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HEALTH AND FOOD SAFETY DIRECTORATE-GENERAL

Food chain stakeholder and international relations
Food safety programme, emergency funding

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REPORT OF THE

"African swine fever"
TASK FORCE SUB-GROUP

Meeting held in

Kiev

Ukraine

1-4 August 2016

REPORT OF THE

MEETING OF THE AFRICAN SWINE FEVER SUB-GROUP OF THE

TASK FORCE FOR MONITORING DISEASE ERADICATION

HELD IN KIEV, Ukraine, 1-4 AUGUST 2016

PARTICIPANTS: see Annex I

AGENDA: see Annex II

LOCATION: Ukrainian State Service on Food Safety and Consumer Protection, Hrinchenko str., Kiev 01001 UKRAINE

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1. INTRODUCTION - Objectives of the EU-Task Force sub-group.

The scope of the visit is to share information and experience of the expert members with the hosting colleagues as well as to give technical support if needed or requested by the visited country. After the visit a report is issued by the experts, based on the information provided on the spot by the country and on the findings verified directly by the experts themselves during the visit.
The main goal of the Task Force is to contribute through expertise and an external independent technical assessment, to the evaluation of strength and weaknesses of strategies and measures in place in the visited country for the control and eradication of the disease concerned.
Conclusions and recommendations are formulated from a general point of view and are proposed in the report with the main purpose to offer a basis for the Veterinary Services of the visited country to reflect on the possible improvement of different aspects of the control and eradication programme for the disease concerned. The country visited may amend the programme according to what is suggested by the Task Force, or it may choose other approaches, also in consideration of social and economic factors that may influence the success of the measures adopted, and which are not in the remit of the Task Force.
Conclusions and recommendations are related to the situation during the visit based on the information provided by the country visited on the spot. Further developments of the country's situation may be the subject of a following visit aimed to get updated information and new feedback from the Veterinary Services.

The reports of the TF are published on the following website: http://ec.europa.eu/dgs/health_food-safety/funding/cff/animal_health/vet_progs_en.htm

2. Report of the meeting of the ASF Task force held in Kiev.

The scope of the meeting was:

- To discuss with UA veterinary service their ASF eradication programme and suggest further amendments in order to revise the strategy in place to stop the spread of the disease and to control it, to ensure also the protection of the EU territory and

- Assess the capacity of UA national reference laboratory to better understand the detailed investments requested by UA for EU funding.

The main findings, conclusions and recommendations are reported below.

2.1. Domestic pigs

Ukraine has about 7.6 million of pigs, 3.75 million of them are kept in commercial enterprises that are registered by the veterinary service, the remaining (about 3.85 million) are in private households. This last data is based on official statistics, collected at territorial level by the local Authority. Criteria used to differentiate commercial holdings from private households have not been clearly explained. Pig movements in backyards are not registered.

2.1.1. Epidemiological background

Since 2012, 78 ASF cases (total in domestic pigs and wild boar) have been reported, the outbreaks can be grouped in four main clusters of infection. In 2016, the ASF epidemiological situation in Ukraine has worsened and the geographical area of spread has enlarged: the disease has been reported in the North, South and West part of the country; up to now 14 out of 25 oblasts have been affected by ASF. Up to August 2016, 17 cases (9 in backyards, 6 in commercial farms and 2 in wild boar) have already been reported and, at the time of the visit, new cases have been confirmed.

A risk assessment to determine the main ASF risks for domestic pigs and wild boar has not been conducted. However, low biosecurity in backyards, illegal sales (sometimes also in illegal markets) and human factors are considered to be the main risk factors for the introduction and spread of ASF. It was also stated, that the occurrence of the disease was most probably linked to vehicles (trucks, cars) which are on transit through Ukraine coming from neighbouring countries. However, this hypothesis is not supported by the current geographical spreading pattern of the disease.

