

Part 2. Biosafety

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RISK ANALYSIS AND MOLECULAR EPIDEMIOLOGY ASPECTS FOR EMERGENT DISEASES OF ANIMALS (AFRICAN SWINE FEVER, BRUCELLOSIS, AVIAN INFLUENZA AND NEWCASTLE DISEASE)

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Summary. The risk analysis is the one of crucial points of the modern approach for the epizootic situation assessment and biorisks management system. Most important for epizootic situation control and international trade management is the system of transboundary diseases distribution risk determination. This allows creating forecasts regarding diseases introduction and distribution among the countries and territories and to develop appropriate strategies of the diseases control and minimize trade-associated diseases related risks. The paper involves description of the basic definitions in area of the risk assessment, strategies and approaches for its determination and analysis. The role of the molecular genetics tools are described from the position of effectiveness for risks forecasting in wildlife and animal husbandry.

Keywords: risk analysis, molecular epidemiology, emergent diseases, animals

The globalization of the modern world, increasing volumes of international trade operations, transport people, animals, agricultural products considerably exacerbates the risk of occurrence and spread of infectious diseases, as well as the distribution of pathogens that cause them. This explains the fact that the biological safety is a key component of national security.

The issues of biosafety in the world dealing with international organizations: World Health Organization (WHO), the Office International des Epizooties — World Organisation for Animal Health (OIE) and the World Organization for Agriculture and Food Organization (FAO).

Adopted by the WHO, OIE and FAO concept 'One health' (One Health — health care consumers by producers and product safety), which is currently sold as a common strategy that consolidates effort veterinary and humane medicine in the areas of assessment and management of biological and nutritional risks resulting from the emergence and spread of infectious diseases, disorders of feeding, animal keeping, processing and quality control and safety of agricultural products.

The main objective of veterinary and humane medicine in the context of the implementation of Biosecurity is to identify and minimize biological *threats* (*hazards*) and risks associated with infectious diseases and their agents.

Risk is the potential situation of losing or gaining of something (Kungwani, 2014). The **risk** could be

also determined as the likelihood that harm will occur. The definition of the risk is strongly aligned with the term hazard — something that has the potential to do harm. The minimization of the possible harm effect is associated with risk management.

Most crucial of the effective risk management is the assessment of risks, that includes the determination of quantitative or qualitative estimation of risk related to a well-defined situation and a recognized hazard (threat).

An acceptable risk is a risk that is understood and tolerated usually because the cost or difficulty of implementing an effective countermeasure for the associated vulnerability exceeds the expectation of losses.

Most risks in veterinary medicine concerning animal health and agricultural products quality and safety are associated with infectious diseases. These are the most crucial and require complex control and prevention. Especially in cases of the emergent and zoonotic diseases, associated with possibility and probability of affection of public health and economical situation in countries and regions.

The practices of the biorisk assessment are based on the analysis of risks for potential introduction of the diseases, analysis of the outbreaks occasion risks, analysis of possible affects and the summarization of the biorisks assessment.

The international approach for the risk analysis data collection and use includes determining of the country/territory status in order to:

- identification of the opportunities for transactions of import and export of agricultural products;
- analysis of global epizootic situation on cross-border and other emergent diseases (African swine fever, influenza, Newcastle disease, bluetongue, Shmallenberg virus, foot and mouth disease, etc.);
- control of the ‘program diseases’ (MDR-TB infections, bovine leukemia (in the case of Ukraine), etc.).

Control of diseases in the regions is aimed to protect national export status, that includes identification of the surveillance strategy, analysis of internal (including human-associated) risks, and identification of the prevention strategy.

The legislative background of the biological and epizootical risks assessment internationally is based on three main key points of legal documents (Sundqvist et al., 2013):

1. Legal documents of the World Trade Organization (WTO) includes definition of regions with low prevalence infectious diseases for the purpose of trade operations: Article 5 ‘Assessment of Risk and Determination of the Appropriate Level of Sanitary or Phytosanitary Protection’ and Article 6 ‘Adaptation to Regional Conditions, Including Pest- or Disease-Free Areas and Areas of Low Pest or Disease Prevalence’ of the Agreement on the Application of Sanitary and Phytosanitary Measures (WTO, 1995);

2. Manuals and Codes of the Office International des Epizooties — World Organisation for Animal Health (OIE), standards for international trade in animals and animal products;

3. National regulations (e.g., for Ukraine):

- the Law of Ukraine ‘On veterinary medicine’;
- veterinary legislation;
- ISO, DSTU, and SOU guidelines for the monitoring and diagnosis of infectious animal diseases.

One of the main principals used for field/regional risk assessment in the veterinary field is the principal of the regionalization.

