



AI – Experiences in Belgium with H3 outbreak and autogenous vaccination

How to deal with AI in the future?

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BVPA

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France, The Netherlands (Poulvet
Group): field diagnostics (Nele
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Depoortere, Stefaan Stock)



Diagnostic laboratory and
Contract Research Organisation,
BSL2 isolator facility and
autogenous vaccine producer,
based in Belgium

- Multi age layer farm with free range, 100k birds on site in 3 houses
- Last (routine) screening for AI December 2018, negative
- Increased mortality from January 2019 onwards, 20 to 50 birds per day on site.
- LPAI (H3N1) confirmed in 1 House, with bacterial (Pasteurella, G. anatis, E. coli) and viral (IB) complications – mortality in line with this findings
- No specific clinical picture at necropsy, septicaemia, cachectic birds, ...
- No culling of the farm possible (authorities) and not allowed to keep birds inside according

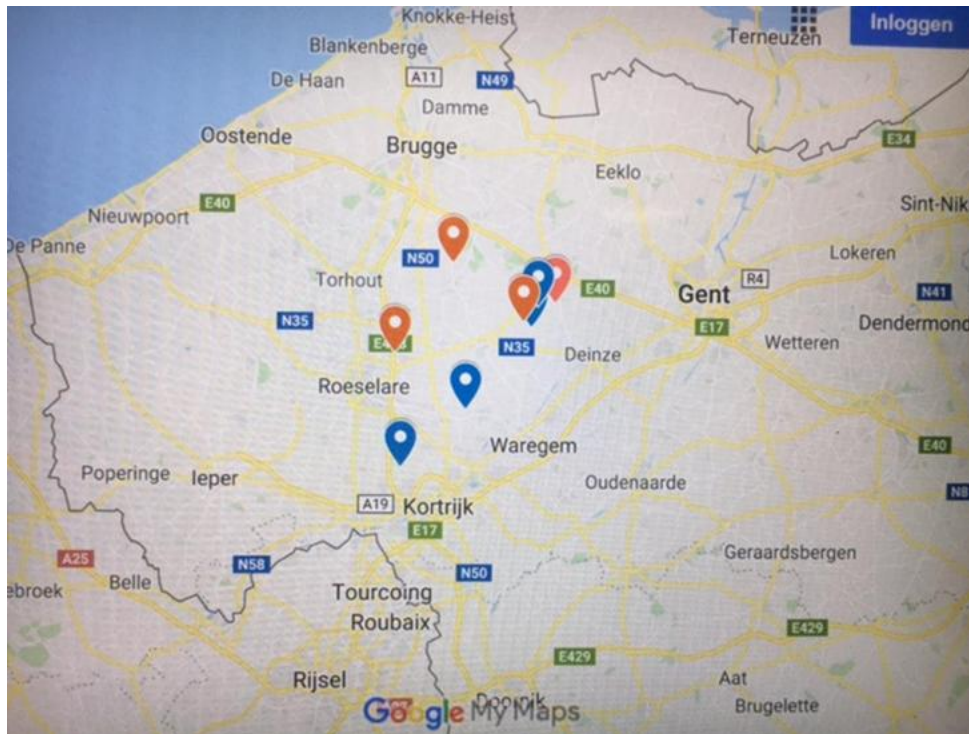
- Early April 2019: in 3 days time, the clinical picture changed in house 2: mortality from 50 to 800+
- Confirmation of LPAI introduced in this second house by qPCR but with much lower ct value (less than 30)
 - Adaptation to chicken as host after 3 months circulating in the three flocks on site: ct values dropped dramatically, very high viral shedding
 - Change in virulence?
 - Combination of both?
 - New co-factor introduced?
 - The clinical picture was very different, all birds having the same picture
- Later research indicated a deletion within the genome of the virus compared to the January Virus
- Clearly involvement of more organs than just respiratory and intestinal tract
 - Muscle tissue, brain and remarkably female reproductive tract (but also roosters died)



