

H3N1 in poultry: symptoms and protection

Introduction: H3N1 in Belgium

In early 2019, Belgium was confronted with an avian influenza outbreak of the H3N1 type in a layer flock. A single farm was affected to start with. At the time the clinical problems were limited. In the course of the second quarter of 2019, the virus was once again detected at the laying farm in question, but with more severe clinical symptoms and a higher mortality rate. In the official IVPI test, which is used to determine whether an AI strain is high-pathogenic or low-pathogenic, the virus was identified to be low-pathogenic. Despite a low IVPI score, in many field cases the clinical impact was high. The large majority of infections in Belgium occurred in adult layers and broiler breeders.

The flocks suffered production losses up to 100 percent and the mortality rate could be as high as 60 percent; more than eighty cases were reported in the end. Limited clinical symptoms were reported in young animals reflecting the low IVPI score. The question arose whether additional infections, such as infectious bronchitis or *E. coli*, could account for the differences in clinical symptoms between the laboratory findings and the field situation.



Detecting infected flocks: serology and PCR testing

Because of the contacts between the Belgian and Dutch poultry sectors, the H3N1 outbreak was considered a risk for the Dutch poultry sector. Diagnostic tests for H3 were developed for the screening of imported poultry. A PCR

test is the most effective method to detect the presence of an H3N1 virus. GD developed an avian influenza H3-PCR immediately upon becoming aware of the outbreak in Belgium. The H3-PCR was tested using samples received

from Belgium, and proved to be highly sensitive and specific. An H3-HI test was also developed for the detection of antibodies in blood. The antigen in this test is based on the H3N1 strain from Belgium.



H3N1 study in young chickens

At the request of the poultry sector, GD has conducted a study into the course of the infection in young animals for the AI strain in question. In the event of an extremely infectious AI strain, expectations were that infection with a minimal volume of virus would result in the virus developing in the animal, with or without clinical symptoms. Moreover, the virus would be shed in high levels, thereby infecting other animals in the flock. In the study, 15 animals were infected

using the H3N1 virus. The virus was administered using the eye drop method, so the virus could spread naturally in the animal. The clinical symptoms proved to be extremely limited in these animals. A single bird will appear somewhat lethargic and feverish, refusing to eat and drink. Therefore, an infection in young chickens can be easily overlooked in the field. Although all animals were infected with the same volume of virus, it turned out that the virus only developed in

four of the fifteen animals, resulting in a positive PCR 7 days after infection, and a positive HI titre 21 days after infection (titres between 5 and 7). Therewith it was discovered that young animals require a high dose of virus to get infected. This might also mean that transmission within a flock of young birds is limited and part of the flock may not be infected.

Pathology in adult animals (layers)

An infectious dose comparable to that administered to the young animals, was administered to a group of 36 SPF laying hens aged 35 weeks. Sick animals were seen from 7 days following infection, and the number of sick animals rapidly increased. The calculated mortality due to the H3N1 infection was almost 60 percent. A striking pathological result in these animals was peritonitis with excessive beige/grey exudate. There was also damage to the oviduct: here too, a large volume of exudate was found. There were no abnormalities in the respiratory system or intestines. From day 4 following infection until the end of the study at 21 days following

infection, the virus remained present and could be transmitted by the animals. Surviving animals were all out of production but had limited or no other symptoms. On post mortem 21 days after infection residual peritonitis was seen in the form of dried fibrin and inflammatory tissue in the abdominal cavity. Furthermore, an increased volume of fluid and flakes (protein and inflammatory tissue) were seen in the oviduct. Based on the findings after experimental infection we are concerned that moulting does not result in full recovery of production. At 21 days after infection all surviving animals had HI titres between 7 and 9.

To summarise

The results of these studies show that this low-pathogenic H3N1 strain requires no other pathogens to cause serious damage in layer flocks. The clinical condition, the necropsy results and the reduced production in the layers match the conditions apparent in the field. In contrast, clinical signs and mortality may be absent or mild in young animals. The spread of the virus within a young flock was not assessed but seems limited. The protection against re-infection is also unclear.