Following a formal request from the importing or exporting country, the following steps are executing in this process, including familiarization with primary information about the country/territory, detection of lack of information and request additional data, visit the site to be tested, determining the risks of imports, the status of ‘free’ area of disease/pathogens, transport/migration routes for risk control, written report on risk assessment preparation, and its sharing, forming of regionalization policy.

The system of the epizootological wellbeing/public health involves multiple approaches, including detailed description of the region and livestock in it, characterization of the region borders, compartmentalization regions and territories, marking of the major and minor settlements, roads, transportation and others, communications, location of the district veterinary service, official laboratory, border guards and transport, other important bodies responsible for the maintenance of livestock, identification of international and inter-regional trade ties, and, finally, specification diseases are risk factors.

The typical risk assessment matrix is demonstrated on the Fig. 1, where the risk category could be ranged from low to extremely high, depending from the probability and severity of the risk.

		PROBABILITY THAT SOMETHING WILL GO WRONG				
		FREQUENT Likely to occur immediately or in a short period of time, expected to occur frequently	LIKELY Quite likely to occur in time	OCCASIONAL May occur in time	SELDOM Not likely to occur but possible	UNLIKELY Unlikely to occur
SEVERITY OF RISK	Category					
	CATASTROPHIC May result in death	E	E	H	H	M
	CRITICAL May cause severe injury, major property damage, significant financial loss, and/or result in negative publicity for the organization and/or institution	E	H	H	M	L
	MARGINAL May cause minor injury, illness, property damage, financial loss and/or result in negative publicity for the organization and/or the institution	H	M	M	L	L
NEGLECTIBLE Hazard presents a minimal threat to safety, health and well-being of participants; trivial.	M	L	L	L	L	

Figure 1. Risk assessment matrix (from <https://q9cqualityconsulting.com/2011/09/22/risk-probability-can-we-do-better/comment-page-1/>)

The regional risks estimation is passed on another plot, which involves multiple factors of risk, and its possibility and probability. The risk possibility score calculated by the points. The high possibility is estimated as 3 points, low — 1 point, and rare — 2 points. Assumed scores could be subdivided to 5–7 categories of risks from the low to extremely high (Table 1).

Table 1 – Transboundary risks estimation matrix

Risk factor	High level	Midlevel	Low level
Existing of the disease in the wildlife	?	?	?
Existing of the disease in domestic animals	?	?	?

Existing of the transmission vectors	?	?	?
Existing of the transboundary transmission ways (migratory wildlife)	?	?	?
Transport communications (migration of people and trade)	?	?	5–7 risk categories

African swine fever. Illustration of this risk assessment model could be described on the example of African

swine fever (ASF). ASF was reported in Ghana, Nigeria, Lithuania, Latvia, Belarus and Poland and the Russian Federation in 2015 (2007 in Russia recorded more than 450 outbreaks of disease among domestic pigs and 200 cases among wild boars) (Fig. 2).

In accordance with the EU Directive 2016/464 with ASF were affected multiple EU regions, including: Estonia (28 affected regions), Latvia (16 regions), Lithuania (11 regions), Poland (14 regions, 48 points). In the mentioned regions held zoning and implemented quarantine measures. The Supervisory zone is also referred about Sardinia (Italy), where ASF outbreak was recorded among wild and among domestic pigs (EC, 2016).

