/Ck/HK/NT71/03	<1	<1	0	0
A/Ck/HK/AP111/03	<1	0	0	1.0
A/Ck/HK/YU250/03	<1	0	0	0
A/Falcon/HK/D0028L/04	<1	0	0	0
A/Ck/AH/85/04	2.8	1.0	<1	0
A/Ck/PP/BPPV3/04	2.5	1.0	1.0	2.3
A/VN/3046/04	1.0	0	<1	<1
A/Thai/1 (Kan-1)/04	2.3	<1	<1	<1
High pathogenicity group				
A/Ph/HK/NT123/03	0	<1	0	<1
A/Ck/HK/SSP94/03	0	0	0	<1
A/Ck/HK/WF27/03	3.0	<1	1.0	<1
A/Ck/VN/133/04	2.5	2.3	<1	1.0
A/Ck/VN/48C/04	3.5	2.3	1.0	1.0
A/Dk/VN/40D/04	1.0	0	0	<1
A/Pigeon/HK/WF32/03	<1	<1	0	<1
A/S.Ck/HK/YU17/03	<1	2.5	0	
A/Ck/HK/YU46/03	<1	<1	<1	<1
A/Ck/HK/SSP171/03	<1	<1		1.7
A/Ck/HK/SSP7/03	<1	2.9		2.7
A/Mal/VN/16D/03	1.0	2.5	2.5	
A/Ck/VN/C58/04	<1	0	<1	<1
A/VN/1203/04	<1	<1	<1	<1
A/Dk/Thai/71.1/04	2.0	0		0

2.3 Volume of surface water, *W*

Following virus shedding in water, the virus is diluted to a certain extent. The dilution is highly dependent on the size of the surface water as well as of the flow rate. Here, it was assumed that the virus was rapidly dispersed leading to homogeneous suspensions. For the calculations, three types of water were considered as was done by Schijven *et al.* (2005) for spread of foot-mouth and disease virus through surface water (***Table 5***). Three dilution scenario's were followed.

Table 5 Dimensions of receiving surface waters (Schijven et al., 2005)

Size	Flow rate ($m^3\ day^{-1}$)	*W* (liter day^{-1})	Width (m)	Depth (m)	*L* (m)
Small	8.6×10^4	8.6×10^7	10	1.5	5.8×10^3
Medium	2.2×10^6	2.2×10^9	50	2.6	1.7×10^4
Large	2.3×10^7	2.3 10^{10}	× 125	3.8	4.8×10^4

L is characteristic length (one-day flow) of river.

2.4 Inactivation of AIV, μ

It is important to know at which rate AIV inactivates in surface water.
Fecal-oral transmission of AIV within wild waterfowl populations is thought to occur via contaminated water (Hinshaw *et al.*, 1979; Stallknecht *et al.*,1990). Stallknecht *et al.* (1990) tested inactivation of five AIVs in sterile glass-filtered distilled water (pH 7.3) at 4, 17 and 28 °C (***Table 6***). Linear regression adequately explained inactivation results 66% - 97% of the variation in all cases. These results are supportive of the possibility that AIVs are able to survive through the winter in surface water and reinfect ducks returning to breeding areas in spring.

Table 6 summarizes estimates of the inactivation rate coefficient (μ) of A/duc/Memphis/546/74 influenza virus in fecal material and river water at 4 °C and 22 °C (Webster *et al.*, 1978). It shows that inactivation of this virus is higher at the higher temperature, similarly low at 4 °C in fecal material and river water, but higher in river water than in fecal material at 22 °C.
Considering the values given in ***Table 6***, where it appears that at low and moderate environmental temperatures virus inactivation is commonly less than a factor of ten combined with the probable large variation in shed virus concentrations, it is clear that virus concentrations in surface water may remain relatively high for at least several days. Also considering continuous intake of water for drinking water production, virus inactivation in surface water was not included in the risk estimation.

2.5 Drinking water treatment, *R*

In the Netherlands, about one third of the drinking water is produced from surface water and two thirds from groundwater. In this risk assessment, we regard drinking water produced from intake of surface water. Especially storage reservoirs are of concern, because these waters are frequently visited by water fowl that may contaminate these reservoirs with AIVs.

Table 6 Inactivation rate coefficient μ of AIVs

Virus	Experimental conditions	Value		Reference
		Day^{-1}	$Log_{10}\ day^{-1}$)	
A/duck/Memphis/546/74 (Hav3Nav6)	Fecal material, pH 7.7, 4 °C, 32 days	0.32	0.14	Webster *et al.*, 1978
A/duck/Memphis/546/74 (Hav3Nav6)	Fecal material, pH 7.7, 22 °C, 8 days	1.3	0.57	
A/duck/Memphis/546/74 (Hav3Nav6)	Mississippi River water, 4 °C, 32 days	0.30	0.13	
A/duck/Memphis/546/74 (Hav3Nav6)	Mississippi River water, 22 °C, 4 days	2.5	1.1	
A/gadwall/LA/17G/87 (H3N8)	17 °C	0.071	0.031	Stallknecht *et al.*, 1990
	28 °C	0.21	0.092	
A/blue-winged teal/LA/44B/87 (H4N6)	17 °C	0.064	0.028	
	28 °C	0.17	0.075	
A/mottled duck/LA/38M/87 (H6N2)	17 °C	0.064	0.028	
	28 °C	0.15	0.065	
A/blue-winged teal/188B/87 (H12N5)	17 °C	0.11	0.048	
	28 °C	0.45	0.20	
A/green-winged teal/LA/169W/88 (H10N7)	17 °C	0.94	0.41	
	28 °C	1.4	0.59	
	4°C	0.0092	0.004	
AIV H7N2	SPF chicken manure,			Lu *et al.* (2003)
	56 °C	290	130	
	37 °C	0.38	0.17	
	15-20 °C	0.27	0.12	
	4 °C	<0.27	<0.12	
	Experimental field chicken manure,	430	190	
	56 °C	4	1.7	
	37 °C	4	1.7	
	28-30 °C	1	0.43	
	15-20 °C	<0.3	<0.13	
	4 °C			
	Commercial field chicken manure,			
	56 °C	580	120	
	37 °C	1	0.43	
	28-30 °C	12	5.2	
	15-20 °C	<3	<1.3	
	4 °C	<0.3	<0.13	

Similarly, birds contaminate these reservoirs with campylobacters (Schijven, 2003). Concentrations of human enteroviruses in surface water in the Netherlands range from 0.01 to 100 per liter (Lodder and de Roda Husman, 2005) . Maximum allowable enterovirus concentrations in drinking water for compliance to a 10^{-4} risk is 10^{-6} per liter (De Roda Husman and Medema, 2005). Therefore, 4-8 log_{10} reduction of the virus concentrations is needed.

Goyal *et al.* (1980) described a membrane filter adsorption-elution method for concentrating viruses from water samples. Commonly a pH of 3.5 is applied, but AIVs are extremely sensitive to this low pH, therefore a new method was developed. AIVs could be adsorbed efficiently to Zeta Plus filters at pH 6. From this study it can be deducted that AIVs probably

have a neutral surface charge at pH 6 – 7. Given this relatively high isoelectric point, one may surmise that these viruses attach relatively easily to solid surfaces, e.g. in porous media such as sand in slow sand filters and in groundwater. The data on inactivation (***Table 6***) show no greater stability than enteroviruses. Based on the surface charge, and similar size and inactivation rate of AIVs and enteroviruses , it is safe to assume that AIVs are reduced by drinking water treatment at least as much as enteroviruses.

For our purposes, it was assumed that drinking water treatment applied in the Netherlands reduces virus concentrations by an average of 8 $\log_{10}$ and a probability of less than 0.1% per year that no viruses are removed at all. Furthermore, it is assumed that reduction of virus concentrations by drinking treatment is lognormally distributed. In addition, infection risks were calculated for a given treatment from no removal up to 12 $\log_{10}$ removal.

2.6 Numbers of chickens per farm in the Netherlands, *F*

Data on the number of chicken farms and numbers of chickens per farm in the Netherlands for the year 2004 were selected using Statline from Statistics Netherlands (www.cbs.nl) and are presented in ***Table 7***. This entails all chickens (broilers and egg layers). The total number of chickens amounts to 86 million. The upper class limit of the highest class (100000) is an assumption. From these data, a distribution of 10000 possible farms (is number of drawings in Monte Carlo simulations) was constructed by weighted drawing according to class size and width.