- Few days later, in radius of few kilometer, but all western from farm 1, 3 new cases
- All cases with a very typical peritonitis and oophoritis in most cases, with egg yolk splashed in the peritoneum and a purulent aspect combined with excessive cavity fluids present
 - All these farms confirmed with LPAI
 - Exactly same picture as at farm 1!
 - But clinical picture did not match,
 - (Bacterial) co-factor involved?
 - Intoxication?
- Cases not connected except location, vet practice and dead bird collection
 - Different layer (2), breeder (2) organisations
 - Different veterinarians from same practice
 - Farms very close to each other, with other (broiler) farms in between

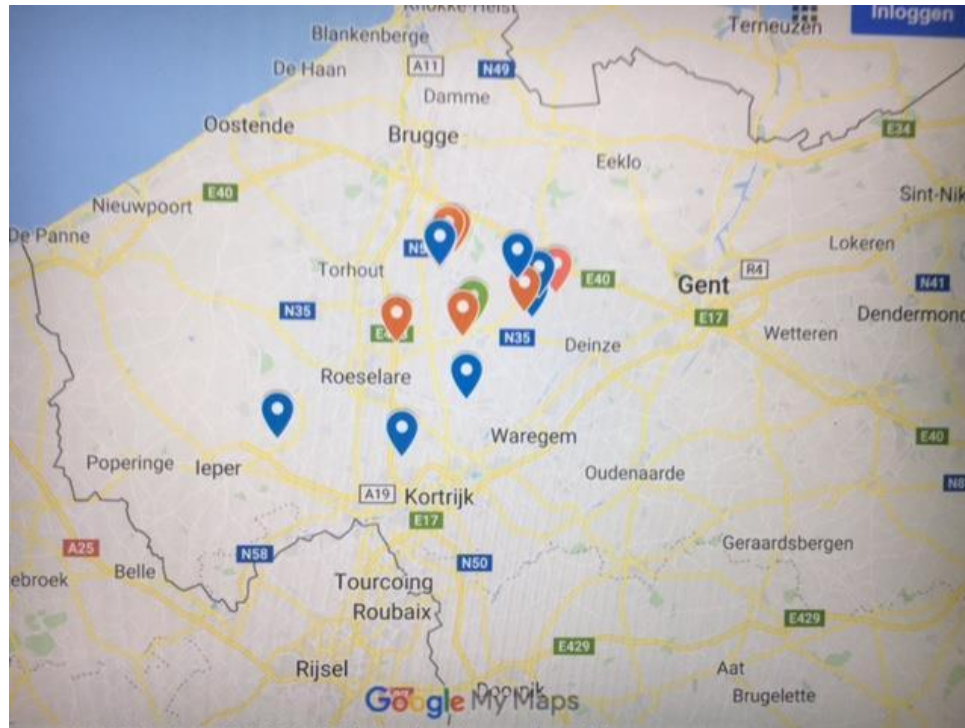
Further spreading

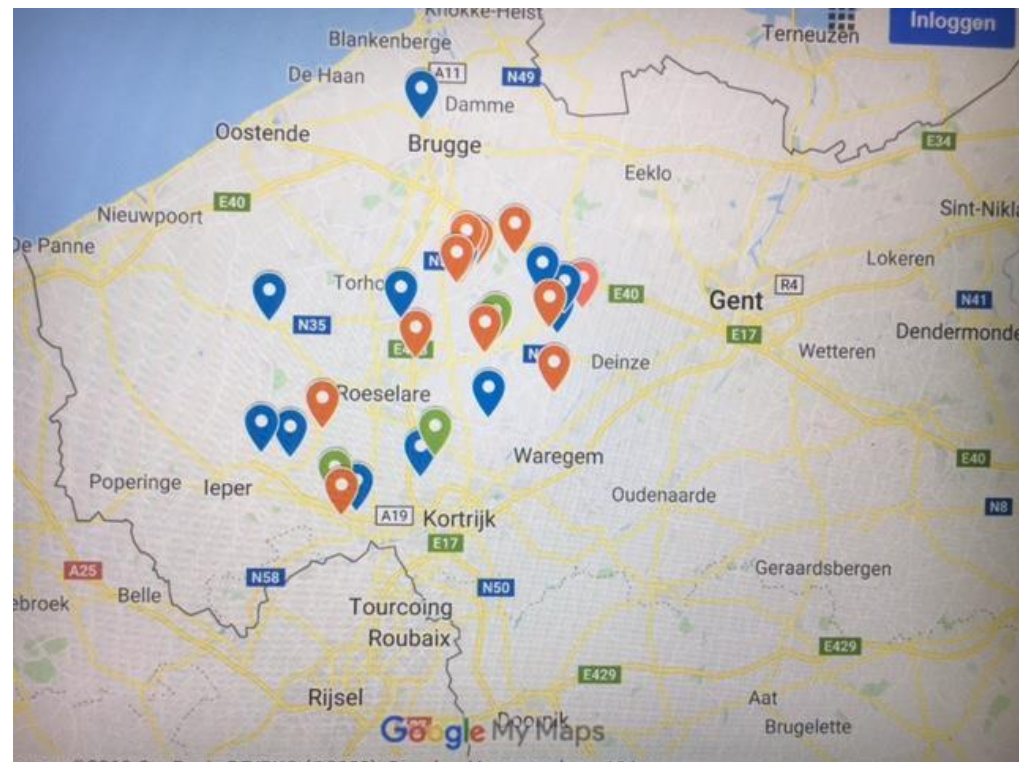
- Few days later outbreaks in more farms
 - Only broiler breeders, layers!