2.1.2. Surveillance in domestic pigs

The present ASF surveillance regime for domestic pigs is set up by the Central Veterinary Administration based on the inputs received from the Oblast and Rayon.
Surveillance in backyards relies on disease reporting whilst in the commercial holdings, the surveillance program foresees:
1) passive surveillance,
2) bio-security checks (4-year),
3) active surveillance: serological testing. In case of active surveillance, no specific indication on which animals have to be sampled is given, however in case sick or dead animals are found, 10% of them should be checked and tested for ASF.
It was reported that in 2016, 17.000 samples were tested for ASF, about half of them because of passive surveillance.
There is a system in place for the daily and monthly diseases reporting, which occurs by telephone, from the field to the Central Administration.

2.1.3. Principles of ASF control in domestic pigs

Control and eradication measures for ASF in Ukraine are based on the following principles:

- The place where a case or outbreak of ASF has been confirmed is declared as outbreak centre**.** It can be a backyard holding, an entire village, or a commercial farm. Within the outbreak centre all pigs are culled followed by cleaning and disinfection.

- Around the outbreak centre a protection zone is established. The radius of this zone can vary between 3 and 20 – 30km. All pigs within this zone will be slaughtered, no movement of pigs out or into the zone is allowed. Commercial farms which are located in the protection zone can be excluded from slaughtering.
- Around the protection zone a surveillance zone is established. The radius of this zone can vary between 20 and 150 km. No movement of pigs out or into the zone is allowed without veterinary authorization.
- Pigs in the restricted zones (protection and surveillance) are not tested or clinical checked for ASF.

2.1.4. Awareness campaigns

Awareness campaigns were organized at field level by the National Laboratory, it included simulations and pig operators were involved. The awareness campaign was part of a project on early detection, carried out in Ukraine in the period 2013 – 2015. The project was financially supported by the Food and Agriculture Organisation of the United Nations (FAO).

2.1.5. Conclusions and recommendations.

Surveillance:

- The ASF surveillance currently in place in Ukraine is not based on scientific ground. Therefore, the surveillance results for domestic pigs and wild boar, most probably do not reflect the real epidemiological situation (particularly in the backyards)
- The current level of active surveillance is so low that the early detection of ASF virus will fail.
- It would be more profitable if the active surveillance would be replaced by an effective passive surveillance based on a well structured dead animal reporting system fully implemented in all the pig breeding systems, including the back yard.

Disease control:

- The proportionality and the effectiveness of the eradication measures conducted within the protection and surveillance zones should be re-evaluated, taking into consideration the epidemiological particularities of the ASF and risk patterns.
- Control tools already available should be used by the veterinary services for surveillance and control purposes (GIS).

- Recommendations provided by the World Organisation for Animal Health (OIE), FAO and the Global Framework for the Progressive Control of Transboundary Animal Diseases (GF-TADs)'s expert mission team, carried out in September 2015, have not been implemented.

- To be able to realize an effective eradication strategy against ASF it is necessary that Ukrainian Veterinary Services work to implement and/or strengthen the following areas:
  - Epidemiological surveillance;
  - Disease control;
  - Animal identification system;
  - Traceability system;
  - Animal movement control system;
  - Communication of epidemiological information;
  - Inspection and certification;

- All pig holdings should be registered and since backyards are playing a role in the current ASF epidemic in Ukraine, under the local circumstances, they are representing a serious risk for the spread of ASF. Taking also into consideration that they represent more than half of the pig population of the Country. Therefore, the legal base should be established into the National Legislation to ensure also for backyards:
  1) registration/identification,
  2) animal movement control and
  3) traceability.