Table 7 Number of chickens per farm and number of farms in the Netherlands (CBS, 2004)

Number of chickens per farm		
Lower class limit	Upper class limit	Number of farms
1	199	354
200	399	76
400	599	20
600	999	24
1000	1999	60
2000	2999	67
3000	4999	115
5000	9999	278
10000	14999	268
15000	17499	113
17500	19999	111
20000	24999	185
25000	34999	281
35000	49999	254
50000	74999	267
75000	100000	296
	Total	2769

2.7 Daily drinking water consumption per chicken, *V*

Broiler fowl have been bred for very high weight gain rates and are actively growing for their entire economical, and very short (6-10 weeks) lives. Broiler fowl weigh 1.5 to 2.5 kg and laying hens, depending on the breed, 1.5 to 4.5 kg. They have the highest known ratio, 0.5, of weight gain/food intake rate of any livestock; a ratio of 1 kg of weight gain for 2 kg of feed. Both types of chicken eat between 70 and 200 g per day and 85 to 115 g/day for the average chicken. Broiler chickens also have a very high water consumption rate since water requirements are known to be correlated with the amount of dry matter metabolized. Both types of chicken drink between 150 to 450 mL per day. This is about 170 mL/kg for the broilers and 120 mL/kg for the layers (Anonymous, 1968, 1974, 1978, 1982, 1984; Brady, 1964; Cole, 1962; Hart, 1974; Richey *et al.*, 1961).

For the risk assessment, it was assumed the chickens drink 150 to 450 ml of unboiled drinking water per day and that this drinking water consumption is uniformly distributed.

2.8 Infectivity of AIV

Webster *et al.* (1978) refers to some older studies, where it was found that after one egg (chicken embryo) passage, influenza viruses from mammals or birds are usually filamentous with some spherical particles.
Webster *et al.* (1978) purified Hav7Neq2 influenza virus from fecal material from a feral mallard duck and found roughly spherical particles of 80 to 120 nm, with little heterogeneity and pleomorphism in size and morphology. However, a Hav3Nav6 virus was found to be extremely heterogeneous in size and shape. Thus, some strains of duck influenza virus are fairly uniformly spherical after isolation from their natural host and others are filamentous and heterogeneous. To our understanding, this observation implies that within the same strain, infectivity of AIV may be highly variable.

In many studies, pathogenicity of AIVs was studied by inoculating ducks or chickens with AIVs intraveneously, intratracheal or orally with high doses of $10^4 - 10^{8.5}$ EID_{50}, whereby commonly all were infected and fell ill (*e.g.* Hulse-Post *et al.*, 2005; Lee *et al.*, 2005; Lu *et al.*, 2003; Sturm-Ramirez *et al.*, 2004, 2005; Webster *et al.*, 1978). No data were found where ducks or chickens were inoculated with low doses. Therefore, no data are available to derive a dose-response relationship for chickens and AIVs.
However, Lu *et al.* (1999) and Nguyen *et al* .(2005) inoculated lightly anesthetized six- to ten-week-old BALB/c mice intranasally with 10^0 to 10^7 EID_{50} H5N1 and H5N2 viruses and determined MID_{50} (mouse infectious dose whereby 50% becomes infected (***Table 8***). Assuming an exponential dose-response relation, infectivity *r* was calculated from the MID_{50}-values as given in Table 8. The infectivities of both the H5N1 and H5N2 viruses isolated from birds in Vietnam LBM were approximately 10000-fold lower than that of the 1997 H5N1 strain, ranging from 1.1×10^{-5} to 0.22 (Ngyuen *et al.*, 2005). It indicates reduced ability of the former to infect mice (Nguyen *et al.*, 2005). The infectivity data from Lu *et al.* (1999) fall within the same range. Apparently, infectivities amongst AIVs vary widely. For our risk assessment we therefore calculated infection risks of chickens for a number of distinct values of *r*'s encompassing this range, namely 1, 0.1, 0.001, 0.0001 and 0.00001. Assuming infectivity of AIVs for humans is much lower than for chickens, a risk of infection for humans was calculated for an *r* of 0.00001.

Table 8 Infectivity of H5 AIVs in mice (Nguyen et al., 2005)

Virus	Log_{10} MID_{50}	$r = \ln 2 / MID_{50}$	Reference
HK/483 (H5N1)	2.2	4.4×10^{-3}	Lu *et al. (1999)*
HK/485 (H5N1)	1.1	5.5×10^{-2}	
HK/156 (H5N1)	3.2	4.4×10^{-4}	
HD/486 (H5N1)	1.2	4.4×10^{-2}	
X-31 (H3N2)	0.7	1.4×10^{-1}	
HK/483/97 (H5N1)	0.5	2.2×10^{-1}	Nguyen *et al.* (2005)
Gs/VN/113/01 (H5N1)	4.3	3.5×10^{-5}	
Dk/VN/342/01 (H5N2)	4.8	1.1×10^{-5}	

3. Results

3.1 Shedding of AIV by a duck, *N*

Figure 1 gives the distribution of *N*, the number of shed virus per day by a duck. The mean concentration is 2.0×10^6 viruses/day, the 95%-interval is $1.8 \times 10^3 - 1.4 \times 10^7$ viruses/day and the maximum is 2.5×10^9 viruses per day. This implies that *N* is often near 10^3 and sometimes near 10^7 viruses/day.

3.2 AIV concentration in surface water, *N/W*

Figure 2 gives the histogram of the virus concentration in the smallest surface water with the least dilution. Table 9 gives the mean AIV concentration for the three types of surface water. Homogeneous distribution was assumed. The shed virus may therefore be transported in the surface waters over 6, 17 and 48 km in one day (***Table 5***). It is realized that the distribution may instead be heterogeneous, whereby concentrations in the intake water for drinking water production may be highly variable.

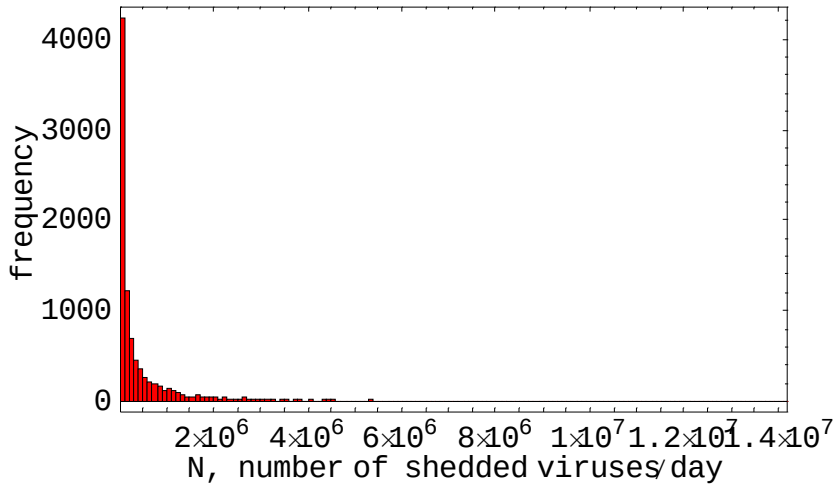

Figure 1 Distribution of number of shed viruses per day by a duck in river water.

Table 9 AIV concentration in surface water

Surface water	Mean virus concentration (N/l)	95%-interval
Small	2.3×10^{-2}	$2.1 \times 10^{-5} - 1.6 \times 10^{-1}$
Medium	9.1×10^{-4}	$8.3 \times 10^{-7} - 6.4 \times 10^{-3}$
Large	8.8×10^{-5}	$8.0 \times 10^{-8} - 6.2 \times 10^{-4}$

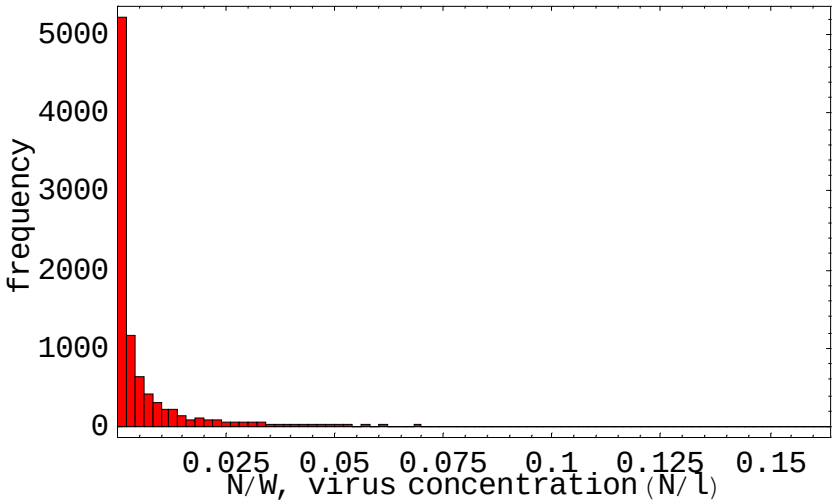

Figure 2 Virus concentration distribution in small surface water

3.3 Drinking water treatment, *R*

Figure 3 gives the distribution of the drinking water treatment, *R* with average 8.0 $\log_{10}$ and 95%-interval 4.5 to 11 $\log_{10}$.