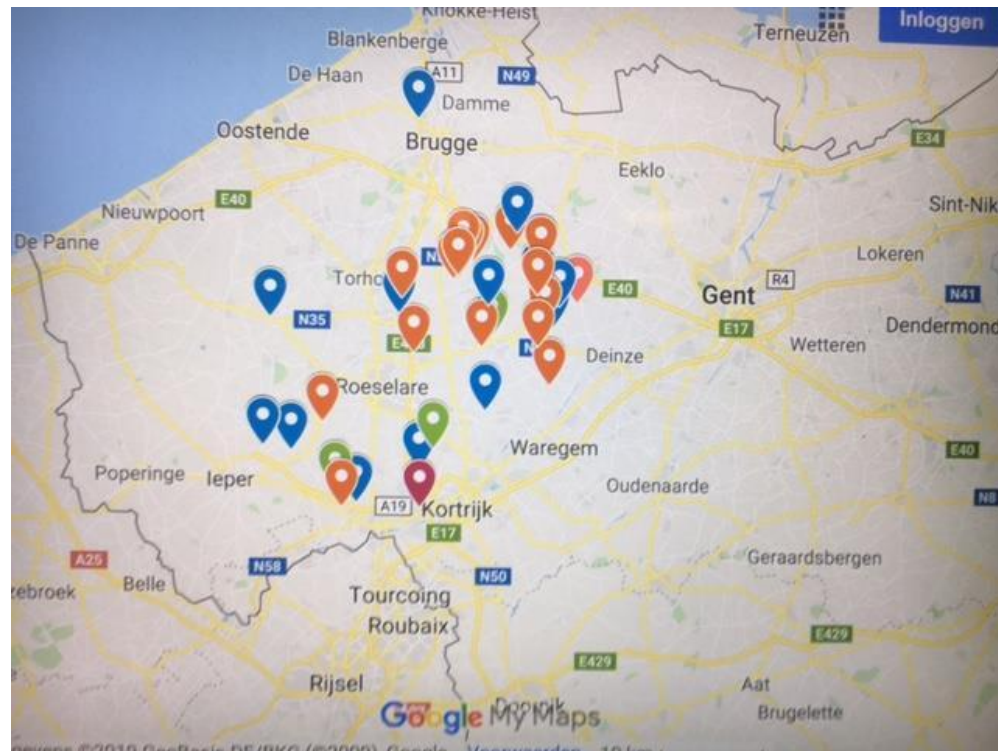


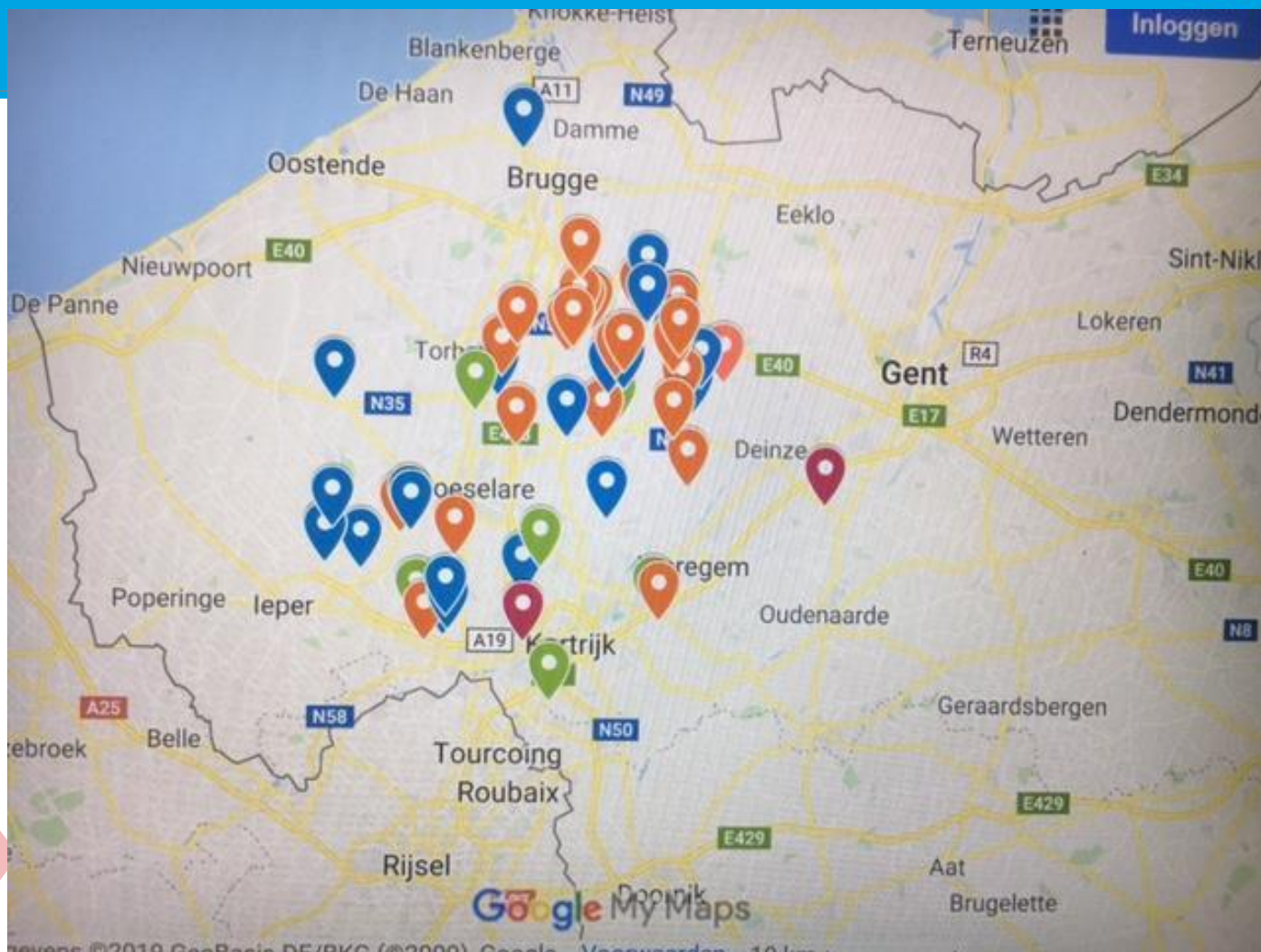
	nr farms
22/04	6
2/05	14
28/06	82

Total cost today estimated 25M €

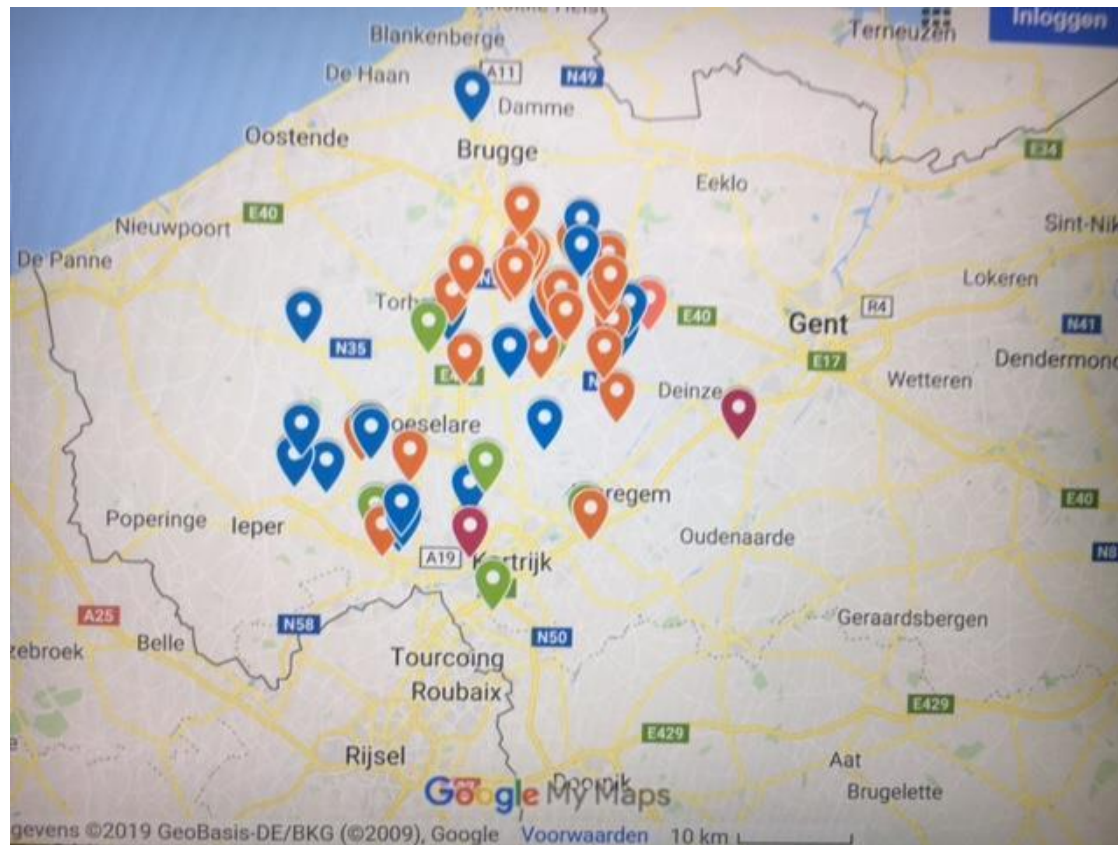






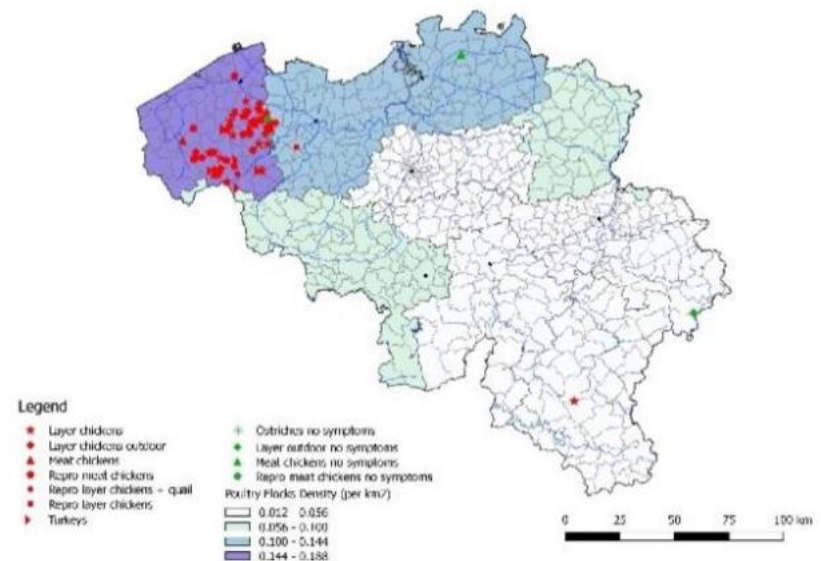


After 5 weeks first farm East of Farm 1



Situation to date (Nov 2019)

- 82 farms infected, most with high mortality (over 60%), drop in egg production (to 0%), egg defects
- Most farms are located in West-Flanders, in very dense poultry production area, and often close to highway and major transport routes. Farm 1 was the most eastern located farm for many weeks during outbreak, only 2 farms further east during outbreak. No spreading to other dense areas.

