



SEROLOGICAL EVIDENCE OF EXPOSURE TO INFLUENZA A IN DIFFERENT AGE CATEGORIES IN PIG FARMS IN SERBIA

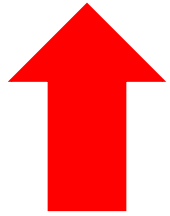
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INTRODUCTION

➤ Respiratory disease of pigs most often caused by the H1N1, H1N2, and H3N2 subtypes of influenza A virus



MORBIDITY

↓ mortality



Direct and indirect losses increased when coinfection with other respiratory pathogens occurs



Disease of public importance



Significance of disease monitoring

INTRODUCTION

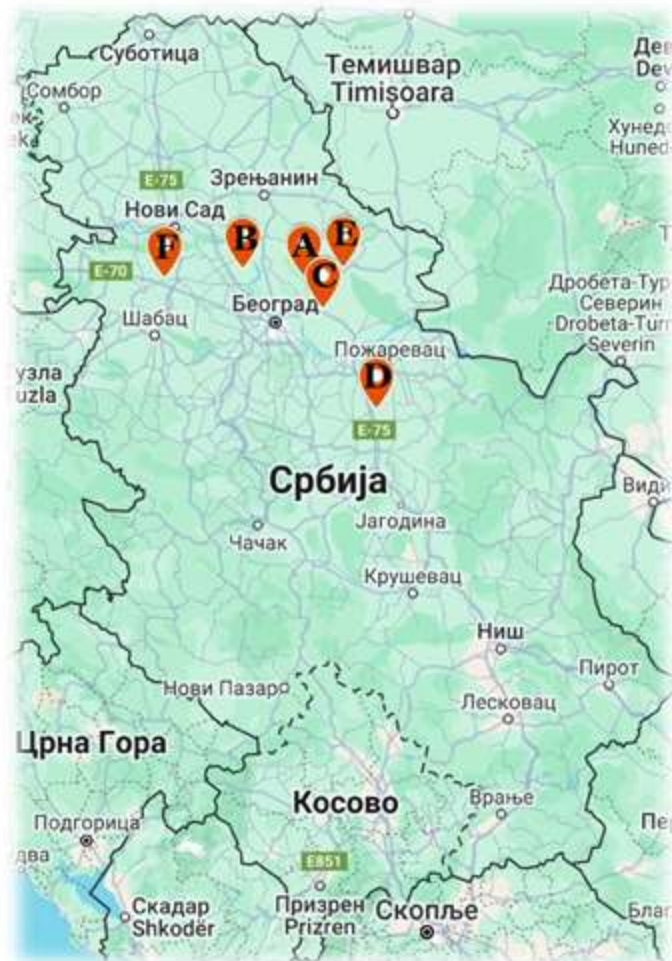
Serological examination in a vaccination-free herd is the first choice method for confirmation of virus circulation and prevalence determination

- In laboratory conditions first antibodies can be measured three days after infection, maximum level reach between 14 and 21 day
- The most important are antibodies against HA, NA, NP and M protein
- Developed immunity is long-lasting and provides protection from infection with antigenically similar strains
- Laboratory methods in use:
 - ✓ ELISA - convenient for large number of samples - screening of the herd
 - ✓ Virus Neutralization Test, Inhibition of Hemagglutination - serotyping, antibody titer

MATERIAL AND METHODS

➤ ELISA

- Examination of 625 blood sera from pigs raised on 6 commercial farms (A, B, C, D, E, F) collected during 2021-2023 period (random sampling and selection of the samples)