3.4 AIV concentration in drinking water, $N/W*10^{-R}$

From the virus concentrations in surface water and the efficiency of drinking water treatment, the virus concentrations in drinking water were calculated. ***Figure 4*** shows the drinking water concentration in the scenario with the small sized surface water and ***Table 10*** gives the values for each of the three surface waters. Note that the virus concentration in drinking water is lognormally distributed and that the mean concentration is near the upper limit of the 95%-interval.

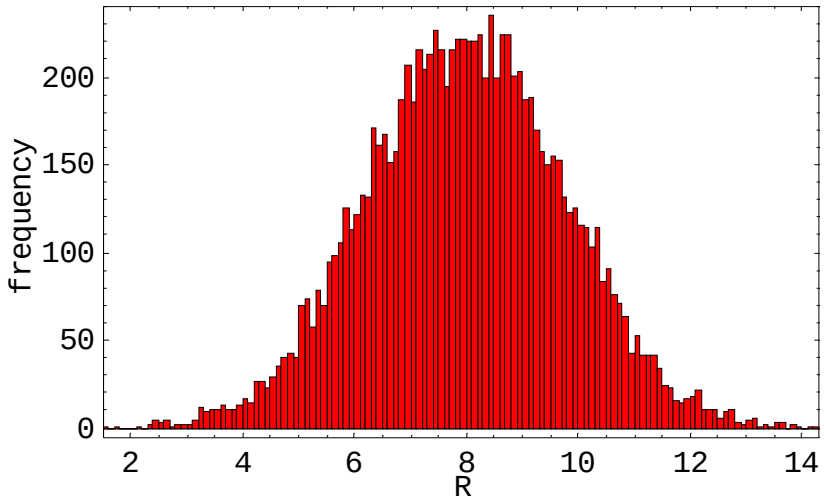

Figure 3 Distribution of log10 reduction of virus concentration by drinking water treatment

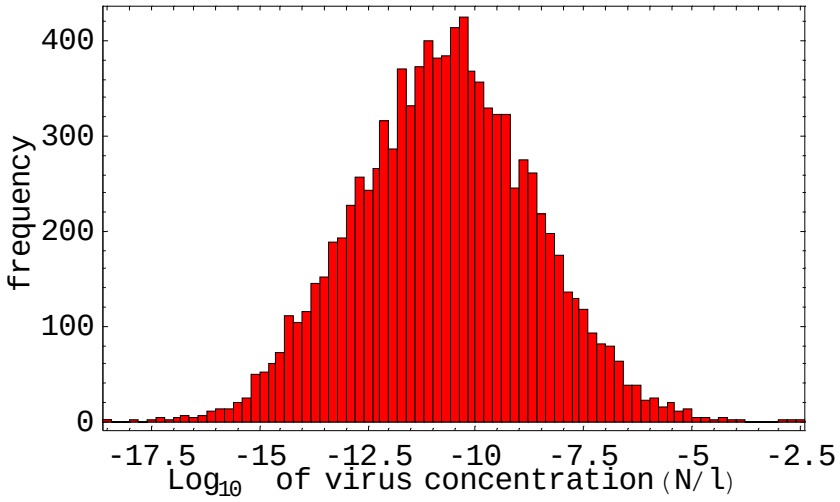

Figure 4 Distribution of log10 of virus concentration in drinking water in the scenario with small sized surface water

Table 10 AIV concentration in drinking water

Surface water	Mean virus concentration (N/l)	95%-interval
Small	7.6×10^{-7}	$2.1 \times 10^{-15} – 1.7 \times 10^{-7}$
Medium	3.0×10^{-8}	$8.1 \times 10^{-17} – 6.6 \times 10^{-9}$
Large	2.9×10^{-9}	$7.8 \times 10^{-18} – 6.4 \times 10^{-10}$

3.5 Numbers of chickens per farm in the Netherlands, *F*

Figure 5 shows distribution of the numbers of chickens per farm in the Netherlands. The average value is 17361 and the 95%-interval is 41 – 92794.

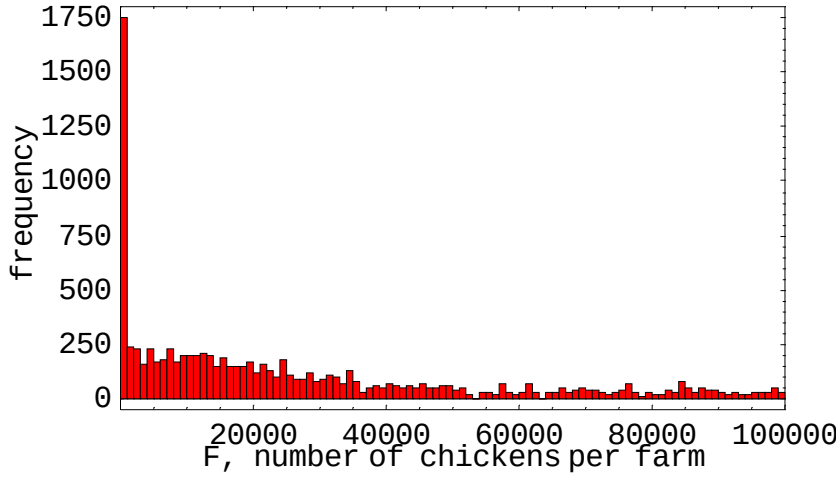

Figure 5 Distribution of the numbers of chickens per farm in the Netherlands in 2004

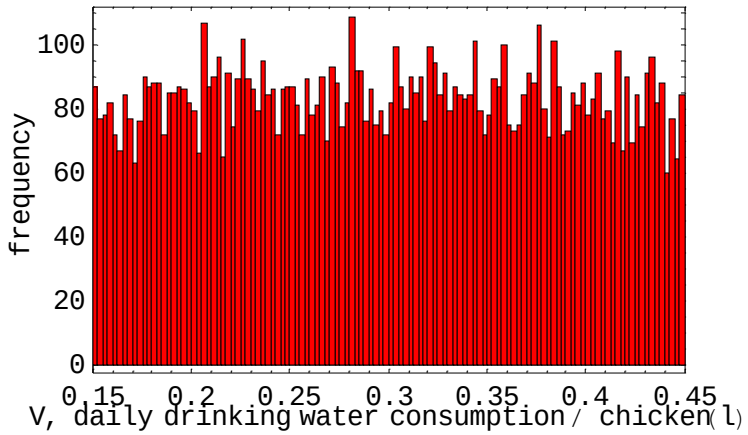

Figure 6 Distribution of daily drinking water consumption per chicken (liter)

3.6 Daily drinking water consumption per chicken, *V*

Figure 6 shows the uniform distribution of the daily drinking water consumption per chicken, with an average of 0.30 liter and a 95%-interval of 0.16 – 0.44 liter.

3.7 Infection risk of per individual chicken, p_i

Figure 7 shows the infection risk p_i per chicken for drinking water from the small surface water, for *r* equal to 1. Note that in the case of *r=1*, the risk of infection equals the risk of exposure. The average value and 95%-interval for all three surface waters are given in ***Table 11***. Note that also the mean p_i is higher than the upper limit of the 95%-interval, but of the same order in magnitude. It also shows a large uncertainty (95%-interval) for p_i and that p_i is proportional to *1/W*. In the worst case assumption of *r=1*, and the small surface water scenario, mean p_i is very low (2.3×10^{-7}).
Figure 8 shows p_i for as a function of r (10^{-5} – 1) for all three surface waters. It appears that a 5 $\log_{10}$-difference in infectivity *r* of the AIV results in a 5 $\log_{10}$-difference of p_i.
Dependent on the applied range of dilution of AIV in surface water, *1/W*, and the infectivity, *r*, of that virus, the mean daily risk of infection, p_i, of an individual chicken that ingests contaminated drinking water ranges from 8.9×10^{-15} – 2.3×10^{-7}.