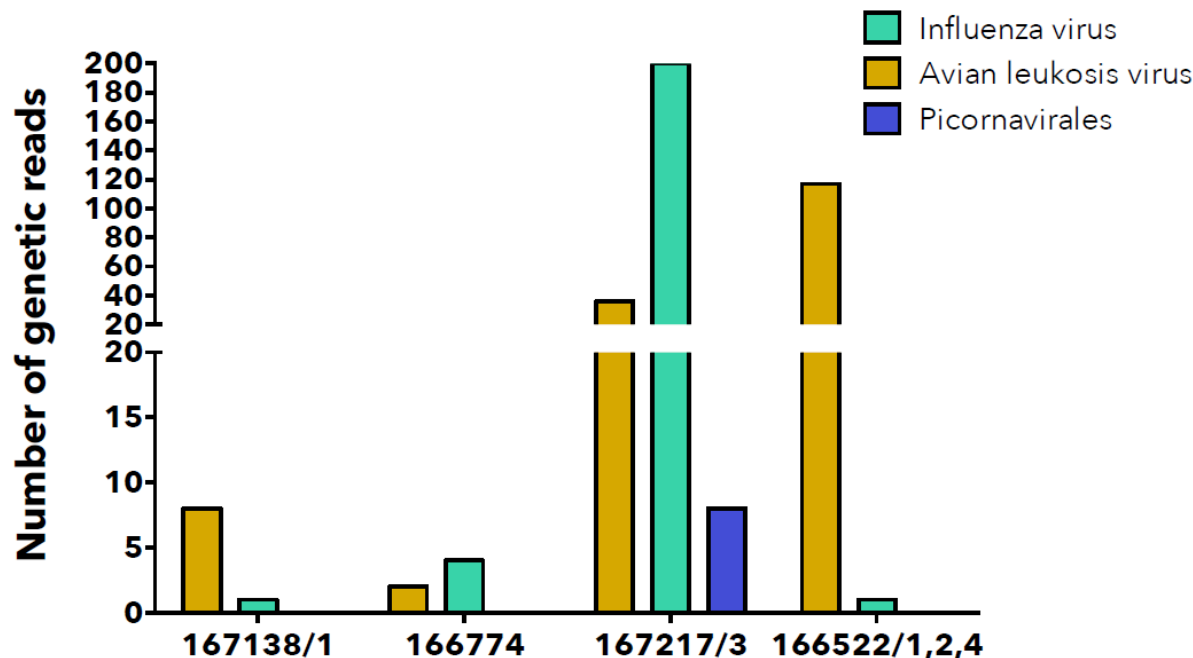


Situation to date

- Mainly layers and breeders in production are affected
- Some of the farms affected highest level of biosecurity
- Discussion around importance biosecurity / aerogenic spreading: reference lab stated (and states) repeatedly no possibility of transmission through air, so veterinary inspectors point to lack of biosecurity as reason for spreading, losing valuable time

Investigation of Co-factors

- All information on vaccination schedules, age, hatchery, feed was gathered
 - No clear links could be found
- Co-infections
 - Multiple samples were tested for the presence of pathogens like IB, ILT, ORT, MG, MS, TRT, Coryza
 - No link could be defined
- 4 samples were selected for NGS sequencing (MinION)







After 21 days recovery of flock (no mortality and ct >42)
egg peritonitis > 80% of animals
prognosis very bad for flock

- Strains isolated from affected farms
- First doses were ready within 3 weeks after decision was made to start production at Poulpharm
- Advantages
 - Autogenous gives homologous protection, very efficacious (see further)
 - Vaccination reduces risk for infection to get started in flocks that come in contact with virus (ref. http://europa.eu/rapid/press-release_MEMO-06-92_en.pdf)
 - No matter if infection is through poor biosecurity or aerogenic
 - Vaccination reduces pathology on farms that might still get infected. Ethically big advantage, but also financially (less culling needed)
 - Vaccination reduces the viral production
 - Spreading within flock and between flocks is slowed down, giving monitoring programs and biosecurity measures time to help controlling the infectionthisch :