64 sow sera



33 boar sera



508 sera from weaners

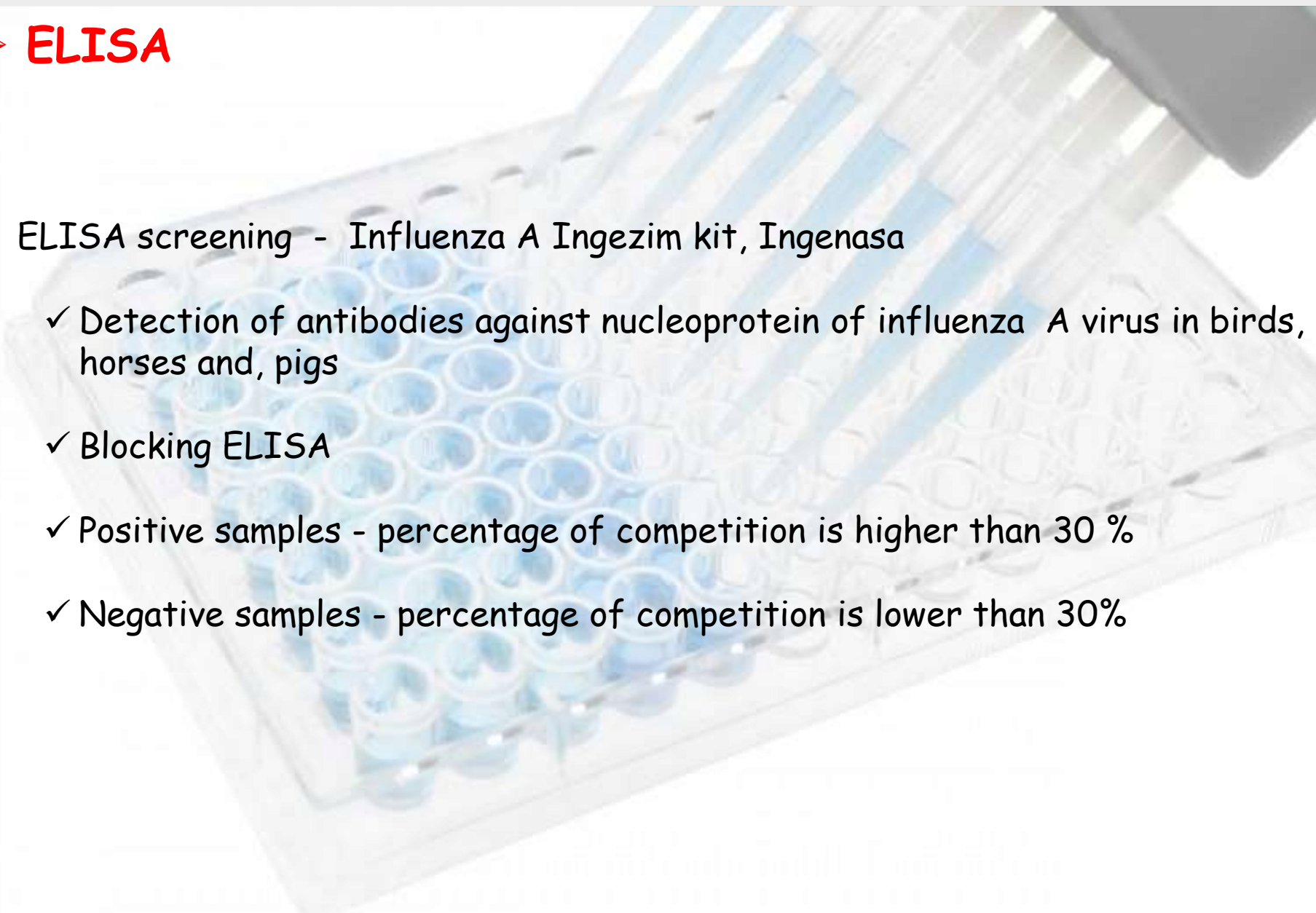


20 sera from suckling pigs

MATERIAL AND METHODS

➤ ELISA

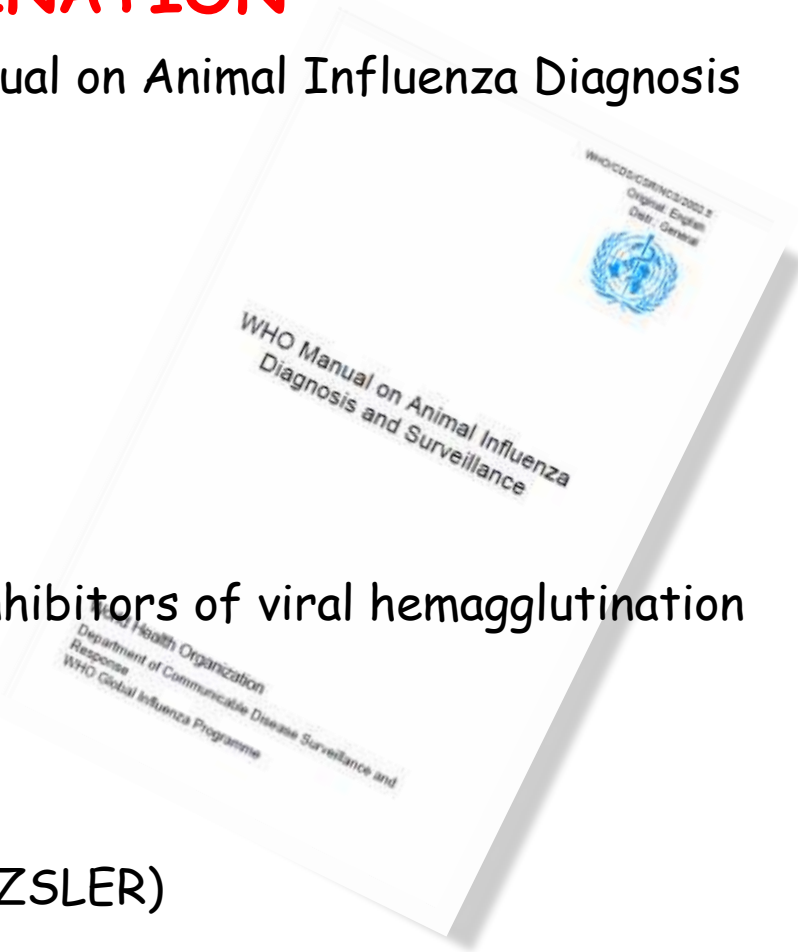
➤ ELISA screening - Influenza A Ingezim kit, Ingenasa