2.2. Wild boar population.

Ukraine with its estimated 44796 wild boars (official census dated 28 February 2016) has a rather low wild boar population density. The species is mainly distributed in the forested Northern and Western Oblasts of the country. In the South and South-East part of the Country, the wild boar population density is lower and fragmented. The wild boar hunting season lasts from August to the end of January. Wild boar population census is carried out in February using one or more unstandardized methods. Each hunting ground conducts its own census and reports to the Oblast Forestry authorities and to the national level. This statistics is then used to calculate a hunting ground specific hunting bag limits. According to size and habitat characteristics of each hunting ground, a minimum and optimal number of wild boars are determined. State Forestry Committee regulates wild boar hunting bag across all the hunting grounds irrespective of their ownership. No hunting is allowed if population density is below 0.3 head/km2 (3/1000 ha). When this threshold is reached, hunters are normally allowed to take no more than the estimated annual increase rate, usually 20-30 % of the population size estimated in February. In most of the country the wild boar population density is below the 0.3 head/km2, thus relatively few hunting grounds are allowed to hunt wild boar and mainly in the North and West of the country. A proportion of hunting grounds have been privatized (either by a single persons or by hunting clubs), while the remaining areas are owned and managed by the state and NGOs (usually the local members of Ukrainian Hunting and Fishing Association). Such a situation results in evident differences in several management activities including reliability of censuses, artificial winter-feeding strategies, collection of the hunting bag data etc., and finally in ASF surveillance. The competent authorities reported at least 20% of poached animals.

2.2.1. ASF early detection and surveillance.

Due to the very low number of wild boars found dead (about 10 during 2016 but specific data are lacking) ASF detection is mainly based on active surveillance. Few dead animals, all ASF positive, have been found across the Country despite the fact that the sole tested representative sample highlighted a 2,5% virus prevalence (Kiev region/oblast, Borovary district). Passive surveillance is poorly implemented since the usual wild boar hunting regime will be disrupted once ASF is detected. In the ASF at risk oblasts all the hunted wild boars have to be tested whereas 10% of the hunted wild boars are tested in the remaining areas. In the infected hunting grounds, culled domestic animals are not tested so that virus prevalence is not estimated. In 2015 about 4000 out of 13663 hunted wild boars have been sampled. The efficiency of the active surveillance is limited due to the absence of an efficient early detection strategy, difficulties in taking samples, dispatching them to the laboratories and lack of reagents. The reported data do not distinguish found dead from hunted wild boars and ASF cases are reported together with outbreaks in domestic pigs. As a result, the real epidemiological situation of ASF in wild boar cannot be assessed quantitatively. At present

it is still not clear if the virus is endemic in the wild boar populations or the detected cases are isolated epiphenomenon from domestic pigs.

2.2.2. ASF management in wild boars.

There is no a clear procedure to design the wild boar infected area. Usually the infected area overlaps the hunting ground in which ASF virus has been detected. The Country adopts the wild boar "depopulation" policy. The policy is applied mainly at the level of the infected hunting ground. When ASF is detected in a hunting ground, all the wild boars have to be culled. However legislative discrepancies are evident: the hunting legislation considers "depopulation" as the minimum optimal wild boar density for a specific hunting ground; the "Anti-epizootic Commission" defines "depopulation" as the culling of the whole wild boar population in the infected hunting ground. As a matter of fact, depopulation is hardly achievable and rarely attempted.

- ***Biosecurity during hunting***

Biosecurity measures during hunting are poorly implemented. In infected hunting grounds, biosecurity measures are not applied since all the culled animals are considered infected and thus disposed. In ASF free hunting grounds, due to the absence of the virus, biosecurity measures are not implemented.

- ***Wild boar feeding***

Artificial wild boar feeding is authorized and largely practiced.

2.2.3. Conclusions and Recommendations.

- The reporting system should clearly distinguish wild boar cases from domestic pigs outbreaks. It should be clearly reported if wild boar cases have been detected in found dead wild boar or in hunted animals.
- Passive surveillance in wild boar should be enhanced on both infected and risk areas. It is recommended to increase the level of passive surveillance in infected areas and in areas at risk of being infected in both domestic pig and wild boar.
- Active surveillance in at risk or free areas has a very low probability of detecting the virus, despite any sampling effort. Collecting samples of 10% of shot animals is meaningless for any purpose (early detection or surveillance).
- Active surveillance in infected areas, based on testing all shot animals, should be carried out (both Antigen and antibodies detection), virus prevalence should be accurately estimated.
- In case of infection in wild boar, the infected area should not be established based on administrative borders (hunting grounds, rayon etc.) but considering the epidemiological situation, habitat characteristics, and the geographical distribution of the wild boar.
- The aim of "wild boar depopulation", including its technical meaning should be clearly defined (EG: density per km^2 etc.).
- Due to the reported difficulties in depopulating infected hunting grounds, alternative strategies, country tailored, aimed in eradicating ASF in wild boar populations should be explored.
- In wild boar infected areas, wild boar management has to take into account the minimum biosecurity measures in order to avoid the possible further spread of the virus through contaminated material.