■ Disadvantages

- Cost
- No guarantee on efficacy
- No time to work out ideal doses and vaccination schemes
- Safety needs to be tested
- Legal status not clear everywhere
 - But for non H5 and non H7 no international restrictions
 - Should this be reconsidered, also for H5/ H7?
 - Definition of autogenous is not clear, epidemiological link should be enough

- Rationale experiment
 - Consensus on potential impact of vaccination on reduction of symptoms
 - Much less consensus on impact on viral production and shedding
- Barely literature to be found on efficacy vaccination especially if not H5 H7
- Many opinions but limited facts known
- Government rather negatively opinion on vaccination, questions on efficacy (false safe feeling) and impact monitoring (but qPCR should do the job)
- Veterinarians need to be able to advice farmers if cost is justified

- 18 week old layers
- 2 x vaccinated, with 2 week interval with H3N1 field isolate, unvaccinated control group
- Intranasal challenge with homologous field strain 28 days post first vaccination
- Research questions
 - Is vaccine safe (no symptoms, or viral infection)
 - qPCR presence of viral RNA and virus isolation at different timepoints of low, medium and high vaccine dose
 - Can vaccination contribute to epidemiological control of H3N1?

Trial performed at Poulpharm

- Poulpharm was founded in 2006, 45 FTE, based in Izegem, Belgium
- Independent research partner for veterinarians, pharmaceutical and nutritional industry
- Laboratory facilities
 - Virology, parasitology, bacteriology
- Animal trial facilities at 3 sites