- ✓ Detection of antibodies against nucleoprotein of influenza A virus in birds, horses and, pigs
 - ✓ Blocking ELISA
 - ✓ Positive samples - percentage of competition is higher than 30 %
 - ✓ Negative samples - percentage of competition is lower than 30%
- 
- A 96-well ELISA microplate is shown in the background, with a pipette dispensing liquid into the wells. The plate is white with blue wells, and the pipette is grey and black.

MATERIAL AND METHODS

➤ INHIBITION OF HEMAGGLUTINATION

- Titer and subtype determination (WHO manual on Animal Influenza Diagnosis and Surveillance)
- Material: 64 ELISA positive sera
 - ✓ 33 sera from sows
 - ✓ 12 sera from boars
 - ✓ 17 sera from weaners
 - ✓ 2 sera from suckling piglets
- Serum preparation (removal of non-specific inhibitors of viral hemagglutination and naturally occurring agglutinins)
 - ✓ starting dilution 1:10
- Reference viruses and sera (OIE ref. lab - IZSLER)

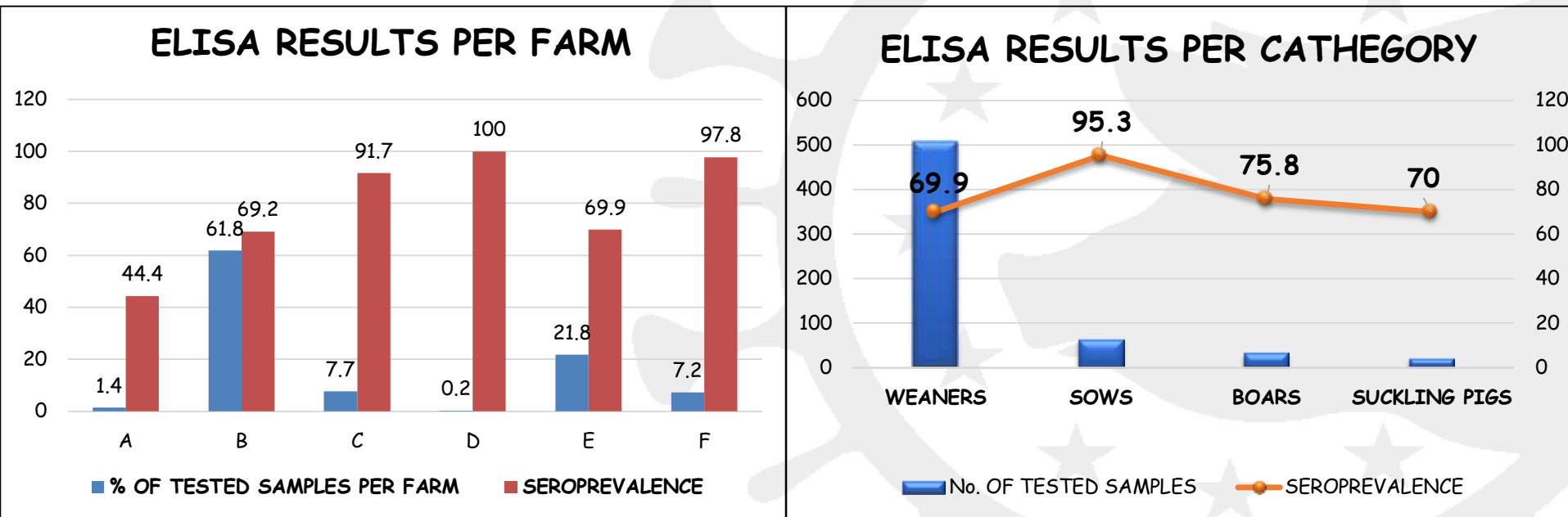


Reference strain	Subtype	Lineage
A/sw/It/282866/13/H1N1pand (H1N1pdm09)	H1N1	A(H1N1)pdm09
A/sw/311368/13/H1N1 (H1avN1)	H1N1	Eurasian avian-like H1avN1
A/sw/It/311349/13/H3N2 (H3N2)	H3N2	A/swine/Gent/1/1984-like H3N2
A/sw/284922/09/H1N2 (H1huN2)	H1N2	A/swine/Scotland/410440/1994-like H1huN2