- In ASF infected hunting ground winter-feeding should be minimized and exclusively aimed in attracting wild boar to the shooting spots.

3. Visit to the Ukraine National reference laboratory for African swine fever.

3.1. Description of the laboratory facilities, management and main findings.

A visit to the National Reference Laboratory (NRL) for ASF located at the State Scientific and Research Institute of Laboratory Diagnostics and Veterinary Sanitary Expertise, in Kiev was carried out on 3th August, 2016. Brief presentations were given by the Director of the Institute (Julia Novojutshay),by the Deputy Director for Scientific Support of Diagnostics of Animal Infections Diseases, and by the Head of the Research Department of virologicals (Oleg Nevolko).

A visit to the facilities was conducted by the Deputy Director, Head of Diagnostic Department (Oleg Nevolko) and the Head of the PCR Department (Lyudmila Maruschak). Different issues were reviewed concerning the facilities, available rooms for ASF diagnosis, personnel resources, laboratory equipment, consumables and reagents, ASF diagnostic techniques, sampling management and traceability, and quality management, throughout the review of the following aspects:

a) Infrastructure, equipment, consumables and personal resources;
b) ASF diagnosis;
c) Quality management system with regards to ASF diagnosis;
d) Diagnostic laboratory network at national level.

Before the visit, a questionnaire prepared by the European Union National Reference Laboratory for ASF (EURL, INIA-CISA), was sent to the Veterinary services and filled out by the Ukraine´s ASF NRL (see annex III). It aimed to better identify potential gaps and needs that could have an impact on the reliability and timeliness of the ASF diagnosis.

a) Infrastructure: Organization, equipment, consumables and personal resources.

The National Reference Laboratory for ASF doesn´t use a specific space for ASF topics. ASF diagnosis is done in the same area as several others infectious diseases. It comprises several laboratories at the Biosafety level 2 facilities, involving two main departments:

The Department in charge of molecular techniques (PCR-polimerase chain reaction Department) with the suitable equipment and material to conduct routine PCR assays (conventional and real time PCR). A correct workflow is followed in the molecular laboratory which minimizes contamination and ensures good laboratory practices with pre- and post-PCR areas physically separated. Four qualified staff people are directly involved in performing the PCR tests, including sampling preparation, nucleic acid extraction and amplification. Characterization of selected virus isolates following the international standards is also performed.

The Virology Department is in charge of antibody detection techniques as well as virus detection techniques based on the detection of the viral antigen. The department includes ten members of staff, two of them with enough expertise on ASF antigen and serological detection techniques, including serological confirmatory techniques, immunoblotting and indirect immunoperoxidase test. The overall staff is also dealing with the diagnosis for other infectious diseases. The

department has good general infrastructure and appropriate facilities for diagnosis and it is well organized in terms of workflow.

Both departments are supported by a registration service in charge of reception, registration and monitoring traceability of the samples received. An identification code is attributed to the samples at entrance on the basis of the diagnostic requested, assuring the traceability of samples. A professional software is being introduced - Laboratory Information System (LIMS - Laboratory Information Management System).

Due to the lack of a specific biosecurity area (BSL3), it is not possible to perform virus isolation. Suitable equipment (such as several laminar flow cabins, one freezer, spectrophotometer, a PCR equipment and a sequencer (ABI3130), in certain cases of around 10 years old, and a limited amount of consumables to perform routine assays, is currently available .

To be noted that there is only one old real time PCR equipment (Rotor Gene 300, 36 samples per round, from 2006) and if the PCR equipment fails, the ASF diagnosis by PCR will be not possible and the conventional PCR that shows a limited sensitivity for the detection of genotype II ASFV (the one currently circulating in eastern Europe) will be not useful.