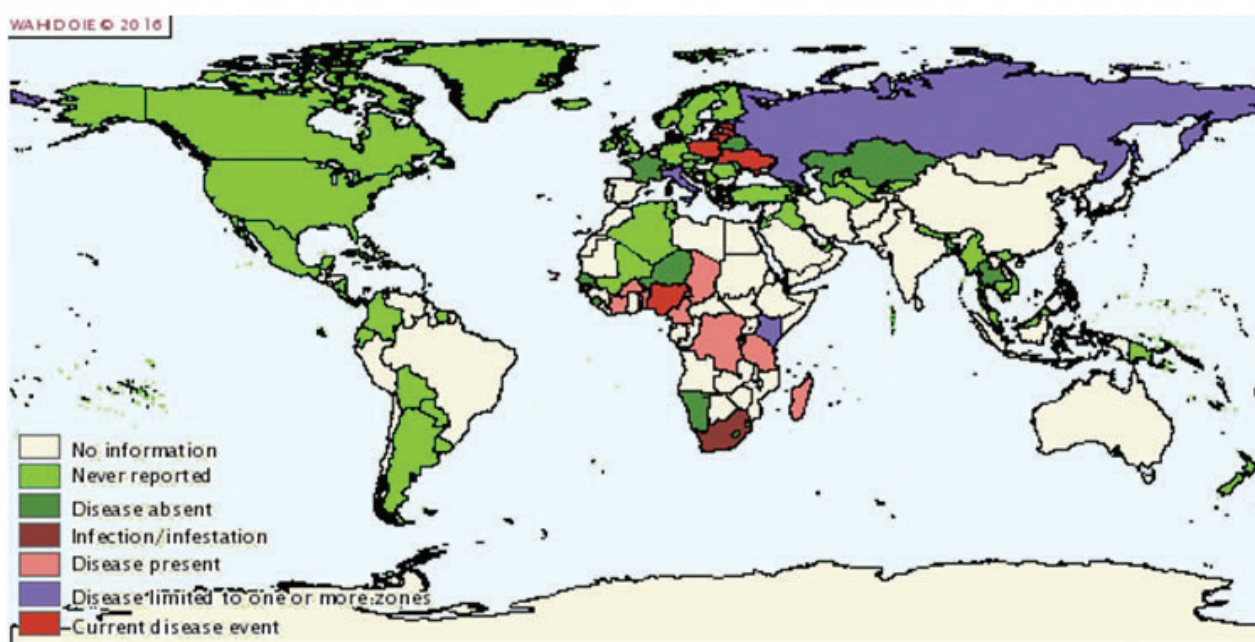


Figure 2. Epizootic situation for the ASF in the World (from http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/Diseasedistributionmap)

The genetic relationship of the virus indicates the presences of 22 genotypes are roughly divided into two genetic lineages. The marker genotype-specific area is the p72-region. The progenitors of the first genetic line are clusters of pathogens from genotype I, which circulated in Africa and Europe in 50–60 years, and the second — 8 genotype isolates Caribbean in 70s.

Today on the territory of Kenya and Uganda, according to the monitoring of populations of ticks and warthog circulating virus genotypes IX and X.

The ‘molecular watch’ demonstrates development of the ‘modern’ 2nd genotype population progenitor-relation from the old African population of the virus. This agent population was introduced via Mideast to Caucasus region, affected Georgia, Armenia, Azerbaijan and Russia. After that in has been introduced in Ukraine

(2012), Belarus (2014), and other countries (Fig. 3) (Gallardo et al., 2015).

The molecular tools used for the characterization of the ASF agent demonstrate similarity among isolates that proves its transboundary dissemination between countries: from Georgia and Armenia to Azerbaijan and Russia, and from Russia to Ukraine, Belarus. These three countries are the partitions of the way of ASF distribution to Poland and Baltic states (Fig. 4) (Gallardo et al., 2014).

Using of the epizootical statistics data and previously described risks assessment matrix we can calculate the transboundary introduction risks for re-introduction of ASF virus in Ukraine from the Eastern border (Table 2). Calculation of the summary of risk points (n = 14) the risk could be characterized as extremely high confirmed risk.

The correlation among molecular characteristics and relative homology of virus circulating in neighbor countries could be the confirmation of risk existence and source of disease introduction.

Table 2 – Transboundary risks estimation for ASF introduction from Russia for Eastern Ukraine

Risk factor	High level	Midlevel	Low level
Existing of the disease in the wildlife	+		
Existing of the disease in domestic animals	+		
Existing of the transmission vectors	?		
Existing of the transboundary transmission ways (migratory wildlife)	+		
Transport communications (migration of people and trade)		+	3 + 3 + 2 + + 3 + 2 = 14 Extreme risk (confirmed)

Brucellosis. Brucellosis is especially contagious infectious zoonotic disease that affects different types of animals and people. Its agent belongs to the genus *Brucella*, which includes 9 species. The most severe forms of the disease causing by *Brucella melitensis*, *Brucella abortus*, and *Brucella suis*. Brucellosis occurs in acute or chronic forms of especially dangerous epizootic and epidemiological point of view is latent *Brucella*-keeping. Patients affected the reproductive organs of animals, at least — other organs and tissues. Economic losses caused by brucellosis in the mass abortion, birth of weak calves and non-viable, stable formation of infertility in adult animals (OIE, 2016).

The source of pathogen infection is the sick animals and *Brucella*-carrier. The reservoir of the pathogen is represented by wildlife (wolves, hares, wild boar, elk, deer), pets (dogs, cats), synanthropic rodents and birds, as well as blood-sucking insects and mites.

Factors of transmission are abortion-foetus, exudates and isolating sick animals, milk, contaminated genetic resources (semen, embryos), livestock products, feed, water, manure or litter, veterinary equipment and instruments.

Brucellosis is transmitted alimentary, contact, sexual and ways aerogenic (Stegniy et al., 2015).