RESULTS AND DISCUSSION

➤ ELISA

- Based on the ELISA results, influenza A virus circulated in each tested farm
- Out of 625, 455 samples reacted positive (overall seroprevalence 72,8%)



- Seroprevalence in sows - Croatia reported the highest seroprevalence in this category as well (61,4%) - *Jungić et al. (2021)*

RESULTS AND DISCUSSION

- Recent small-scale serological investigation of backyard pigs from 69 holdings with low biosecurity in Serbia revealed an absence of seroconversion in all tested animals - *Milićević et al.* (2023)



RESULTS AND DISCUSSION

➤ IHA:

- 52 sera from pigs that originating from 4 farms gave positive reaction with used reference viruses
- Positive: 28 sows, 10 boars, 12 weaners and 2 suckling pigs
- Lower sensitivity in comparison with ELISA (81,25%) - reference antigens isolated in 2009 and 2013, potentially antigenically different from circulating strains
- Same result regarding comparison of two tests in investigations in Croatia and Slovenia (presence of false negative reactions in IHA) - *Jungić et al. (2021)*, *Plut et al. (2023)*

RESULTS AND DISCUSSION

➤ IHA:

- Using IHA circulation of H1N1 and H3N2 subtypes revealed on 4 farms (B, C, D, E)
- None of the samples gave positive reaction with H1N2 reference strain - same as in research of *Plut et al. (2023)* in Slovenian pig herds

RESULTS PER FARM AND SUBTYPE

Farm (total No. of tested per farm)	Category	Tested per category	Neg	Pos	Positive per subtype (%)		
					H1N1	H3N2	H1N2
B (15)	sow	1	0	1	1(100%)	1(100%)	0
	weaner	12	3	9	7(70%)	4(40%)	0
	suckling pig	2	0	2	1(50%)	1(50%)	0
C (24)	sow	12	2	10	10(100%)	5(50%)	0
	boar	12	2	10	7(70%)	6(60%)	0
D (1)	sow	1	0	1	1(100%)	1(100%)	0
E (24)	sow	19	3	16	15(93.75%)	8(50%)	0
	weaner	5	2	3	4(80%)	2(40%)	0
Total		64	12	52	44 (84.6%)	28(55.8%)	0

RESULTS AND DISCUSSION

➤ IHA:

- 33 positive to H1avN1, 39 positive to H1N1pdm09, 29 positive to H3N2

SEROLOGICAL RESPONSE IN RELATION TO USED REFERENCE STRAINS

Serology response to:	No. of positive samples (%)	No. of positive per strain/strains (%)		
		H1avN1	H1N1pdm09	H3N2
1 strain	18 (34,62%)	2 (11,1%)	8 (44,45%)	8 (44,44%)
2 strains	20 (38,46%)	H1avN1+H1N1pdm09	H1avN1+H3N2	H1N1pdm09+H3N2
		14 (70%)	3 (15%)	3 (15%)
3 strains	14 (26,92%)	H1avN1+H1N1pdm09+H3N2		
		14 (100%)		

RESULTS AND DISCUSSION

➤ IHA:

➤ Serological response in relation to the category and reference strain

	Category				total
	sows	boars	suckling pigs	weaners	
No. of tested	33	12	2	17	
No. of positive (%)	28 (84.8)	10 (83.3)	2 (100)	12 (70.6)	
Present antibodies	No. of positive (the highest detected titer)				
H1avN1	0	1(1:10)	0	1(1:10)	2
H1N1pdm09	6 (1:160)	1 (1:10)	0	1 (1:10)	8
H3N2	1 (1:160)	3 (1:160)	1 (1:40)	3 (1:20)	8
H1avN1+H1N1pdm09	7	2	1	4	14
H1avN1+H3N2	2	0	0	0	3
H1N1pdm09+H3N2	1	2	0	0	3
H1avN1+H1N1pdm09 +H3N2	11	1	2	0	14

CONCLUSION

➤ SEROLOGICAL METHODS FOR SIV DIAGNOSTIC ARE:

- Cost-effective
- Easy to perform on a large-scale
- Do not require specific biosafety measures
- Not time-limited, as antibodies are long-living

With some restrains...

OUR RESEARCH CONFIRMED

- ✓ ↑ seroprevalence in commercial farms independently from age category
- ✓ Circulation of H1N1 and H3N2 SIV in four out of six analyzed farms
- ✓ Majority of the tested animals was in contact with two or three different strains of swine influenza A viruses, with domination of H1N1pdm09 strain

Thank you for your attention!



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