Some other equipment is quite old, has some operational problems or doesn´t even exist and needs replacement (such as a freezer and a fridge, equipment for DNA extraction, microcentrifuge for eppendorf tubes, or vortex for shaking tubes, and single chanel pippetes Pipetboy Acu or equivalent for sample preparation, multichannel pippetes, and a tissue homogenator).

Provision of kits for virus and antibody detection have not been provided in 2016 and reagents (such as primers, DNA extraction kits, reagents, and PCR kits) are provided in very low quantity and this would negatively affect the diagnosis of ASF. The funds allocated by the State for the laboratory in 2016 have not been provided.

Procurement of consumables, new staff for contract or new equipment seems to be not a problem (available in one/ three months for equipment). Maintenance of equipment is well organized by the companies.

b) ASF diagnosis

Expertise and diagnostic techniques: The NRL has qualified expertise and staff is well trained to use international validated techniques, as shown by the satisfactory results obtained annually in the Annual Inter-laboratory Comparison Tests (ILCT, prepared by the EURL). The NRL usually uses commercial kits (PCR, Antigen ELISA, ELISA for Antibody detection, and serological confirmatory tests Immunoblotting-IB- and Indirect Immunofluorescence –IPT). The use of the appropriate reagents and control reference sera for both ELISA and IPT tests (provided by the EURL), ensure the confident diagnosis of the disease by antibody detection.

Samples currently analyzed at the NRL: The samples sent to the NRL for suspicion or surveillance are mainly spleen, lymph nodes and sometimes also bones (for analysis of bone marrow), as only dead animals are analyzed. No serum samples are sent to NRL.

Current ASF diagnosis: The laboratory has performed around 3000 virus detection analysis by PCR. out of the total of around 16-17.000 annual analysis performed (the rest is analyzed by the Regional

laboratories by antigen detection technique, FAT). Analysis of samples by antibody detection is not performed, due to a lack of serum samples and of the commercial kits for antibody detection.
ASF virus detection by PCR, currently uses the OIE conventional PCR and a commercial real time PCR the LSI VetMAX African Swine Fever Virus Detection Kit developed by Thermo Fisher.

In addition, the direct immunofluorescence technique (FAT), is performed at the NRL.

c) Quality management system with regards to ASF diagnosis.

All the techniques for virus and antibody detection, including those for serological confirmation are accredited by the National Accreditation Agency in line with the ISO/IEC/17025. Quality management system, internal and external control already exists.

d) Diagnostic laboratory network at national level.

There is a well-established laboratory network at national level, that includes 21 regional laboratories located, and three branches- NRL filial, in 24 regions (Oblasts). These laboratories perform solely the antigen detection technique by direct immunofluorescence (FAT technique) in organs (spleen and lymph nodes, and bone marrow). The Regionals doesn´t perform analysis by antibody detection techniques. The NRL is responsible for advising and training on this technique, and supervise the ASF diagnostic expertise of the regional laboratories. Regionals send suspicious cases to the NRL for confirmation. To date, 79 outbreaks (in wild boar, and domestic pigs) have been confirmed at the NRL since 2012.

3.2. Conclusions and Recommendations.

- The NRL has appropriate facilities for diagnosis, qualified staff, validated ASF techniques and procedures, and use international reference standards and material to perform a correct ASF diagnosis. However;

    - Only old PCR equipment is allocated at the NRL for PCR virus detection and they don´t perform antibody detection techniques due to a lack of serum samples to be analyzed and the lack of kits for antibody detection.
    - Regionals only use FAT technique for antigen detection that has a very limited sensitivity unless antibody detection is carried out in parallel. Therefore some important information is being lost, for a reliable control of the disease.
    - With the current strategy of ASF diagnosis performance, both at NRL and at Regionals laboratories, it is not possible to get a confident diagnosis and the information required to have the real picture of the epidemiological situation of the country.
    - It is not possible to perform virus isolation to confirm virus presence, due to the lack of a BLS3 area.
    - Funds allocated for purchase diagnostic kits and consumables were not provided this year by the State. As a consequence, the NRL will not be able to perform the requested ASF diagnosis in autumn, due to a lack of consumables and reagents for PCR.

