



VALIDATION OF A MULTISPECIES COMPETITION ELISA FOR DETECTION OF ANTIBODIES AGAINST INFLUENZA A VIRUSES IN AVIAN SPECIES, PIGS AND HORSES

C. van Maanen, Rianne Buter¹, Eric van Esch¹, Vincent Munster²,
Ron Fouchier², Ann Cullinane³ and Sjaak de Wit¹

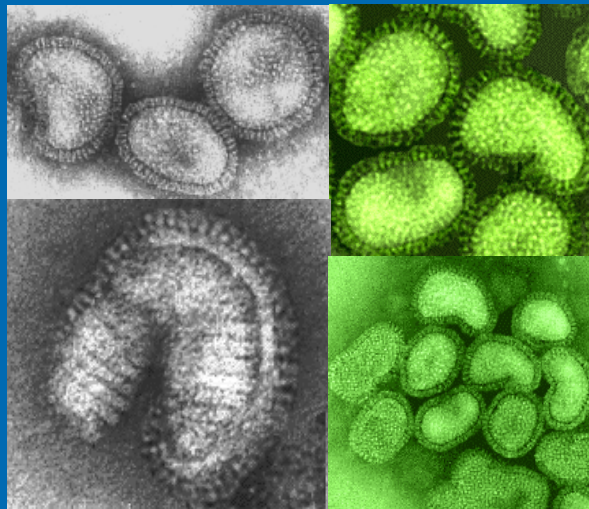
¹GD Animal Health Service, Deventer, The Netherlands

²Erasmus Medical Centre, Department of Virology, Rotterdam, the Netherlands

³Irish Equine Research Centre, Johnstown, Ireland

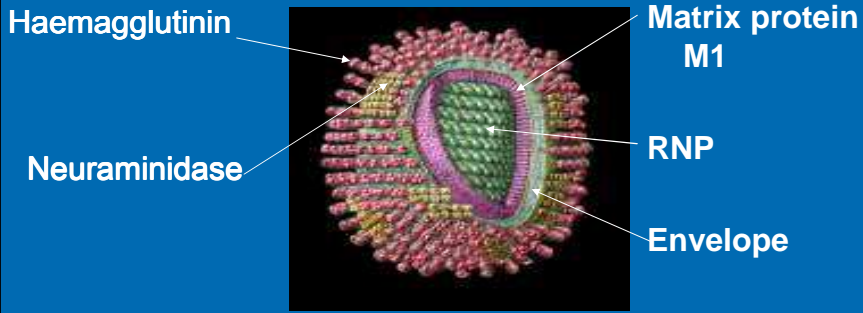


Flu under the microscope





Influenza Virus Revealing Internal Structures



HA subtypes in different animal species

Subtype	Human	Swine	Horse	Bird
H1	Human icon	Swine icon		Bird icon
H2	Human icon			Bird icon
H3	Human icon	Swine icon	Horse icon	Bird icon
H4		Swine icon		Bird icon
H5	Human icon			Bird icon
H6				Bird icon
H7	Human icon		Horse icon	Bird icon
H8				Bird icon
H9	Human icon			Bird icon
H10				Bird icon
H11				Bird icon
H12				Bird icon
H13				Bird icon
H14				Bird icon
H15				Bird icon
H16				Bird icon



N-subtypes in different animal species

Subtype	Human	Swine	Horse	Bird
N1				
N2				
N3				
N4				
N5				
N6				
N7				
N8				
N9				



Free range farm





Dutch H7N7 epidemic 2003

- IVPI: 2.98; Sequence: HP
- H7N7 ; Source most likely reassorted virus from wild ducks (H7N3 with H11N7)



Stamping out of infected flocks

- number of farms 241 (13 backyard)
- number of birds killed 5 million

Preventive culling

- number of farms: about 1200
- number of birds: about 25 million



Serological monitoring avian influenza

- Regular check for the presence of flocks with antibodies against AI
- Control by AHS
- Sampling: responsibility farmer
- Blood test: ELISA or AGPT (only broilers)
- Positive reaction: samples to CVI Lelystad
- Frequency: depends on the type of birds