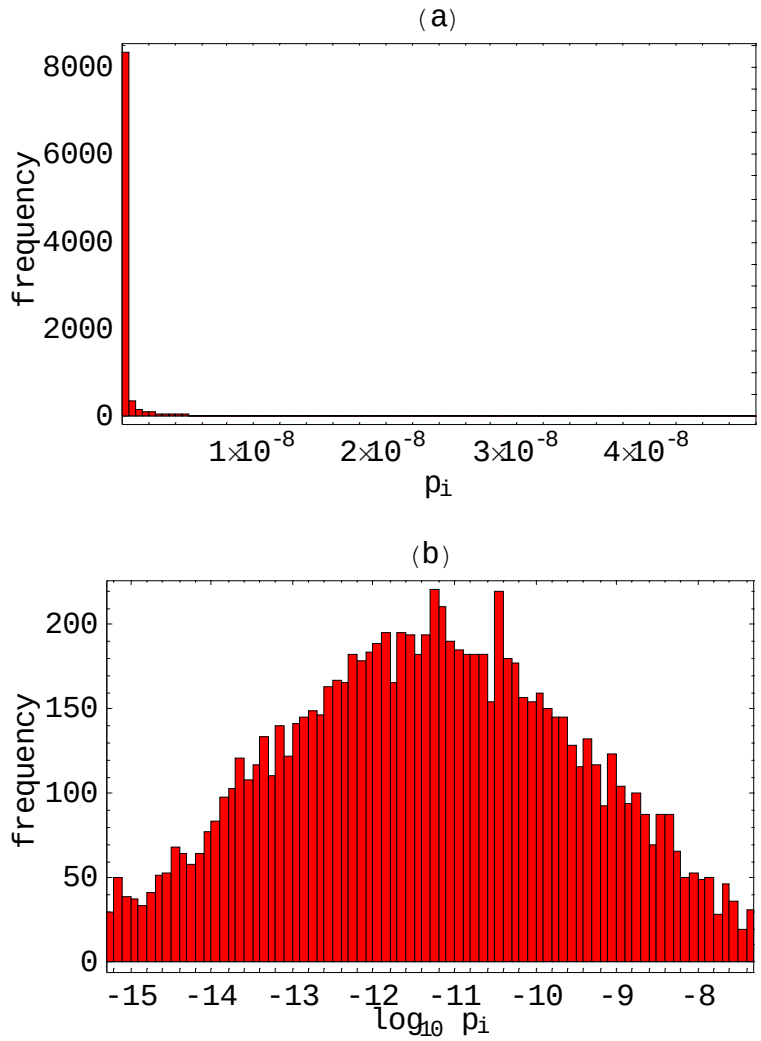

Figure 7 Infection risk p_i (a) en $\log_{10} p_i$ (b) per individual chicken for drinking water from small surface water; r=1.

Table 11 Infection risk p_i per individual chicken for drinking water; r=1

Surface water	Mean p_i	95%-interval
Small	2.3×10^{-7}	$6.7 \times 10^{-16} - 5.4 \times 10^{-8}$
Medium	9.2×10^{-9}	$0 - 2.1 \times 10^{-9}$
Large	8.9×10^{-10}	$0 - 2.1 \times 10^{-10}$

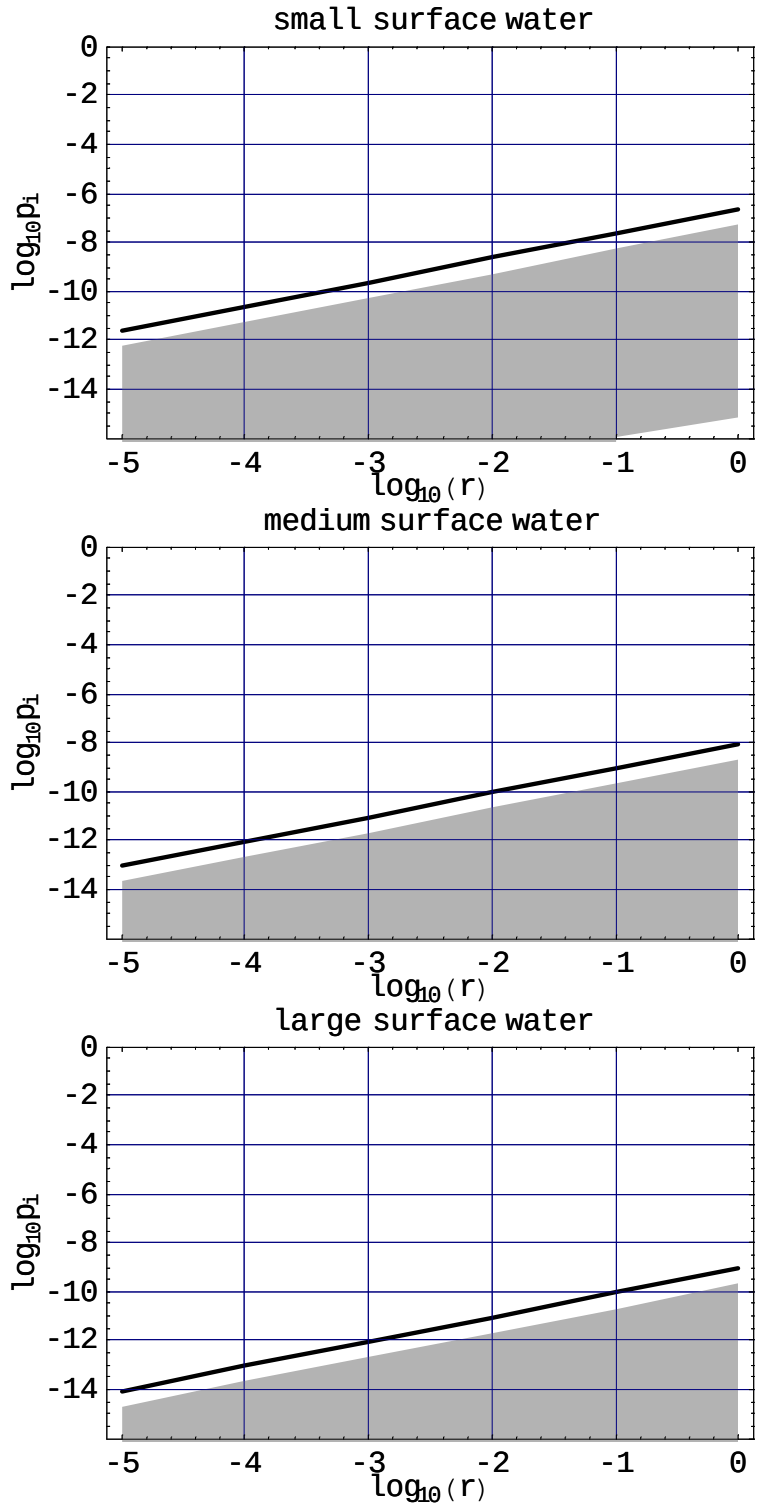

Figure 8 Risk of infection p_i of an individual chicken as a function of virus infectivity r.

3.8 Risk of infecting at least one chicken in a farm, p_F

Figure 9 shows the infection risk p_F for at least one chicken in a farm for drinking water from the small surface water, for r equal to 1. The average value and 95%-interval for all three surface waters are given in ***Table 11***. Note that also the mean p_i is similar in value to the upper limit of the 95%-interval. This risk is four orders in magnitude higher than that for an individual chicken (previous section). The risk of infecting at least one chicken in a farm is of more relevance, because all chickens in a farm will consume the same drinking water, moreover, once one or more of them are infected, the virus may spread efficiently and rapidly by means of secondary infections within the farm. The risk of infection at least one chicken in a farm equals the risk of infecting a farm.

Figure 10 shows p_F for as a function of r (10^{-5} – 1) for all three surface waters. Likewise as for p_i , a 5 $\log_{10}$-difference in infectivity r of the AIV results in a 5 $\log_{10}$-difference of p_F. Dependent on the applied range of dilution of AIV in surface water, $1/W$, and the infectivity, r, of that virus, the mean daily risk of infection, p_F, of at least one chicken in a farm that ingests contaminated drinking water ranges from $4.8 \times 10^{-10} – 9.6 \times 10^{-4}$.

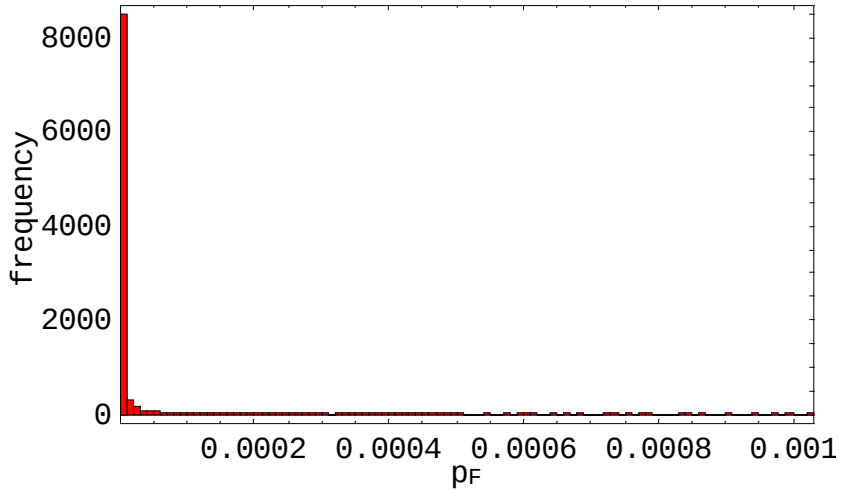

Figure 9 Infection risk p_F for at least one chicken in a farm for drinking water from small surface water; r=1.