- Due to the current situation, the increase of laboratories performing diagnosis by virus detection techniques by PCR should be reinforced. It could be performed by extending the PCR test to at least three or four strategic Regional laboratories. These regional should be

located in/near of the affected oblast at least in the north, south, and west territories to avoid long distance transportation to the NRL.

- To incorporate ASF antibody detection techniques at the NRL as well as at the Regional laboratories. Support and reinforce of a consistent surveillance in all the regional laboratories doing FAT (antigen detection technique) by implementation of antibody detection techniques by ELISA in parallel to FAT for each animal analyzed. It will require simultaneously the collection of serum and blood (in case of wildboar or dead animals) samples for analysis.

- A specific training in antibody detection techniques should be carried out by the Ukraine´s NRL for the Regionals lab staff: it is recommended to attend, previously, a training course for trainers at the EURL for ASF at INIA-CISA, Valdeolmos, Madrid (Spain). In this course at least two people from the NRL with expertise in techniques for ASF antibody detection would be trained for the use of different type of samples such as blood or exudates by its analysis in antibody detection techniques, including OIE "in house" techniques and commercial kits, giving advice about the sensitivity of false positive and negative reactions and a good interpretation of results.

- Reagents for ASF diagnosis, consumables, and equipment is in small quantity or lacking at all and should be provided to perform the ASF diagnosis and go ahead for a reliable diagnosis. Particularly, a new PCR equipment for the NRL is required. A fail in the solely currently old existing could jeopardize the ASF diagnosis.

- A list of reagents, consumables and equipment could be prepared and provided by the EURL, in collaboration with the NRL.

- A new diagnostic strategy should be implemented at the NRL and at the Regionals. However the specific strategy to be implemented will be dependent on the surveillance program in place and the possibility for the provision of enough financial resources for reagents, commercial kits, consumables and equipment at NRL and at the Regionals.

- Characterization of virus circulating in the affected regions (Oblasts) should continue while possible and the EURL for ASF is ready to co-operate.

A warm thank you is extended to the Ukrainian hosts for their great hospitality and willingness to share information. The effort of arranging this meeting is greatly appreciated.

Annex I

MEETING OF THE AFRICAN SWINE FEVER SUB-GROUP OF THE TASK FORCE FOR MONITORING DISEASE ERADICATION HELD IN KIEV, Ukraine, 1-4 AUGUST 2016

PARTICIPANTS

Task Force African swine fever Sub-Group - members

- Dr. Marisa Arias (ASF EURL) Spain
- Dr Bellini Silvia (IZSLER) Italy
- Dr. Vittorio Guberti (ISPRA) Italy

European Commission

- Dr. Valentina PIAZZA Deputy Head of Unit D4 "Food safety programme, emergency funding": (DG SANTE)

Country Representatives (main list)

Ukrainian State Service on Food Safety and Consumer Protection

- Mr Volodymyr Lapa - Head
- Mr Mykola Bilous - Acting Director of Department for Food Safety and Veterinary;
- Mr Oleksii Klymenok - Chief of Unit foe Animal Health and Welfare;
- Ms Olga Shevchenko - Chief of Directorate for International Cooperation.

National Central Laboratory:

- Mrs Julia Novozhytska - First Deputy Director;
- Mr Oleg Nevolko - Deputy Director.

National University of Ukraine on Life and Environmental Sciences:

- Mr Volodymyr Polischuk - National Consultant of FAO, epizootologist

State Agency on Forestry Resources of Ukraine:

-Mr Andrii Shelepylo - Deputy Chief of Unit on Hunting Grounds and Chasing.