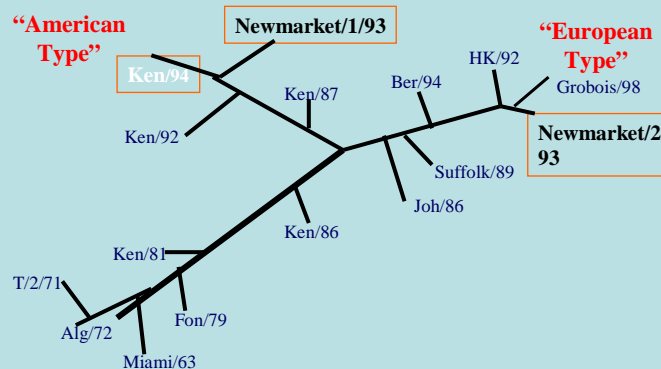
Start monitoring program 2002 Test characteristics indirect ELISA

- Sensitivity field sera 98%
- Specificity: 99.3 to 99.6%
- r and R high

- Major drawback:
 - Only suited for chickens and turkeys

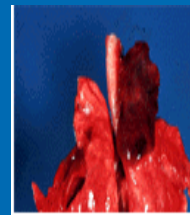
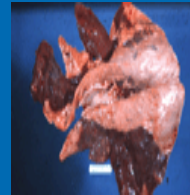


Equine influenza: A equi-2/H3N8

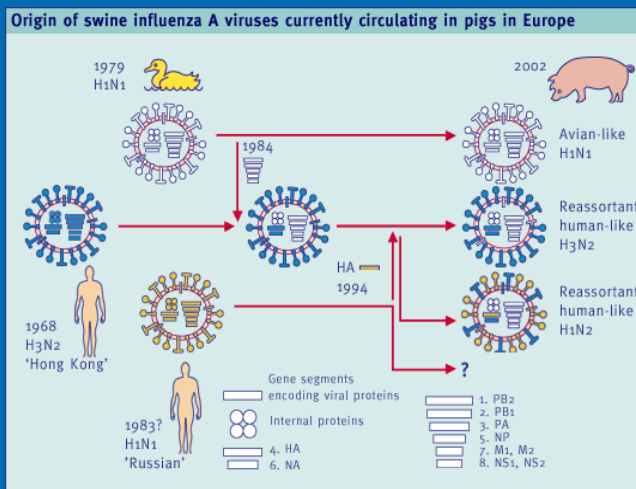




Swine Influenza viruses



Porcine flu in Europe: origin of influenza viruses





Materials & Methods: ELISAs

- FlockChek indirect AI ELISA was routinely used for AI monitoring in poultry
- Good performance, but only useful for poultry and turkeys
- IDEXX AI MultiS-Screen ELISA evaluated for multispecies diagnosis and monitoring of influenza A infections
- ELISA is based on a viral antigen(s) coated to the plate and a HRPO-conjugated monoclonal antibody directed against the nucleoprotein of influenza A virus.



Avian serum panels

- Specificity evaluation:
 - Field sera (n=1011) from AI-free Dutch poultry flocks
 - Serum samples from single reactors in the current monitoring scheme (n=30)
- Sensitivity evaluation:
 - Sequential serum samples (n=478) obtained after infection with H5N2 (LP), H6N2, H7N3 (LP), H9N2 or after inoculation with an inactivated H5N2 vaccine
 - Sera from a H5N2 infected poultry flock (n=20)
 - Sera from a H1N5 infected turkey flock (n=28)
 - Sera (n=57) from different avian species after H5N2 vaccination
 - Sera (n=1921) from different avian species from divergent geographical regions



Porcine serum panels

- Specificity evaluation:
 - Serum samples from CD/CD piglets (n=179)
 - Field sera (n=30) negative (<9) in HI tests against H1N1, H1N2 and H3N2

- Sensitivity evaluation:
 - Field sera (n=120) with well defined HI titres varying between <9 and ≥ 576 .
 - The panel included sera that were positive against only one up to three relevant subtypes.



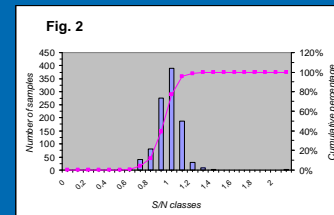
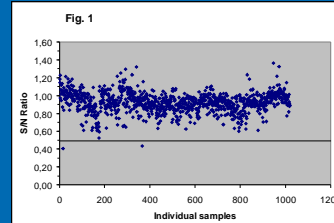
Equine serum panels

- Well defined equine sera (n=100) from vaccination-challenge experiments.
- The status of these sera varied from negative against H7N7, H3N8 American lineage and H3N8 European lineage to (high) positive against one or more of these subtypes.
- Individual sera originating from a monitoring project for equine upper respiratory tract disease (n=147)
- Paired serum samples (n=56), collected in the acute and convalescent phase of upper respiratory tract disease