Figure 3. Molecular profile of ASF in Eurasia (from http://asf-referencelab.info/asf/images/files/WS%20CISA%202014/PPTS/08_Arias_M_ASF_Gen_Activ_URL_2014.pdf)

ASFV: MOLECULAR CHARACTERIZATION

Summary

COUNTRY	YEAR	P72 GENOTYPE	CVR SUBTYPING	INTERGENIC SUBTYPING
Georgia	2007	II	GII-CVR1	GII-IGR1
Armenia	2007	II	GII-CVR1	GII-IGR1
Azerbaijan	2008	II	GII-CVR1	GII-IGR1
Russia Federation	2007-2012 (EURL)	II	GII-CVR1	GII-IGR1
	2012-2015 (Pokrov)	II	GII-CVR1	GII-IGR1 + GII-IGR2
Ukraine	2012, 2015	II	GII-CVR1	GII-IGR2
Belarus	2013	II	GII-CVR1	GII-IGR2
Lithuania	2014-2015	II	GII-CVR1	GII-IGR2
Poland	2014-2015	II	GII-CVR1	GII-IGR2
Latvia	2014-2015	II	GII-CVR1	GII-IGR2
Estonia	2014	II	GII-CVR1	GII-IGR2
	2015	II	GII-CVR1 + GII-CVR2	GII-IGR2
Sardinia	1978-1997	I	GI-CVRIII	
	1997-2015	I	GI-CVRX	

Figure 4. Molecular characteristics of ASF virus, allocated in European region (from <http://www.efsa.europa.eu/sites/default/files/151123-p7.pdf>)

Scientists of NSC 'IECVM' played a key role in the scientific support of the eradication of brucellosis, bovine brucellosis has been completely eradicated in Ukraine in 1975 against the backdrop of its widely spread in Russia, Romania, Hungary and Kazakhstan (Obukhovska et al., 2014).

Today there is a risk of transboundary animal brucellosis entry to Ukraine from countries disadvantaged on the infection (including Russia, Georgia, Turkey, Greece, Serbia, Iran, Mongolia, China) via:

— while export-import operations (via infected breeding or productive animals, animal products or genetic resources);

— as a result of a possible cross-border migration of wildlife.

The surveillance and diagnostics tools were developed in NSC 'IECVM' for the brucellosis control in Ukraine:

— national standard of Ukraine — anti-*Brucella abortus* serum;

— positive and negative control sera set for AT and CFT;

— serological kit for differentiation of *Brucella* cultures,

— RS-antigen for AT,

— serological test kits for diagnosis of infectious epididymitis in rams LCFT and AGID.

To ensure the scientific support of production of biological products for the diagnosis and prevention of brucellosis in the NSC 'IECVM' created the unique

Ukrainian collection of *Brucella* strains (135 strains), including:

- 4 strains of *B. melitensis*;
- 71 strain of *B. abortus*;
- 15 strains of *B. suis*;
- 45 strains of *B. ovis*.

The studies of the molecular genetic characteristics of strains collection are performed using PCR, restriction analysis and sequencing of the genome.

The perspective studies for risk assessment concerning the animal brucellosis are: monitoring in the wild (especially in the areas of risk of transboundary drift illness), and molecular genetic characterization of *Brucella* collection.

Avian influenza and Newcastle disease. The viruses that cause highly pathogenic avian influenza (HPAI) and Newcastle disease (ND) are currently eradicated in poultry in Ukraine. But the often-occurred in EU and Russia, so could be potentially transmitted via wild birds. Especially significance of these diseases demonstrates recent outbreaks of H5N8 in Germany, Italy and other member states of EU. However, the zoonotic threat of their spillover and emergence into poultry remains due to the presence of infected wild birds. Emergence is further supported by the viruses' intrinsic genetic and antigenic variability, which can facilitate host jumping and switching. In addition, global warming and climate change may increase viral transmission among reservoir and non-reservoir hosts (Gerilovych et al., 2008;

Stegniy, Gerilovych, and Stegnyy, 2008; Gerilovych and Potkonjak, 2009; Muzyka et al., 2014).

Historically, these diseases natural nosology areas were concentrated in Southern East Asia, but since last years it was enlarged (Fig. 5), and the new subtype of the highly pathogenic avian influenza virus, H5N8 has been occurred. It distributed in Americas, Africa, and Eurasia. Taking into account the molecular epizootology

profile of HPAI in 2005/2006 and 2008, when the origin of virus was primary from Russia, and next time from EU countries (Fig. 6), and the non-wellbeing status of big amount of EU and CIS countries, the risks of new type introduction in poultry of Ukraine is extremely high. These risks are associated with wild birds, migrating via territory of our state.