Annex II

MEETING OF THE AFRICAN SWINE FEVER SUB-GROUP OF THE

TASK FORCE FOR MONITORING DISEASE ERADICATION

HELD IN KIEV, Ukraine, 1-4 AUGUST 2016

AGENDA

№	Object	Time	
	01-08.2016		
Arrival of TF experts and EU representative			
	02.08.2016		
1	**Venue of meeting:** Ukrainian State Service on Food Safety and Consumer Protection, Hrinchenko str., Kiev 01001 UKRAINE Welcome by the UA CA Introduction by EU representative on the role of the TF and scope of the meeting	09:00-10:00	
2	Presentation by UA (central, regional and local CA) on the ASF situation, on the measures implemented-under implementation to combat ASF in domestic pigs. Provide data. Special attention should be devoted to the measures implemented at the border with EU. ..,	10:00-13:00	
3	Lunch	13.00-14.00	
4	Presentation by UA (central, regional and local CA) on the ASF situation, on the measures implemented-under implementation to combat ASF in wildlife. Data and maps to be provided.. Presence of hunting association representatives/forestry services is recommended in order to discuss also their specific involvement.	14.00-18.00	
	03.098.2016		
	Visit of the National laboratory Explanation from their side on how the diagnosis is organized (central-regional level) Explanation of their needs	09:00-18.00	
	04.08.2016		
1	Meeting of the TF experts (only) to discuss the draft ASF programme and to prepare conclusions and recommendations of the meeting to be presented to UA CA.	09:00-13.00	
	Lunch	13.00-14.00	
2	Presentation of the draft programme and of the main conclusions and recommendations of the meeting to the AU CA	14.00-16.00	
3	Discussion and end of the meeting.	16:00-17:00	

ANNEX III

QUESTIONAIRE,
(As general rule answer the questions in tables with "X")

1.-What is the maximum number of samplesyour laboratory could be tested for ASF diagnosis per week at the NRL laboratory?
<100
100-200
200-500 **X**
500-1000
>1000
Comments:

2.-What is the maximum number of analysis by PCR detection techniques that could be tested each two working days at your NRL laboratory?
<100
100-200 **X**
200-500
500-1000
Other:
Comments:

3.-What is the maximum number of serological analysis (including confirmatory testing) that could be tested eachtwo working daysat your NRL laboratory?
<100
100-200 **X**
200-500
500-1000
Other:
Comments:

4.-INFRAESTRUCTURE.
Which of the following elements are currently available in your NRL laboratory? If it not available, how long will it take to implement it? Indicate an answer to every question:

QUESTION	It is currently available	1-2 weeks	1 month	3 months	6 months	>1 year
Administrative area	X					
Physical area for sample reception	X					
Physical laboratory for sample preparation	X					
Physical laboratory for molecular diagnosis	X					
Physical laboratory for	X					

serological diagnosis						
Laboratory biosafety office						X
Storage facility for i.e. consumables, samples, reagents, etc						X

Comments:

Due to the limited area of laboratory premises there is no separate room for the Office of Biosecurity and storage of suppliers, samples, reagents at the National Reference Laboratory. Their presence in the future is only possible in case of reconstruction or constructs new facilities.

5.-EQUIPMENT

Which of the following elements are currently available in your NRL laboratory? If not available, how long will it take to implement it? Indicate an answer to every question:

QUESTION	It is currently available	1-2 weeks	1 month	3 months	6 months	>1 year
Enough administrative equipment (i.e. phone, fax, computer, etc)	X					
Appropriated standardized equipment for sample preparation	X					
Appropriated standardized equipment for ASFV molecular diagnosis	X					
Appropriated standardized equipment for ASF serological diagnosis	X					
Appropriated equipment for sample storage (i.e. fridge, freezer, etc)	X					
Appropriated cleaning, disinfection and sterilization equipment	X					
Appropriated equipment for safety and waste management						X
Policy for laboratory equipment: procurement, maintenance and replacement	X					

Comments:

Additionally there is a necessity to purchase:

1. Amplifier for real time PCR (present a significant physical and the load is no backup equipment in case of failure of the existing)
2. Equipment for automatic secretion of nucleic acids
3. Spectrophotometer (nanodrop)
4. Freezer -80 ˚□C, volume 400 l
5. Disposer for medicinal wastes, crematorium (for organic wastes)
6. Update the organization equipment (printers, computers)