Results avian panels: specificity

- Specificity 99.8% (CI 99.5%-100%)
- 2/30 (6.7%) of single reactors scored positive in MS-ELISA
- Frequency distributions are shown in Figs 1&2



Results avian panels: sensitivity

AI MultiS-Screen ELISA

AI serotype	Number of ELISA positive sera of the total number of sera x days after inoculation				
	-7	7	14	21	27
H5 (inactivated)	0/12	3/9	9/11	9/11	15/15
% positive	0%	33%	82%	82%	100%
H5N2	0/10	6/10	10/10	9/10	-
H6N2	0/12	9/12	11/13	12/13	13/14
H7N1	0/10	5/8	8/10	9/10	-
H9N2	0/13	6/7	12/12	14/14	15/15
% positive	0%	63%	89%	91%	97%



Results avian panels: sensitivity

AI Indirect ELISA

AI serotype	Number of ELISA positive sera of the total number of sera x days after inoculation				
	-7	7	14	21	27
H5 (inactivated)	0/13	2/14	6/15	13/15	15/15
% positivity	0%	14%	40%	87%	100%
H5N2	0/10	4/9	8/10	8/10	-
H6N2	0/13	7/11	8/13	9/12	10/14
H7N1	0/10	5/9	6/10	9/10	-
H9N2	0/14	12/14	14/14	15/15	15/15
% positivity	0%	65%	77%	87%	87%

www.gddeventer.com

Bird	(n) total	(n) negative	(n) positive	seroprevalence	AI prevalence*
Anseriformes					
Mallard	186	76	110	59%	7,3%
Northern pintail	2	0	2	100%	2,9%
Eider duck	2	1	1	50%	5,4%
Barnacle Geese	108	65	43	40%	0,7%
Charadriiformes					
Shorebirds	1050	1004	46	4%	0%
Red knot	184	97	87	47%	120%
Ruddy turnstone	88	41	47	53%	150%
Black-tailed godwit	74	73	1	1,4%	0%
Ruff	33	33	0	0%	0%
Oystercatcher	32	31	1	3%	0%
Dunlin	20	20	0	0%	0%
Common Redshank	10	10	0	0%	0%
Bar-tailed godwitt	6	6	0	0%	0%
Grey Plover	1	1	0	0%	0%
Shag	5	5	0	0%	0%
Guillemot	41	31	10	24%	0,4%
Brunnichs guillemot	17	10	7	41%	0%
Puffin	16	13	3	19%	0%
Razorbill	2	2	0	0%	0%
Kittiwake	15	8	7	47%	0%



Results porcine panels

- CD/CD sera: specificity 100%
- HI negative field sera: specificity 74%..
- HI positive field sera: sensitivity 89 – 97%

Indicator virus	Total (n)	Positive (n)	Negative (n)	Sensitivity
H3N2	36	32	4	88.9%
H1N1	39	38	1	97.4%
H1N2	39	38	1	97.4%



Results equine panels

- SRH/HI negative sera (n=42): specificity = 95%
- HI positive field sera (n=111): sensitivity = 89%
- Experimental sera: variable sensitivity (40 – 100%)
- False negative ELISA results strongly associated with low HI titres (9/18)
- Paired serum samples: good agreement (kappa=0.77) with HI-test for seroconversion or significant increase in titre



Conclusions poultry and wild birds

- The MultiS-Screen ELISA showed excellent specificity (99.8%) for Dutch poultry and equivalent or better sensitivity than the previously used indirect ELISA (65% of chickens already seropositive 7 days after inoculation)
- The seroprevalence of influenza A virus infections in wild birds varies considerably depending on bird order, species, and temporal and geographical factors.
- The seroprevalence data generated in this study provided, together with available virological data, further insight in the epidemiology and ecology of influenza viruses in wild birds.



Conclusions pigs and horses

- The AI MultiS-Screen ELISA showed excellent specificity (100%) for pigs, based on CD/CD piglet sera, but moderate specificity based on HI negative field sera. Definition of gold standard?
- For pigs the sensitivity of the ELISA varied between 88.9% and 97.4% for the relevant subtypes. Only low HI titres are missed.
- For horses the AI MultiS-Screen ELISA showed a specificity of 95%.
- Sensitivity for recently infected and vaccinated horses and agreement with HI results with for paired serum samples (seroconversions) were good.
- In conclusion, this multispecies ELISA seems fit for purpose for serodiagnosis and seromonitoring of influenza A virus infections in multiple avian and mammalian species.



Acknowledgements

- IDEXX for financing this study.
- Dutch practitioners for their cooperation in the equine influenza surveillance program



Thank you for your attention!