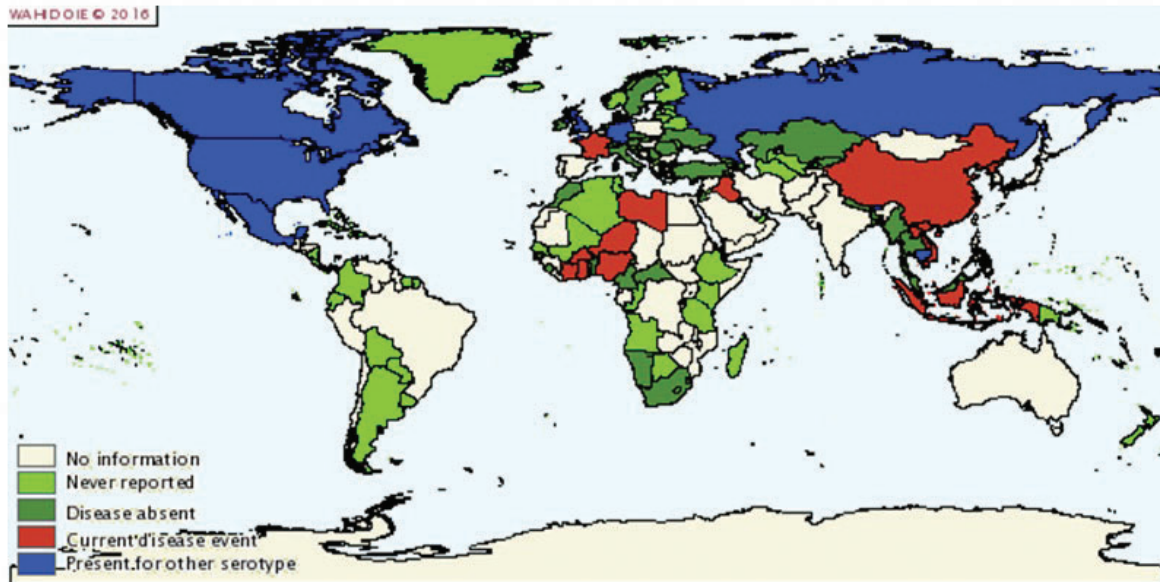


Figure 5. New HPAI epizootic situation (from http://www.oie.int/wahis_2/public/wahid.php/DiseaseInformation/Diseasedistributionmap)

