

## Ukraine Biological Threat Reduction Program (BTRP) Cooperative Biological Research (CBR) Project

*Risk assessment of selected especially dangerous pathogens  
potentially carried by migratory birds over Ukraine*

UP-4 PROJECT OPTION YEAR 2 QUARTERLY REPORT  
for the period 31 October 2019 – 30 January 2020 (Q4)

Prepared for:



Prepared by:

BLACK & VEATCH SPECIAL PROJECTS CORP.



in collaboration with:



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## 1 Project Information

### 1.1 Task Order information

#### 1.1.1 Contract Number

HDTRA1-08-D-0007-0004

#### 1.1.2 Project Title

Biological Threat Reduction Program (BTRP)  
Cooperative Biological Engagement Program (CBEP)  
Phase IIb in Ukraine – HDTRA1-08-D-0007-0004

### 1.2 CBR information

#### 1.2.1 Project number

UP-4 Option Year 2 (OY2)

#### 1.2.2 Project title

Risk assessment of selected especially dangerous pathogens potentially carried by migratory birds over Ukraine

#### 1.2.3 Performance Period

Project Period of Performance – 31 January 2019 – 30 January 2020 (OY2)

Reporting Period of Performance 31 October 2019 – 30 January 2020 (OY2, Q4)

#### 1.2.4 Performing Organization

Black & Veatch Special Projects Corp. (BVSPC)

#### 1.2.5 Teaming Partner

Metabiota, Inc.

#### 1.2.6 Threat Reduction Impacts

- Develop risk maps of highly pathogenic avian influenza virus (HPAIV) and Newcastle disease virus (NDV), which are especially dangerous pathogens (EDPs) carried by wild birds and domestic poultry in Ukraine.
- Provide risk projections and track avian EDPs via Ukrainian and regional biosurveillance programs, with outputs reducing the economic risk posed to the regional poultry industry and offsetting the potential for spillover/trans-border infections in swine and humans.
- Further limit threat posed by these pathogens by developing sequencing capacity for avian EDPs at multiple Ukrainian Institutes, expanding the ability to identify and contain outbreaks.
- Enhance preparedness and response in Ukraine and the overall region by using project data and partner biosurveillance networks to inform public officials.

## 2 UP-4 Project Description

### 2.1 Project Title

**“Risk assessment of selected especially dangerous pathogens potentially carried by migratory birds over Ukraine”**

### 2.2 Research Objectives

#### 2.2.1 Problem Description

Especially dangerous pathogens (EDPs) that cause highly pathogenic avian influenza (HPAI) and Newcastle disease (ND) continue to threaten wild birds and poultry in Ukraine and other countries in Eurasia. There is continued risk of the emergence of HPAIV and NDV and of zoonotic spillover from infected wild birds into the commercial and backyard poultry populations of Ukraine. Avian EDP emergence is driven by the viruses’ intrinsic genetic and antigenic variability, which can lead to infection of new host species (host jumping). In addition, local and regional environmental impacts of agricultural activity may increase the potential for viral transmission in avian reservoir species, and into non-reservoir hosts, including domestic poultry [1-3].

In December 2016-March 2017, the H5N8 subtype of HPAIV re-emerged in Ukraine (Chernivtsi, Odesa, Mykolaiv, and Ternopil Oblasts) infecting wild birds (ducks, geese, and swans) and domestic poultry (chickens, ducks, and geese) with considerable lethality [4-8]. Although detailed epidemiological studies were not performed, estimates of attack rate were approximately 10% in domestic flocks, and over 1000 birds were counted dead – noting that official World Organization for Animal Health (OIE) reports were likely a vast underestimate of the outbreak’s scale (ref. 4-8 and UP-4 project scientists, unpublished observations).

During the first year of the UP-4 project (December 2016-November 2017), 84 positive samples for avian influenza virus (AIV) were detected by polymerase chain reaction (PCR), with 12 identified as H5 subtype. While sequencing is ongoing, it is possible that these isolates are HPAIV subtype H5N8 viruses brought from north-central Eurasia by wild bird migrations [9]. Samples were collected from wild waterfowl in Odesa and Kherson Oblasts in January, March, August, and September 2017. NDV was identified in 11 fecal samples.

The continual threat of the emergence of novel strains of EDPs has highlighted the acute need for analytical biosurveillance of avian species, and genotypic characterization of pathogens, by coordinated research and building of scientific resources in Ukraine and countries in the region.