Table 12 Infection risk p_F for at least one chicken in a farm for drinking water; r=1

Surface water	Mean p_i	95%-interval
Small	9.6×10^{-4}	$1.1 \times 10^{-12} – 1.0 \times 10^{-3}$
Medium	1.8×10^{-4}	$0 – 4.0 \times 10^{-5}$
Large	4.1×10^{-5}	$0 – 3.4 \times 10^{-6}$

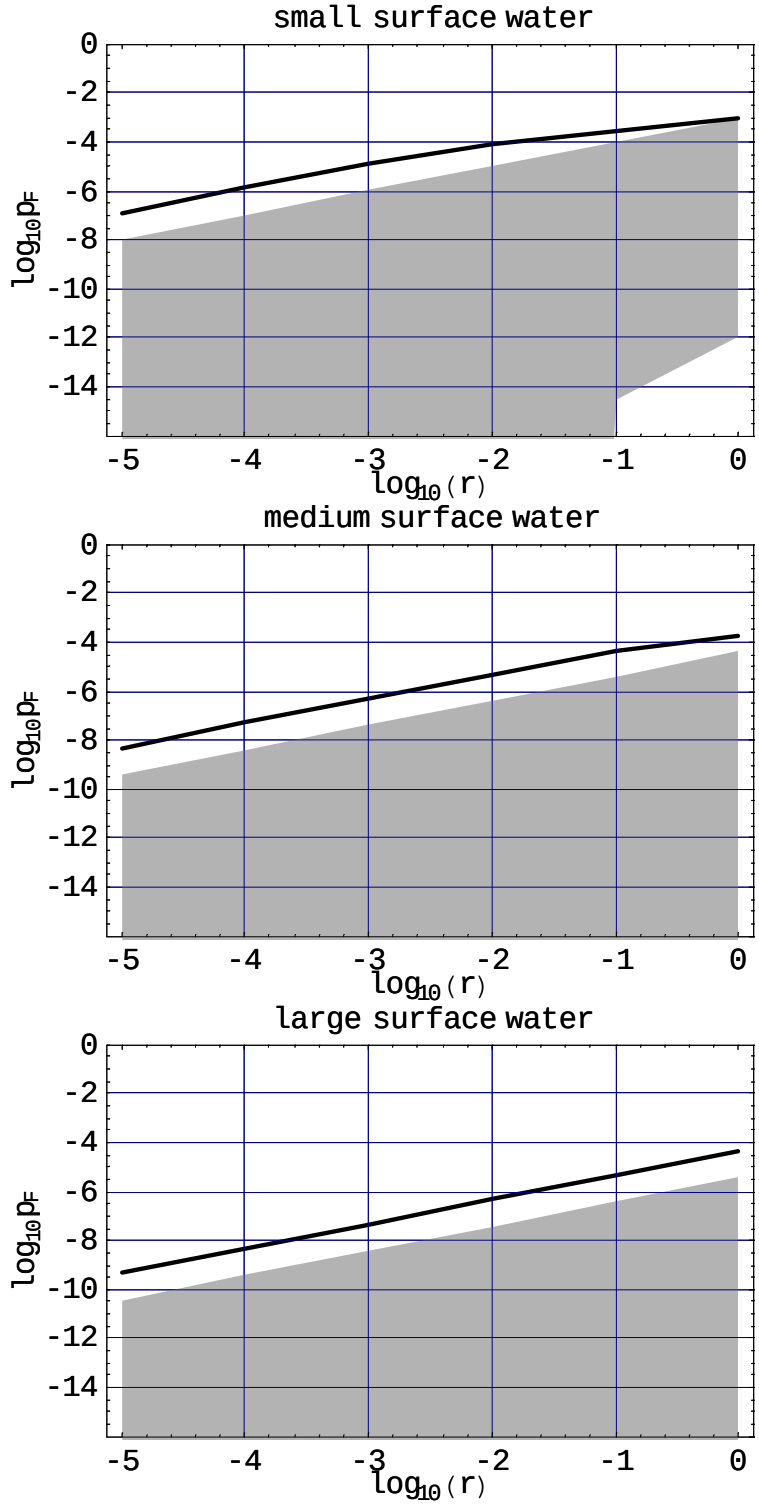

Figure 10 Risk of infection p_F of at least one chicken in a farm wih F chickens as a function of virus infectivity r.

In order to demonstrate the effect of drinking water treatment on p_F, p_F was calculated for a given drinking water treatment, R, from 0 – 12 log10 and as function of virus infectivity, r (***Figure 11***). In the case of no treatment at all, p_F may reach $1.8 \times 10^{-3} – 7.4 \times 10^{-1}$, which means a relatively high risk of infecting a farm, regardless the infectivity of the virus.
E.g. in the case of 4 $\log_{10}$ treatment, p_F may reach $2.1 \times 10^{-6} – 6.8 \times 10^{-2}$, and in the case of 8 $\log_{10}$ treatment, p_F may reach $2.1 \times 10^{-10} – 2.1 \times 10^{-5}$.
If one would want to limit this risk to a maximum of 10^{-6}, then a drinking water treatment of 3.4 – 8.4 $\log_{10}$ would be needed, largely depending on the infectivity of the virus.
Figure 12 demonstrates the effect of farm size and virus infectivity on p_F. If *r=1*, p_F ranges from $2.5 \times 10^{-3} – 2.5 \times 10^{-7}$ and if r=10-5, p_F ranges from $2.5 \times 10^{-7} – 2.5 \times 10^{-12}$.

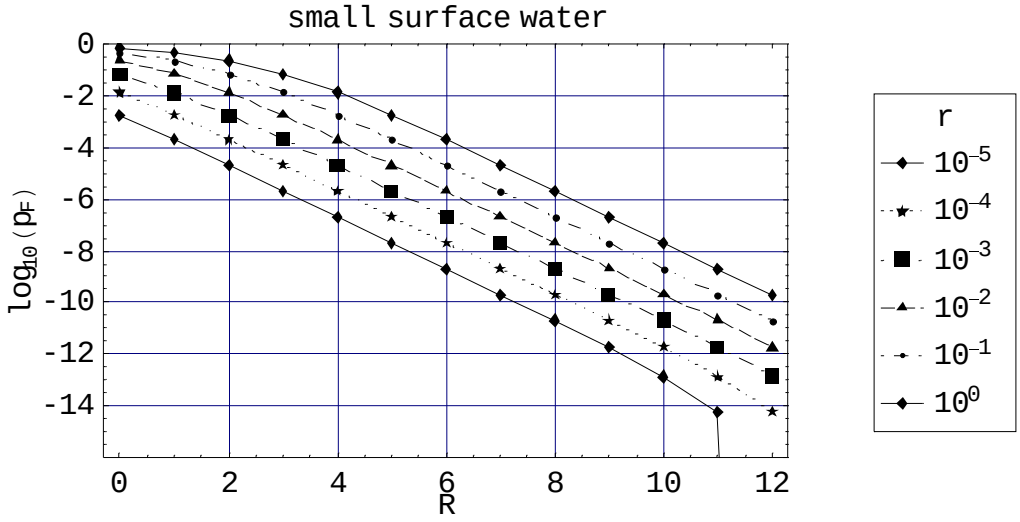

Figure 11 Mean risk of infection, p_F, of at least one chicken in a farm as function of drinking water treatment, R, and virus infectivity, r.

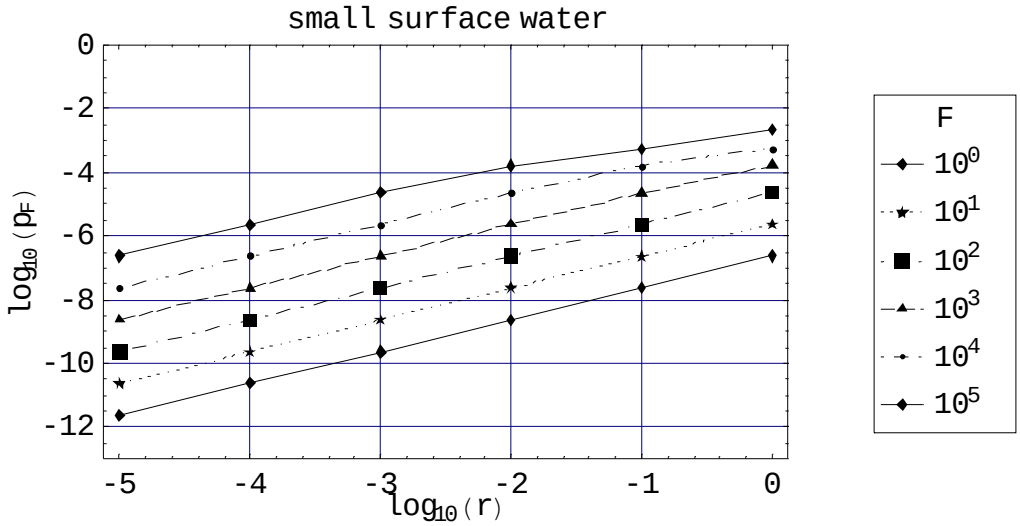

Figure 12 Mean risk of infection, p_F, of at least one chicken in a farm as function of farm size, F, and virus infectivity, r.