Animal health barometer for poultry 2nd quarter 2019

VETERINARY DISEASES		1 st QUARTER 2019	2 nd QUARTER 2019	TREND (OVER 2 YEARS)
Article 15 GWWD (Health & Welfare Act) diseases (diseases named in articles 3 and 7 of the 'Rules for prevention, control and monitoring of infectious animal diseases and zoonoses and TSEs')				
Avian influenza in the Netherlands (H5/H7) <small>(Source: GD, WBVR, national government)</small>	HPAI (H5/H7):	Not detected	Not detected	↓
	LPAI (H5/H7):	Not detected	Not detected	↓
	Serology: (Antibodies for H5/H7)	2 flocks	1 flock	↓
Avian influenza in Europe (H5/H7) <small>(Source: OIE)</small>	HPAI (H5/H7):	Bulgaria: H5N8 Russia: H5	Bulgaria: H5N8	↓
	LPAI (H5/H7):	Denmark: H5 and H7N7	Denmark: H5	↓
ND in the Netherlands <small>(Source: GD, OIE)</small>	Commercial poultry	Not detected	Not detected	-
ND in Europe <small>(Source: GD, OIE)</small>	Commercial poultry	Not detected	Not detected	↓
<i>M. gallisepticum</i> ^A <small>(Source: GD)</small>	Serological monitoring by GD:			
	Reproduction sector:	0 farms	0 farms	-
	Rearing layers:	0 farms	0 farms	-
	Layers:			
	- not vaccinated and infected:	0 farms	1 farm	↓
	- vaccinated and infected:	4 farms	5 farms	↑
	Turkeys:	0 farms	0 farms	-
	Reports in EWS^c based on positive serology and/or voluntary PCR testing:			
	Layers:	5 farms	5 farms	-
	<i>M. synoviae</i> ^B <small>(Source: GD)</small>	Serological monitoring and/or dPCR by GD: % of positive farms versus farms tested		
Grandparent stock (incl. pullets) (meat):		0%	0%	-
Broiler breeder pullets:		2%	2%	↓
Broiler breeders:		14%	17%	↓
Layer grandparents (incl. pullets for parents and grandparents):		0%	0%	-
Layer breeders:		11%	9%	↑
Layer pullets:		13%	12%	-
Layers:		77%	72%	-
Turkeys:		18%	11%	↑
Salmonellosis (non-zoonotic salmonella): <small>(Source: GD)</small>				
<i>Salmonella arizonae</i>	N/A	N/A	N/A	N/A
<i>Salmonella Gallinarum</i> (SG)	Not detected	Not detected	-	-
<i>Salmonella Pullorum</i> (SP)	1 case detected in backyard chickens	-	↑	↑
Article 100 diseases in poultry (compulsory notification)				
Campylobacteriosis	No data available	-	-	N/A
Salmonellosis (zoonotic salmonella) (at the flock level) <small>(Source: NVWA)</small>				
S. Enteritidis	Reproduction:	1 flock	1 flock	-
	Layer pullets:	0 flocks	0 flocks	-
	Layers:	10 flocks	5 flocks	↓
S. Typhimurium	Reproduction:	0 flocks	1 flock	↑
	Layer pullets:	0 flocks	0 flocks	-
	Layers:	0 flocks	0 flocks	-

Table continuation

VETERINARY DISEASES		1 st QUARTER 2019	2 nd QUARTER 2019	TREND (OVER 2 YEARS)
Other OIE-list poultry diseases in the Netherlands subject to compulsory notification				
Avian chlamydia (Source: GD)		Not detected by GD	Not detected by GD	-
Gumboro (IBD) (Source: GD; EWS)	Reported in EWS^C: Broilers:	11 farms	6 farms	↑
Infectious bronchitis (IB) (Source: GD)	Types most commonly detected by GD: Broilers:	D388	D388	↑
	Layers:	4-91/D181/D388	4-91/D181/D388	-/↑/↑
Infectious laryngotracheitis (ILT) (Source: GD; EWS)	Reported in EWS^C: Parent stock (layer):	-	1 farm	-
	Layers:	-	2 farms	↓
	Broilers:	1 farm	1 farm	-
Turkey Rhinotracheitis (TRT) (Source: GD)	Detected by GD: Parent stock (layer):	1 farm	-	-
	Broilers:	2 farms	3 farms	-
Other poultry diseases				
Coryza (<i>Avibacterium paragallinarum</i>) (Source: GD; EWS)	Reported in EWS^C: Layers:	5 farms	7 farms	↑
	Backyard poultry:	2 cases	2 cases	↑
Erysipelas (<i>Erysipelothrix rhusiopathiae</i>) (Source: GD)	Detected by GD: (new infections): Layers:	2 farms	1 farm	↑
<i>Pasteurella multocida</i> (Source: GD)	Detected upon necropsy: Layers:	-	1 farm	-
	No reports to the NVWA			
Histomonosis (Source: GD)	Detected by GD: Reproduction (meat sector):	5 farms	4 farms	-
	Layers:	1 farm	1 farm	-
	Turkeys:	1 farm	-	↓

A Based on serological monitoring

B Based on serological monitoring and/or the DIVA M.s.-PCR

C Early Warning System

- ↑ Increase or strong increase
- ↑ Limited increase
- Situation unchanged
- ↓ Limited decrease
- ↓ Decrease or strong decrease



Animal health monitoring

Since 2002, GD has been responsible for animal health monitoring in the Netherlands, in close collaboration with the veterinary sectors, the business community, the Ministry of Agriculture, Nature and Food Quality, vets and farmers. The information used for the surveillance programme is gathered in various ways, whereby the initiative comes in part from vets and farmers, and partly from GD. This information is fully interpreted to achieve the objectives of the surveillance programme – rapid identification of health problems on the one hand and monitoring trends and developments on the other. Together, we team up for animal health, in the interests of animals, their owners and society at large.