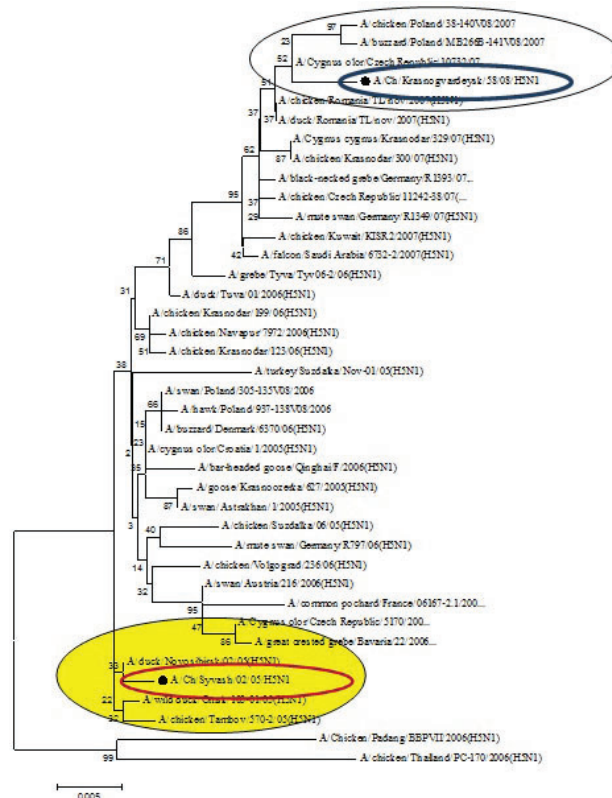


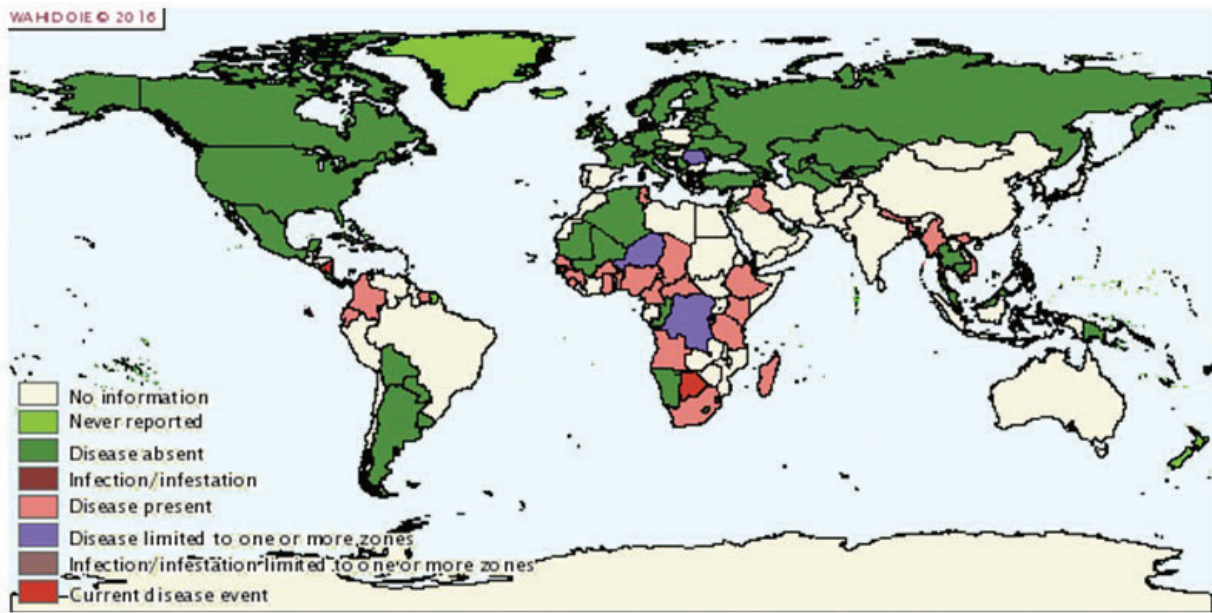
Figure 6. Phylogenetic relations of Ukrainian isolates (•) of HPAI with EU and Russian isolates

World epizootic situation concerning Newcastle disease also demonstrates, that disease endemic area from Southern-East Asia was moved to African countries (Fig. 7).

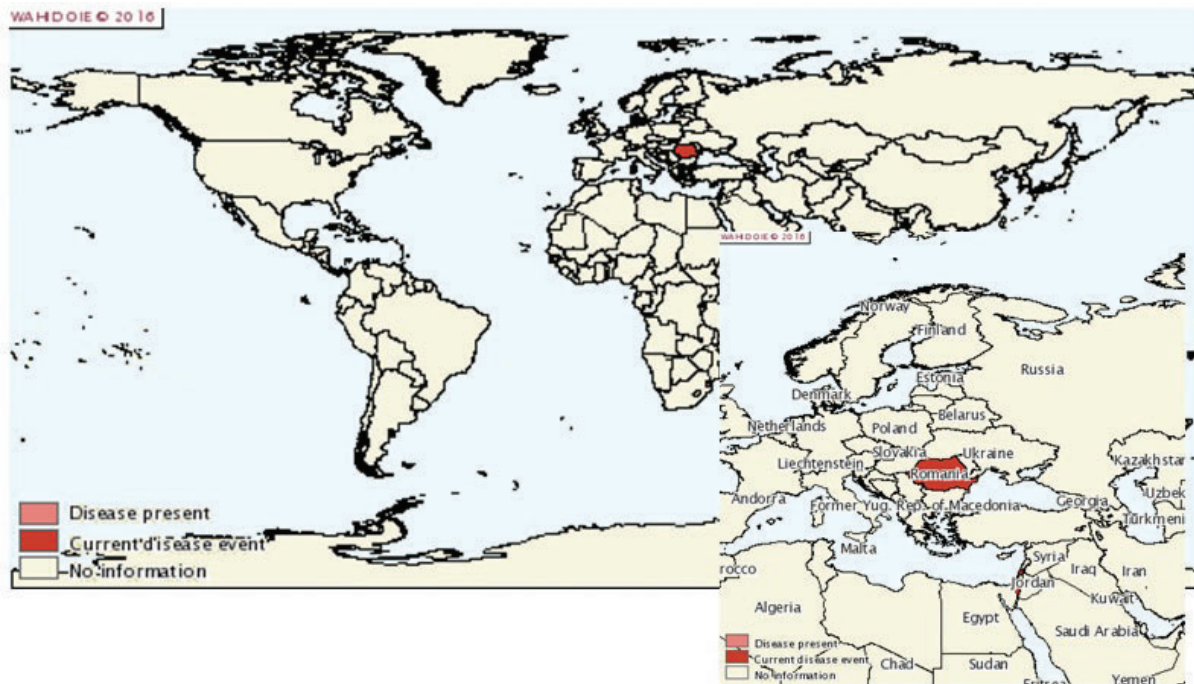
The molecular epizootology data collected by NSC 'IECVM' demonstrate circulation of 1st (historically), 2nd, 4th, and 5th genotypes (according Aldous et al., 2003) of NDV in Ukraine. These viruses

have Western European, Eastern European and Asian origin (Fig. 8).