### **2.2.2 Research Goals**

Comprised of a base year, Option Year 1 (OY1), and Option Year 2 (OY2), research project UP-4 aims to assess the ecologic, epizootic, and epidemiologic risk of infectious diseases transmitted by migratory birds associated with major flyways in Ukraine. The Quarter 4 (Q4) Report describes highlights of project implementation in UP-4 OY2, which encompasses the following Aims and Tasks:

**AIM 1. Conduct longitudinal biosurveillance of EDPs in migratory birds and domestic poultry at key interface sites in Ukraine and countries connected by migratory flyways.**

- Task 1.1. Longitudinal avian surveillance and detection of avian EDPs.
- Task 1.2. Data capture for data sharing and Avian Virus Risk Mapping analysis.
- Task 1.3. Tracking and analysis of bird migrations and transit of avian EDPs.
- Task 1.4. Engage regional partners through protocol and data sharing and workshops.

**AIM 2. Deploy PCR diagnostics, virus genome sequencing, and Geographic Information System (GIS) data sharing resources to build a collaborative Avian Virus Risk Map for Ukraine and countries in the region.**

- Task 2.1. PCR diagnostics and sequencing analysis of avian samples for AIV, NDV, and HPAIV.
- Task 2.2. Full genome sequencing of selected high-risk AIV and NDV strains.
- Task 2.3. Build an integrated, GIS-based Avian Virus Risk Map for Ukraine and the region.

### **2.2.3 Study areas**

The following administrative geographic regions were selected for these studies:

- Southern Ukraine:
  - Odesa Oblast (Tuzlovska group of estuaries and Danube River, lakes region that encompass various migration corridors, and Kiliiskyi rayon; where deaths of 1,099 heads of poultry were registered in January 2017 and HPAI H5N8 was confirmed by the State Scientific Research Institute of Laboratory Diagnostics and Veterinary Sanitary Expertise [SSRILDVSE] and reported to the OIE).
  - Kherson Oblast (Henichesk rayon, Kakhovka rayon, and Kalanchak rayon where HPAIV was detected and reported to the OIE in November and December 2016).
  - Moreover, HPAIV outbreaks were detected in Odesa and Kherson Oblasts in 2005-2006 (H5N1) and 2016-2017 (possibly H5N8; OIE, 2016). The National Scientific Center Institute of Experimental and

Clinical Veterinary Medicine (IECVM) isolated multiple low pathogenicity influenza viruses and NDV isolates in 2010-2016 at these locations.

- Northern Ukraine:
  - Chernihiv Oblast (Ripky rayon, Chernihiv rayon/Mena ornithological sanctuary, and Ichnia rayon, which are situated at the intersection of several migratory routes).
  - HPAIV outbreaks were detected in the Chernihiv region in 2016-2017 (possibly H5N8).

#### **2.2.4** Expected Impact

Through examination of the ecologic, epizootic, and epidemiologic risk of avian disease transmission from wildlife to poultry and humans, the UP-4 projects (base year, OY1, and OY2) will provide a foundation of knowledge permitting a more current and robust evaluation of risk focused on the most prevalent risk factors in Ukraine. In UP-4 OY2, research activities centered on Ukraine and regional partners, will advance ongoing efforts in longitudinal avian ecology analysis, biosafe sample collection, diagnostic capabilities, data management and analysis, and reporting. Data and database development will support the forecasting and contingency plan for the National Veterinary and Health Authorities of Ukraine concerning selected EDP risks in wildlife.

#### **2.2.5** Project Participants

- National Scientific Center Institute of the Experimental and Clinical Veterinary Medicine (IECVM)  
Address: 83 Pushkinska Street, 61023 Kharkiv, Ukraine  
Principal Investigator: Dr. Borys Stegnyy, Director
- The State Scientific Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE)  
Address: 30 Donetska Street, 03151 Kyiv, Ukraine  
Principal Investigator: Dr. Maryna Sapachova, Head of Department
- Ukrainian Anti-Plague Research Institute (UAPRI)  
Address: 2/4 Tserkovna Street, 65003 Odesa, Ukraine  
Principal Investigator: Dr. Oksana Yurchenko, Acting Head of Laboratory of Indication of Especially Dangerous Biological Pathogenic Agents
- Institute of Veterinary Medicine (IVM)  
Address: 30 Donetska Street, 03151 Kyiv, Ukraine  
Principal Investigator: Dr. Sergiy Nychyk, Director

- University of Alaska-Anchorage (UAA)/University of Alaska-Fairbanks (UAF)  
Address: 3211 Providence Drive, Anchorage, Alaska 99508-4614  
Subject Matter Expert (SME): Dr. Eric Bortz, Associate Professor, UAA  
Department of Biological Sciences