Figure 13 and ***Figure 14*** show the relation of p_F with drinking water treatment, R, and virus infectivity, r, for a given farm size in the range of 1 to 10^5. These calculations show what level of drinking water treatment would be required for a certain farm in order not to exceed a chosen level of infection risk, depending on virus infectivity, r. These estimates are based on the scenario, where the virus is shed in the small surface water. Obviously, in the case of a factor of ten more dilution, 1 $\log_{10}$ less virus removal by drinking water would be required.

Table 13 Required drinking water treatment, R, log_{10}, where $p_F < 10^{-6}$ per day

Farm size, F	Drinking water treatment, R, $\log_{10}$	
	$r = 10^{-5}$	$r = 1$
1	0	4
10	0.5	5
100	1	6
1000	2	7
10000	3	8
100000	4	9

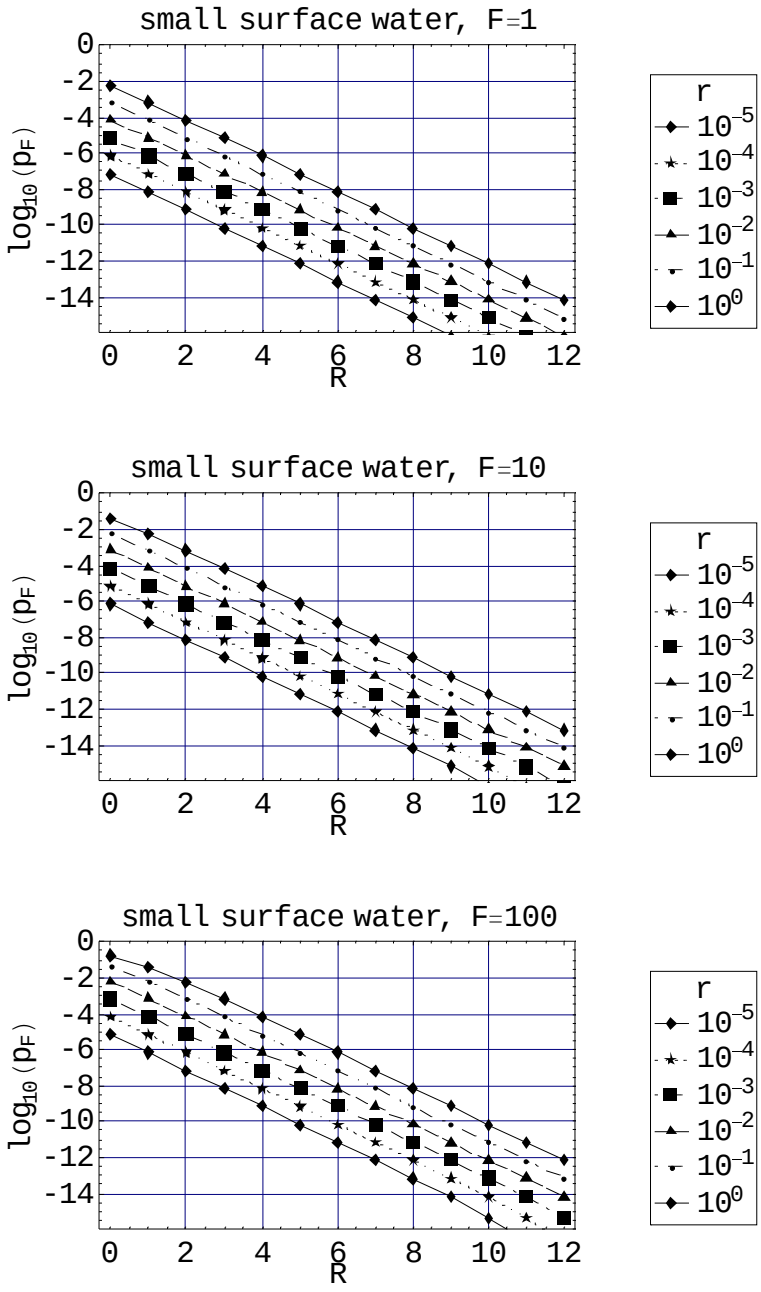

Figure 13 Mean risk of infection, p_F, of at least one chicken in a farm, F=1, 10, 100, as function of drinking water treatment, R, and virus infectivity, r.

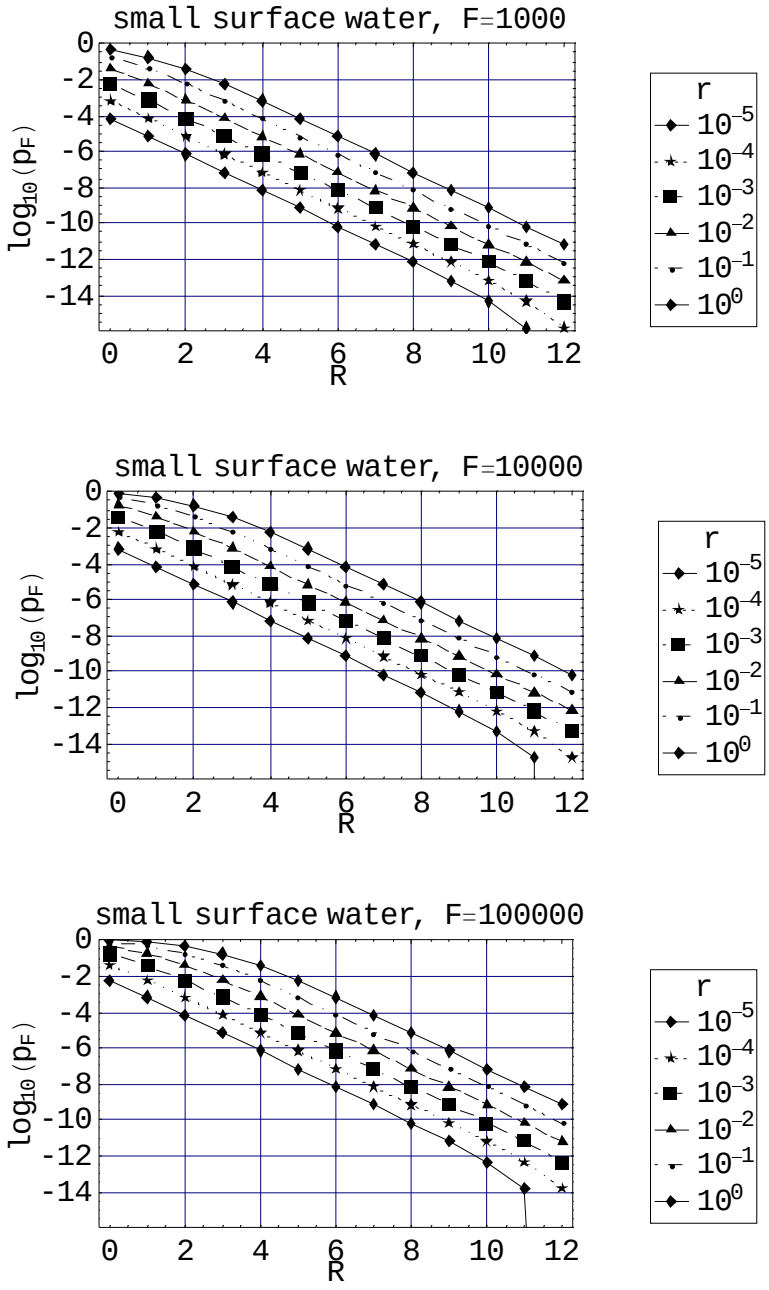

Figure 14 Mean risk of infection, p_F, of at least one chicken in a farm, F=1000, 10000, 100000, as function of drinking water treatment, R, and virus infectivity, r.