Migratory crossways of the wild birds can introduce 'African' viruses to our state. Potential of this action was demonstrated in our previous study concerning other paramyxoviruses. Also, as potential harm of Newcastle disease virus introduction could be determined Romania.



a



b

Figure 7. Epizootic situation concerning Newcastle disease in the World and EU countries: a — 2015, b — 2016 (from http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/Diseasedistributionmap)

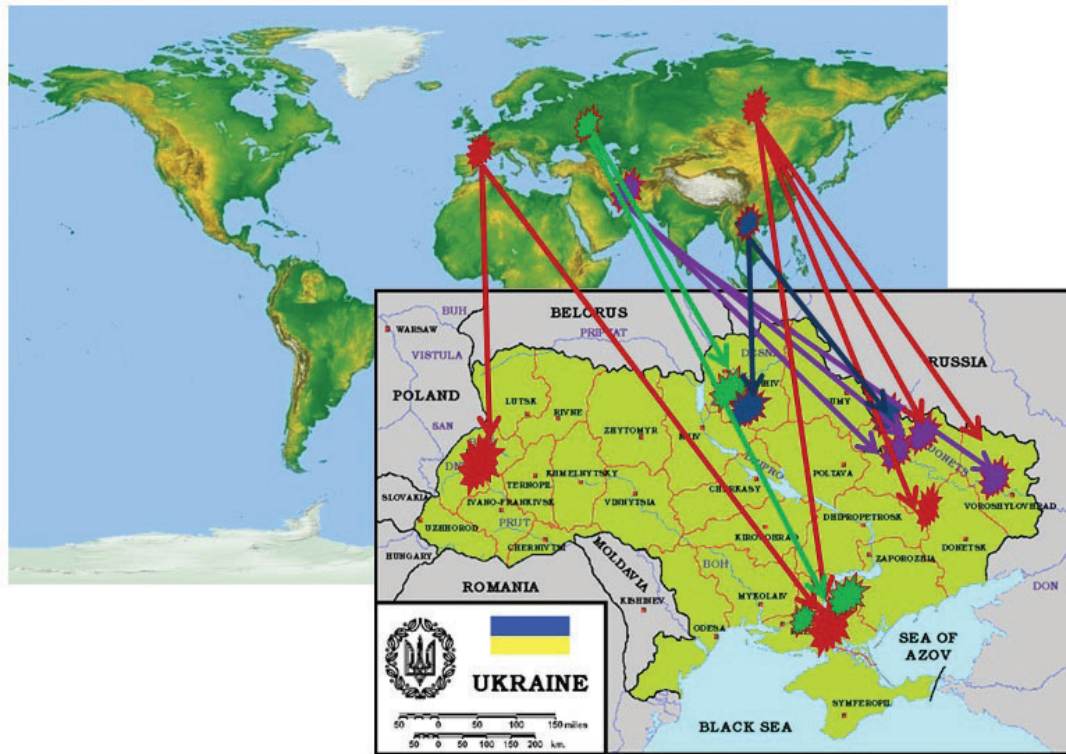


Figure 8. Origin of Ukrainian strains of Newcastle diseases from different genotypes

Conclusion. The epizootical risks for emergent diseases transboundary introduction could be estimated by the complex risk factor assessment. The existing risk could be ranged to 5–7 categories of risks, including low, moderate, and high clusters. The extremely high risks of ASF virus introduction are existed for Eastern Ukraine from Russia. Moderate to high-level risk of transboundary animal brucellosis entry to Ukraine from disadvantaged on the infection of countries (including Russia, Georgia, Turkey, Greece, Serbia, Iran, Mongolia,

China) exists. High-level risks are estimated concerning avian especially dangerous pathogens introduction to Ukraine via migratory birds. Using of the molecular genetics tools allow to determine viruses and bacteria host and geographical origin. This could be used in identification of the risk source and let enhancing of the existing surveillance with more target capacity to develop effective countermeasures against emergent animal diseases.