AI trial: 32 isolators BSL2

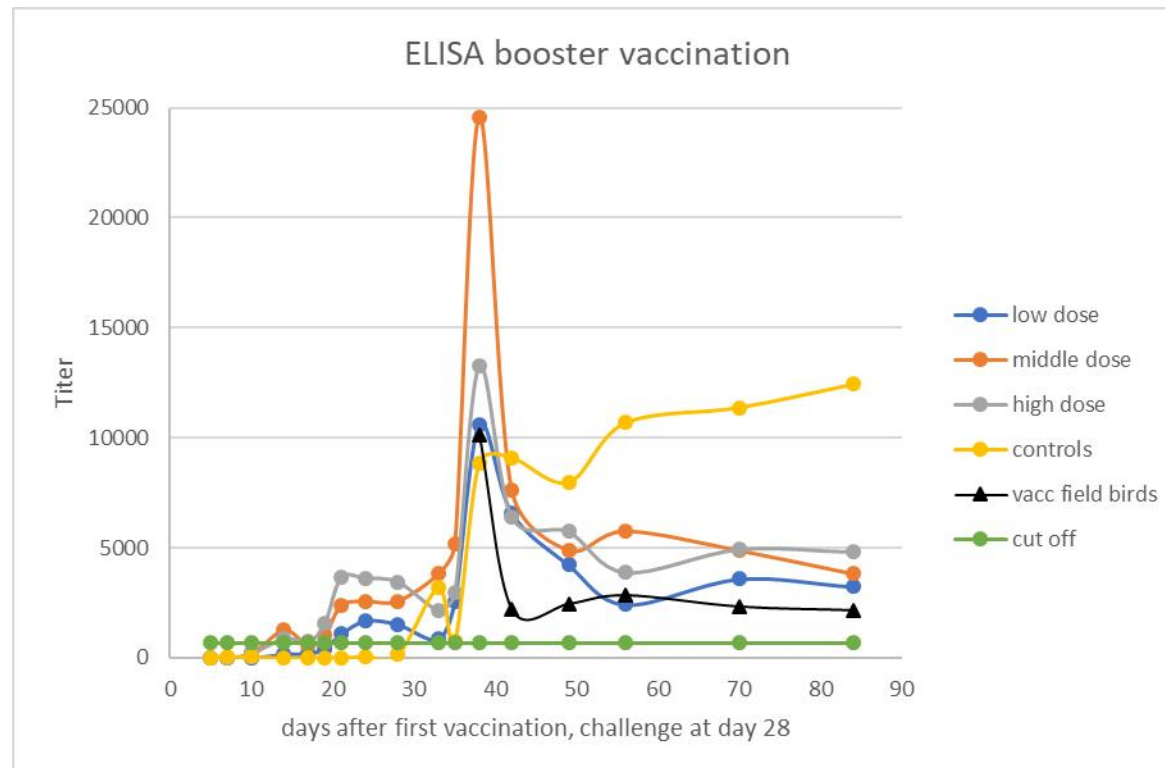


Results lowest dose group versus controls

lowest ct												
	2 dpc		4 dpc		7 dpc		10 dpc		14 dpc		18 dpc	
qPCR (tracheal)	ct	pos/total	ct	pos/total	ct	pos/total	ct	pos/total	ct	pos/total	ct	pos/total
H3 vaccinated (primer + booster, 2 weeks apart)	>42	0/8	>42	0/8	>42	0/8	>42	0/8	>42	0/8	>42	0/8
Control	36,09	1/6	36,02	2/6	36,45	4/5*	35	2/5	>42	0/5	>42	0/5
	2 dpc		4 dpc		7 dpc		10 dpc		14 dpc		18 dpc	
qPCR (cloacal)	ct	pos/total	ct	pos/total	ct	pos/total	ct	pos/total	ct	pos/total	ct	pos/total
H3 vaccinated (primer + booster, 2 weeks apart)	40,28	1/8	>42	0/8	>42	0/8	38,97	1/8	>42	0/8	>42	0/8
Control	28,24	2/6	30,5	3/6	25,05	5/5*	32,41	4/5	36,41	2/5	34,5	2/5
dpc = days post challenge												
*1 bird culled due to picking												

- Infectie was effective, all control animals positive 7 dpi with lowest ct value of 25.05
- Challenge was close to what was seen in field flocks early in infection before symptoms
- Very strong to complete reduction of qPCR presence of H3N1
 - Cloacally 2 x a very weak positive (2dpc and 10 dpc)
- Tracheal completely negative for all of sampling dates (no tracheal take)
- Virus isolation
 - Controls: 80% of birds positive on virus isolation cloacally (different timepoints), 50% for tracheal isolation
 - Vaccinated group: remained negative for the duration of the trial both cloacally and tracheally

- All birds seroconvert but 20-30% of animals are below recommended cut-off ELISA kit Biochek
- First positive according to supplier 1 animal on 8, 17 days after first vaccination



- Vaccine is safe
- Vaccine is very efficacious in terms of reducing viral spreading and shedding: cloacal and tracheal no take at all of virus
- Virus re-isolation after challenge remained completely negative - infection dies in vaccinated birds
- Vaccination is a very useful tool both ethically , economically and epidemiologically

- Classified as LPAI -> co-factors were searched but no single co-factor could be found

- Very important in discussion on measures to be taken by authorities: European laws don't allow for compensation for LPAI

- No use was made of the “unknown disease” culling options, against advice of the field vets that indicate that this was an unknown pathology with dramatic impact on health and epidemiologically not behaving like normal LPAI

- Trials for elucidating pathology: too little and too late. One trial at GD confirmed the pathology with the H3 strain as only etiologic agent. Still today reference lab searching for co-factor

-Need to have a good discussion with OIE and regulators: change rigid classification of LPAI and HPAI, with dramatic consequences in disease control

- Veterinarians can not do their job if this classification remains, and/or if reference centers wait too long to classify as unknown disease entity

- Current policies of massive killing and/or no action taken impossible to explain to public- big threat for image poultry industry

- More free range in future will allow more similar cases to happen – society is changing!

-Vaccination as early as possible in the infection should be standard?

- On multi age farms vaccinate other flocks on farm as quickly as possible to prevent mutation and adaptation
- Vaccinate neighboring farms to limit risk virus can escape after adaptation and high shedding (asap)
- Homologous autogenous vaccination is demonstrated to be very efficacious, not sure if commercial vaccines could be a good alternative, more research needed
- New legislation EU can cover epidemiological link, for LPAI should be geographical link

-Field Poultry veterinarians (WVPA) crucial role to play in critical phase of outbreaks, and in policy making

- We have noticed that reference lab, governmental bodies have no room for interpretation if there are errors within legislation
- Academia too far from field to pave the way, can play supportive role though

Thanks for your attention



Questions?

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