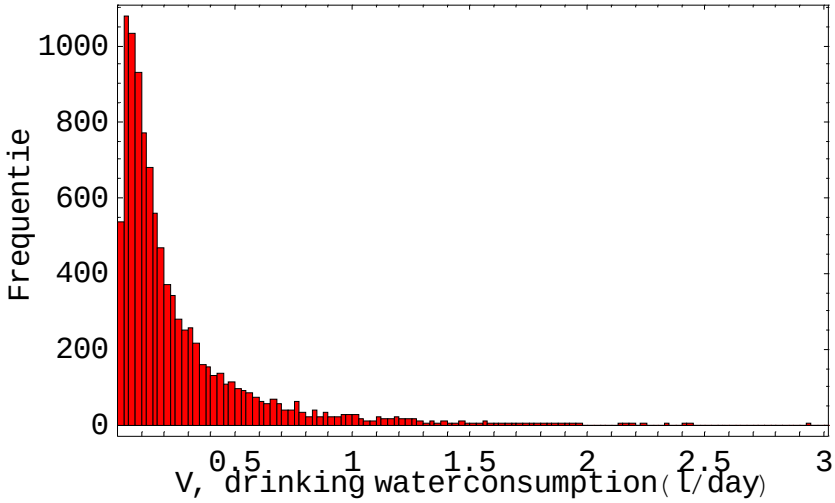

Figure 15 Human daily consumption of unboiled drinking water in the Netherlands (Teunis et al., 1997)

3.9 Human drinking water consumption, *V*

Figure 15 shows the distribution of the daily consumptions of unboiled drinking water by humans in the Netherlands, with an average of 0.27 liter and 95%-interval of 0.017 – 1.27 liter per day.

3.10 Risk of infecting one human, infectivity $r=10^{-5}$

Figure 16 shows the estimated daily risk of infection for an individual human, assuming a virus infectivity, r of 10^{-5}. In that case, the mean value is 1.8×10^{-12}, and the 95%-interval $0\text{-}3.5 \times 10^{-13}$, which is negligibly low.

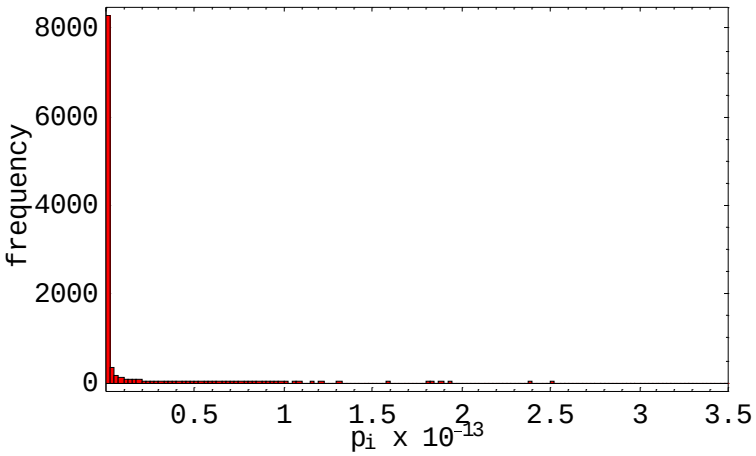

Figure 16 *Infection risk p_i per individual human for drinking water from small surface water; $r=10^{-5}$.*

4. Discussion

In the present study, risks of infection of chickens and humans were estimated due to consumption of drinking water that was assumed to be contaminated with H5N1 AIV. It was assumed that an infected duck was shedding this virus in surface water and that a fraction of this virus was able to pass drinking water treatment and reach a chicken or human. This risk assessment was conducted in order to evaluate the potential transmission of AIV to poultry and humans via drinking water. Despite the lack of data and the large uncertainties involved, this quick microbiological risk assessment allowed us to draw conclusions about the risks for chickens and humans associated with the possible exposure to H5N1 AIV via water.

It was found that the mean daily risk of infecting an individual chicken lies between $8.9 \times 10^{-10} – 2.3 \times 10^{-7}$, depending on the assumed virus infectivity from $10^{-5} – 1$. The wide range reflects the large uncertainties in the estimates of the number of viruses shed in the surface water and the infectivity of the virus. Nevertheless, these estimates suggest a low risk of infecting an individual chicken. However, the risk of infection with highly pathogenic avian influenza virus of at least one chicken in a farm is more relevant, because once one chicken in a farm has been infected, the whole farm must be considered lost, either due to deaths caused by the infection or destruction to prevent further spread of the virus. This infection risk was found to be four orders in magnitude higher than the risk of infection of an individual chicken. Especially large farms, and even more in the case of a highly infectious virus, may run a high risk. The risk of infecting at least one chicken in a farm as a function of farm size, drinking water treatment and virus infectivity was estimated in order to gain insight into what level of drinking water treatment is required in order not to exceed a certain level of infection risk. As an example these treatment values were given for a daily risk level of 10^{-6}. In that case and for the most infectious virus (r=1) at least 4 $\log_{10}$ drinking water treatment is required for individual chickens and at least 9 $\log_{10}$ for chicken farms with 100000 chickens. To put it simply, a ten times larger farm is at a ten times higher risk. If one wants a ten times lower risk level, the drinking water treatment needs to be ten times more efficient.

This risk assessment demonstrated that especially the numbers of viruses shed into the water and the infectivity of the virus determine the risk of infection. In the present study it was assumed that one duck was contaminating surface water. However, because ducks are commonly present in flocks (and with other water fowl), it is plausible that more than one duck may have been infected by direct contact with other ducks and with contaminated surface water and thus be shedding virus too. In addition, it needs to be stressed that the distribution of the virus in the surface water is probably not homogeneous, the concentration of virus in the intake water for drinking water production may therefore be highly variable. Moreover, the shedding of virus in feces may not be a constant event.
As pointed out in section 2.8, no actual dose response relationship for chickens and AIVs exists. Although in many studies, chickens and ducks were inoculated with AIVs in several ways, always high doses were applied. Especially in the case of exposure to waterborne viruses, data on exposure to low numbers of viruses are crucial. Dose-response data from infecting mice (Lu *et al.*, 1999; Nguyen *et al.*, 2005) demonstrated that infectivity between highly pathogenic AIV strains may differ widely. In the risk assessment, a dose-response model with infectivity r as a constant was applied, but probably r is very heterogeneous, as indicated by changes in pathogenicity and the observed pleomorphism of AIVs. Therefore, an important lesson that is learned from the current study is that researchers need to put effort

into quantifying numbers of viruses that are excreted by an infected organism and, if possible, determine a dose-response relationship for a wide range of doses in order to reduce the large uncertainties encountered in evaluating the importance of transmission of such agents as H5N1 AIV via water. Nevertheless, the current data for AIV allowed to conclude that highly efficient drinking water treatment is required in case high numbers of highly infective virus are shed into surface water, but the actual estimated risk is very uncertain. On the basis of this evaluation it is recommended to remain alert on the spread of AIVs by water fowl in neighboring European countries.

Assuming a low infectivity of 10^{-5} of AIV for humans, the daily risk of infection of humans was estimated to be on average 1.8×10^{-12} with an 95%-interval of $0 – 3.5 \times 10^{-13}$, which is very low. It may be reasonable to assume that AIVs are orders of magnitude less infectious to humans than to birds because of the species barrier. The risk of infection of humans would be 10^{-6} per day if exposure in combination with infectivity would be 10^{6} times higher. If this were to occur for a whole year, which is very unlikely, then an annual risk in the order of 10^{-4} per person per year would be reached. The latter risk level is the maximum level for infection by waterborne pathogens such as enteroviruses, according to the Dutch legislation for drinking water (Staatsblad, 2001).
Exposure of 10^{6} times higher would be *e.g.* achieved if concentrations in surface water were 1000 times higher, combined with a 1000 times less efficient drinking water treatment, or if drinking water treatment is not more than 2 $\log_{10}$ reduction. These are unlikely scenarios. Obviously, failures, especially total failures in drinking water treatment are unwanted at all times. Thus, one may conclude that H5N1 infection of humans in the Netherlands from efficiently treated and produced drinking water is negligible. Efficient and robust drinking water treatment may be determined by means of a risk analysis for enteroviruses that is required from the Dutch drinking water companies already by law and may be warranted by means of proper operational management using water safety plans (WHO, 2004). To protect public and animal health, it is recommended to strictly adhere to all measures already prescribed for prevention of the spread of pathogenic avian influenza viruses (Bosman *et al.*, 2004).

In this study, the source of contamination of drinking water with AIV was surface water. Contaminated surface water may reach ground water, *e.g.* at locations were river bank filtration is applied. But in those cases, drinking water treatment as applied in the Netherlands, applying multiple treatment steps is efficient. Other ways of ducks contaminating natural groundwater in the Netherlands are unlikely.