## 2.3 Technical Approach

### 2.3.1 Methodology

Expanded investigation of avian EDPs in UP-4 OY2 will continue long-term surveillance, detection, and risk analysis of AIV, HPAIV, and NDV using methods developed and implemented in the base-year study and OY1. Bird observations, viral detection data, and GIS methods are being used to analyze ecological impacts on virus prevalence and genotypes. Detailed ornithological observations will be conducted, enumerating the numbers, species, and migratory behavior of wild birds particularly in the mass gathering sites of wild birds in southern Ukraine during spring and fall migrations. Laboratory diagnostics will be based on PCR methods validated in the UP-4 base year. Based on UP-4 findings, diagnostic PCR will be expanded to include additional pathotyping primer sets for differentiating emerging HPAIV H5 and H7 strains and virulent NDV. Positive samples will be selected for nanopore sequencing of full genomes using the Oxford Nanopore Technologies (ONT) MinION platform.

An Avian Virus Risk Map will be designed based on biosurveillance, GIS data, host species, ornithological observations, migration data, virus genome sequences, PCR diagnostics and pathotyping, phylogenetics, and molecular structure analyses. These data will be layered with environmental data and analyzed in the ArcGIS and QGIS platforms using geostatistical density mapping, and ML algorithms [10].

Biosurveillance in specific regions of Ukraine will provide a third year of longitudinal data for analytical mapping of EDP incidence in wild birds and interfaces with domestic poultry. Ultimately, these biosurveillance efforts will facilitate development of rapid disease reporting and biosecurity control strategies, which will contribute to limiting the spread of avian viruses in wild birds as well as the spillover and transmission of AIV and NDV among domestic poultry.

### 2.3.2 Description of Technical Approach

Technical approaches and methodology remain the same as the project's base year and act synergistically across all major Tasks. Principally, biosurveillance of avian EDPs will entail expert ornithological analysis, environmental (feces) sample collection, and PCR diagnostics for AIV, HPAIV, and NDV. These activities, conducted in specific regions of Ukraine, will provide a third year of longitudinal data for analytical mapping of EDP incidence in wild birds and interfaces with



domestic poultry. Additional pilot projects will function as data multipliers, adding depth to this study through:

- Analysis of special bird populations: Colony birds, waders, and poultry
- Virus genotyping by full genome virus sequencing and annotation
- A workshop for avian data analysis, including: Regional data sharing, GIS methods, and live capture bird sampling and migration tagging
- Inclusion of concomitant data collection by international partners

Analyses will then be integrated collaboratively to share data as an Avian Virus Risk Map for Ukraine and the region and published in open access data repositories.

Sample collection, diagnostic testing, sample characterization, genotyping, GIS mapping, data reporting, and other aspects of project work will continue in OY2 according to specific protocols developed within the project's base year and OY1. The protocols were standardized and organized according to the type of field and laboratory work conducted and shared with all institutions involved in the project.

All sampling is being conducted according to established biosafety and biosecurity standards and practices affirmed by regulatory and scientific stakeholders in Ukraine with consideration given to the ecologic-epizootic risk of disease as ascertained through current and historical monitoring of bird populations and avian diseases. Collection of cloacal swabs from live birds will be conducted according to the corresponding biosafety standard operating procedures (SOPs) and Animal Care and Use Committee (IACUC) approval process.

UP-4 scientists will continue diagnostic testing for HPAIV and NDV using quantitative real-time reverse transcription PCR (qRT-PCR) and standardized protocols developed in the UP-4 project. Employment of GIS and modeling technologies will enable spatial representation of the distribution of the studied infections and their causative agents in the defined areas in Ukraine.





**Fig. 16. A preliminary Avian Virus Risk Map for Ukraine. Sites of sampling and a recent (January 2020) HPAIV outbreak in Vinnitsyia are indicated.**

### 2.7.2 Summary of Technical Accomplishments for this Period.

- The ornithological situation in southern and northern Ukraine was estimated, considering weather conditions from November 2019 – January 2020.
- Laboratory testing (PCR and virus genome sequencing) was improved, with full genome sequencing of 3 viruses by MinION in Ukraine (bioinformatics analysis of data is ongoing).
- Field expeditions, with annotated GIS mapping of sampling sites, species identification, and metadata recording, resulted in collection of 991 specimens from wild birds.
- Detailed analysis was performed for 4 samples from UP-4 OY2 MinION sequencing data generated in Ukraine.