References

- Aldous, E. W., Mynn, J. K., Banks, J. and Alexander, D. J. (2003) 'A molecular epidemiological study of avian paramyxovirus type 1 (Newcastle disease virus) isolates by phylogenetic analysis of a partial nucleotide sequence of the fusion protein gene', *Avian Pathology*, 32(3), pp. 239–257. doi: 10.1080/030794503100009783.
- EC (European Commission) (2016) 'Commission Implementing Decision (EU) 2016/464 of 29 March 2016 amending the Annex to Implementing Decision 2014/709/EU concerning animal health control measures relating to African swine fever in certain Member States, as regards the entries for Estonia', *Official Journal of the European Union*, L 80, pp. 36–47. Available at: http://data.europa.eu/eli/dec_impl/2016/464/oj.
- Gallardo, C., Fernández-Pinero, J., Pelayo, V., Gazaev, I., Markowska-Daniel, I., Pridotkas, G., Nieto, R., Fernández-Pacheco, P., Bokhan, S., Nevolko, O., Drozhzhe, Z., Pérez, C., Soler, A., Kolvasov, D. and Arias, M. (2014) 'Genetic variation among African swine fever genotype II viruses, Eastern and Central Europe', *Emerging Infectious Diseases*, 20(9), pp. 1544–1547. doi: 10.3201/eid2009.140554.
- Gallardo, C., Reoyo, A., Fernández-Pinero, J., Iglesias, I., Muñoz, J. and Arias, L. (2015) 'African swine fever: A global view of the current challenge', *Porcine Health Management*, 1, p. 21. doi: 10.1186/s40813-015-0013-y.
- Gerilovych, A. P. and Potkonjak, A. (2009) 'Molecular evolution of Newcastle disease virus in Ukraine', *Contemporary Agriculture: The Serbian Journal of Agricultural Sciences*, 58(1–2), pp. 46–55. Available at: <http://polj.uns.ac.rs/wp-content/uploads/arhiva-savremena-poljoprivreda/2009Savremenapoljoprivreda12.pdf>.
- Gerilovych, A. P., Smietanka, K., Stegnyy, B. T. and Minta, Z. (2008) 'Comparative study of Highly pathogenic avian influenza strains isolated in Ukraine in 2006 and 2008', *Veterinary Medicine [Veterynarna medytsyna]*, 91, pp. 18–21.

Kungwani, P. (2014) 'Risk management — An analytical study', *IOSR Journal of Business and Management*, 16(3), pp. 83–89. doi: 10.9790/487x-16338389.

Muzyka, D., Pantin-Jackwood, M., Stegny, B., Rula, O., Bolotin, V., Stegny, A., Gerilovych, A., Shutchenko, P., Stegny, M., Koshelev, V., Maiorova, K., Tkachenko, S., Muzyka, N., Usova, L. and Afonso, C. L. (2014) 'Wild bird surveillance for avian Paramyxoviruses in the Azov-Black Sea region of Ukraine (2006 to 2011) reveals epidemiological connections with Europe and Africa', *Applied and Environmental Microbiology*, 80(17), pp. 5427–5438. doi: 10.1128/aem.00733-14.

Obukhovska, O., Stegny, B., Babkin, A., Ibatullin, I., Stegny, M., Shutchenko, P. and Orlov, S. (2014) 'Identification of false-positive results of serological tests of Brucellosis in cattle', *Brucellosis 2014 International Research Conference (Berlin, Germany, 9–12 September 2014): Abstracts*. p. 123. Available at: <http://www.bfr.bund.de/cm/349/brucellosis-2014-proceedings.pdf>.

OIE (World Organisation for Animal Health) (2016) *Terrestrial Animal Health Code*. (1-2 Vols). Paris: OIE. ISBN 9789295108011, 9789295108028. Available at: <http://www.oie.int/en/international-standard-setting/terrestrial-code/access-online/>.

Stegny, B., Obukhovska, O., Bashenko, M., Mandygra, A., Gerilovich, A. and Zavgorodny, A. (2015) 'Development

of Ukraine national standard for National *Brucella* strain collection maintenance in accordance with European requirements for biosafety and biosecurity', *18th Annual Conference of the European Biosafety Association (Vienna, Austria, 21–24 April 2015): Book of abstracts*. p. 112.

Stegny, B. T., Gerilovych, A. P. and Stegny, A. B. (2008) 'Molecular characterization of seed strains for bivalent vaccine against Newcastle disease and Highly pathogenic avian influenza', *Contemporary Agriculture: The Serbian Journal of Agricultural Sciences*, 57(3–4), pp. 9–14. Available at: <http://polj.uns.ac.rs/wp-content/uploads/arhiva-savremena-poljoprivreda/2008Savremenapoljoprivreda34.pdf>.

Sundqvist, B., Bengtsson, U. A., Wisselink, H. J., Peeters, B. P. H., Van Rotterdam, B., Kampert, E., Bereczky, S., Olsson, N. G. J., Szekely Björndal, Å., Zini, S., Allix, S. and Knutsson, R. (2013) 'Harmonization of European laboratory response networks by implementing CWA 15793: Use of a gap analysis and an "Insider" exercise as tools', *Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science*, 11(S1), pp. S36–S44. doi: 10.1089/bsp.2013.0020.

WTO (World Trade Organization) (1995) 'Agreement on the application of sanitary and phytosanitary measures (signed 15 April 1994, entered into force 1 January 1995)', *United Nations Treaty Series*, 1867, pp. 493–507. Available at: <https://treaties.un.org/doc/Publication/UNTS/Volume%201867/volume-1867-A-31874-English.pdf>.