Recently, it was estimated that occupational and sport divers in the Netherlands may run a high risk of infection with waterborne pathogens due to frequent and intense contact with water throughout the whole year (Schijven and de Roda Husman, 2004, 2005). It was estimated that the volume of fresh surface water that occupational divers may swallow during a single dive is on average 4.8 – 6 ml and on a yearly basis 41 – 200 ml. Likewise, the volume of fresh surface water that sport divers may swallow is on average 11 ml per single dive and 230 ml per year. Divers may be directly exposed to contaminated surface water. In the case of an infectivity r of 10^{-5}, the associated risk of infection would be in the order of 10^{-8}. Likewise, one may surmise, that a recreational bather, that would swallow 10 ml of bathing water that is similarly contaminated with AIV would be exposed to the same extent as a diver. Similar risks may also apply to other recreational water use, such as by surfers and kayakers. In addition to exposure to AIV contaminated water by ingestion, exposure by inhalation of AIV contaminated aerosols by recreational water users may also play a role.

References

Anon. (Agric.). 1978. Broiler Raising in Canada. Publication 1509. Agriculture Canada.

Anon. (Agric.). 1982. Farm Feed Processing and Handling. Publication 1572 E. Agriculture Canada.

Anon. (N.A.S.). 1974. Nutrients and Toxic Substances in Water for Livestock and Poultry. A Report of the Subcommittee on Nutrient and Toxic Elements in Water. National Academy of Science. National Research Council Committee on Animal Nutrition, Washington, DC.

Anon. (N.T.A.C.). 1968. Water Quality Criteria. Report of the National Technical Advisory Committee to the Secretary of the Interior. Federal Water Pollution Control Federation, Washington, DC.

Anon. 1984. Guide to the Care and Use of Experimental Animals. Canadian Council on Animal Care. Ottawa. Vols. 1 and 2.

Bosman A, Mulder YM, Leeuw JRJ de, Meijer A, Du Ry van Beest Holle M, Kamst RA, Velden PG vand der, Conyn-van Spaendonck MAE, Koopmans MGM, Ruijten MWMM, Executive summary Avian flu epidemic 2003: public health consequences, 2004, Nat Inst Publ Health Environ, Bilthoven, The Netherlands, report 630940003

Brady, S. 1964. Bioenergetics and Growth. Hafner Pub. Co., Inc. N.Y.

CBS, Statline from Statistics Netherlands, 2004, www.cbs.nl.

Cole, H. H. Editor. 1962. Introduction to Livestock Production. W. H. Freeman and Co.

Galama JGM, Vogelpest en oseltamivir: een terugblik. Ned Tijdschrift Geneesk 2003, 47(23) 1100-1103.

Goyal SM, Hanssen H, Gerba CP, Simple method for the concentration of influenza virus from allantoic fluid in microporous filters, Appl Environ Microbiol, 1980, 30(3), 500-504.

Hart, B. T. 1974. A Compilation of Australian Water Quality Criteria. Australian Water Resources Council. Technical Paper Number 7. Canberra.

Hinshaw VS, Webster RG, Turner B, Water-borne transmission of influenza A viruses?, Intervirology, 1979, 11, 66-68.

Hulse-Post DJ, Sturm-Ramirez KM, Humberd J, Seiler P, Govorkova EA, Krauss S, Scholtissek C, Puthavathana P, Buranathai C, Nguyen TD, Long HT, Naipospos TSP, Chen H, Ellis TM, Guan Y, Peiris JSM, Webster RG, Role of domestic ducks in the propagation and biological evolution of highly pathogenic H5N1 influenza viruses in Asia, PNAS, 2005, 102(30), 10682-10687.

Jong MD de, Hien TT, Avian Inluenza (H5N1), J Clin Virol, 2005, in press.

Lee CW, Swaybe DE, Linares JA, Senne D, Suarez DL, H5N2 avian inlfuneza outbreak im Texas 2004: the first highly pathogenic strani in the United States in 20 years? J Virol, 2005, 79(17) 11412-11421

Li KS, Guan Y, Wang J, Smith GJD, Xu KM, Duan L, Rahardje AP, Pathavathana P, Buranthanai C, Nguyen TD, Estoepangestie ATS, Chalsingh A, Auewarakui P, Long HT, Hanh NTH, Webby RJ, Poon LLM, Chen H, Shrotridge KF, Yuen KY, Webster RG, Peirs JSM, Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia, Letter to Nature, 2004, 430, 209-213.

Lodder WJ, Roda Husman AM de, Presence of noroviruses and other enteric viruses in sewage and surface waters in the Netherlands, Appl Environ Microbiol, 005, 71(3) 153-1461.

Lu H, Castro *ET AL.*, Pennick K, Liu J, Yang Q, Dunn P, Weinstock D, Henzler D, Survival of Avian Influenza Virus H7N2 in SPF chickens and their environments, Avian Diseases, 2003, 47, 1015-1021.

Lu X, Tumpey TM, Morken T, Zaki SR, Cox NJ, Katz JM, A mouse model for the evaluation of pathogenesis and inmmunity to influenza A (H5N1) viruses isolated from humans. J Vrol, 1999, 73, 5903-5911.

Nguyen DC, Uyeki TM, Jadhoa S, Maines T, Shaw M, Matsuoka Y, Smith C, Rowe T, Lu X, Hall H, Xu X, Balish A, Klimov A, Tumpey TM, Swayne DE, Huynh LPT, Nghiem HK, Nguyen HHT, Hoang LT, Cox NJ, Katz JM, Isolation and characterization of avian influenza viruses, including highly pathogenic H5N1 from poultry in live bird markets in Hanoi, Vietnam, in 2001, J Virol, 2005, 79(7) 4201-4212.

Richey, C. B., P. Jacobson and C. W. Hall. 1961. Agricultural Engineers Handbook. McGraw-Hill Book Co., Inc. NY.

Roda Husman AM de, Medema GJ, Inspectorate guideline - Assessment of the microbial safety of drinking water, Netherlands Ministry of Housing, Spatial Planning and the Environment, 2005.

Schijven JF, Rijs G, de Roda Husman AM, Verstappen G. Estimation of the risk of infection of dairy cows by food and mouth disease virus spread by way of surface water, Risk Analysis, 2005; 25(1) 13-21.

Schijven JF, Roda Husman AM de. Schatting van de blootstelling van beroepsduikers aan micro-organismen in water RIVM, 2004, rapport 330000006 (in Dutch).

Schijven JF, Roda Husman AM de. Schatting van de blootstelling van sportduikers aan micro-organismen in water RIVM, 2005, rapport 330000009 (in Dutch).

Schijven JF, Schatting van de kans op infectie door Campylobacter via water, H2O, 2003, 19: 27-30.

Staatsblad, Waterleidingbesluit 2001, Staatsblad nr 31(in Dutch).

Stallknecht DE, Shane SM, Kearney MT, Zwank PJ, Persistence of avian influenza viruses in water, Avian Diseases, 1990, 36, 406-411.

Sturm-Ramirez KM, Ellis T, Bousfield B, Bisset L, Dyrting K, Rehg JE, Poon L, Guan Y, Peiris M, Webster RG, Reemerging H5N1 influenza viruses in Hong Kong 2002 are highly pathogenic to ducks, J Virol, 2004, 78(9), 4892-4901.

Sturm-Ramirez KM, Hulse-Post DJ, Govorkova *ET AL.*, Humberd J, Sieler P, Puthavathana P, Buranthanai C, Nguyen TD, Chaisingh A, Long HT, Naipospos TSP, Chen H, Ellis TM, GUanY, Peiris JSM, Webster RG, Are ducks contributing to the endemicity of high;y pathogenic H5N1 influenza virus in Asia? J Virol, 2005, 79(17), 11269-11279.

Teunis PFM, Medema GJ, Kruidenier L and Havelaar AH (1997) Assessment of the risk of infection by *Cryptosporidium* or *Giardia* in drinking water from a surface water source. Water Research 31(6);1333-1346

Teunis, PFM, Havelaar AH, The Beta Poisson dose-response model is not a single-hit model. Risk Analysis, 2000, 20, 513-20.

Tiensin T, Chaitaweesub P, Songserm T, Chaisingh A, Hoonsuwan W, Buranathai C, Parakamawongsa T, Premashtira S, Amonsin A, Gilbert M, Nielen M, Stegeman A, Highly pathogenic avina influenza H5N1, Thailand, 2004, Emerging Infectious Diseases, 2005, 11(11), 1664-1672.

Webster RG, Yakhno M, Hinshaw VS, Bean JW Jr, Murti KG. Intestinal influenza: replication and characterization of influenza viruses in ducks. Virology 1978, 84: 268-278.

WHO Guidelines for Drinking Water Quality. Third edition. WHO, Geneva, 2004. http://www.who.int/water_sanitation_health/dwq/gdwq3/en/index.html