6.-PERSONNEL:

Which of the following elementsare currently available in your NRL laboratory? If not available, how long will it take to recruit specific trained personnel to manage the current ASF situation ?Indicate an answer to every question:

QUESTION	It is currently available	1-2 weeks	1 month	3 months	6 months	>1 year
Enough administrative staff (i.e, purchasing, suppliers contact, etc.)	X					

Enough staff for sample reception and registration	X					
Enough staff for sample preparation	X					
Enough staff for ASFV molecular diagnosis	X					
Enough staff for ASF serological diagnosis	X					
Enough biosafety staff						X
Enough staff for cleaning, desinfection and sterilization						X
Staff for reporting the results	X					
Personnel replacement						X

Comments:

1. In the staff list of the Unit for molecular and genetic studies introduce the position of sanitary specialist

7.-SAMPLE MANAGEMENT

a)Which of the following elements are currently available in your NRL laboratory?andb)Which of the following points you consider are more important for a reliable ASF diagnosis (Indicate a number 1 low ; 2 medium low; 3 medium; 4 quite important; fundamental/great important), you can give a value from 1 to 5 to each one of the questions:

QUESTION	It is currently available	1	2	3	4	5
Availability of sample submission form	X				X	
Standard operating procedure (SOP) for sample processing	X					X
Guidelines for an appropiated trazability of the samples	X				X	
Guidelines for sample analysis priorization	X					X

Comments:

8.-CONSUMABLES AND REAGENTS FOR ASF DIAGNOSIS

Which of the following items are currently available in your laboratory? If not available, how long will it take to implement it? Indicate an answer to every question:

QUESTIONS	It is currently available	1-2 weeks	1 month	3 months	6 months	>1 year
Availability of validated kits and/or reagents for molecular diagnosis	X					
Availability of validated kits and/or reagents for antibody detection (ELISA)				X		
Availability of validated kits and/or reagents for antibody detection (confirmatory test)	X					
Availability of international ASF reference standards.	X					
Availability of consumables and reagents (stock-keeping)	X					
Availability of suppliers management system (suppliers list)	X					
Laboratory procurement (timely distribution) for the	X					

maintenance of appropriated stock of reagents/consumables						

Comments:

1. Due to the absence of the state funding currently there is no sets of reagents for the detection of antibodies using ELISA

9.-METHODS AND PROCEDURES FOR ASF DIAGNOSIS

Which of the following elements and tests are currently available in your NRL laboratory? If not available, how long will it take to implement it? Indicate an answer to every question:

QUESTIONS	It is currently available	1-2 weeks	1 month	3 months	6 months	>1 year
Availability of validated standard operating procedures (SOPs)	X					
Validated ASFV molecular detection test	X					
Validated ASF antibody detection test (ELISA)						
Validated ASF antibody detection test (confirmatory test)	X					
Validated CSF/ASF diiferential diagnosis test	X					
Workflow for ASF diagnosis	X					
Guidelines for the interpretation of the results	X					

Comments:

1. Due to the absence of the state funding currently there is no sets of reagents for the detection of antibodies using ELISA. Upon the condition of financing it is possible within 2 months (3 weeks for purchase, 30-45 days for delivery).

10.-REPORTING SYSTEM

Which of the following elements are currently available in your NRL laboratory? If not available, how long will it take to implement it? Indicate an answer to every question:

QUESTIONS	It is currently available	1-2 weeks	1 month	3 months	6 months	>1 year
Defined flow chart depicting the process from arrival of the samples until diagnosis including reception, preparation, testing and reporting result	X				X	
Guidelines to report the results. Information to the veterinary authorities	X					
Database, standard record-keeping system, recording and reporting tools	X					

Comments:

For managing the laboratory workflows and documents the professional software is being introduced to work - Laboratory Information System (LIMS - Laboratory Information Management System) "Bravo-Lab"

11.-QUALITY MANAGEMENT SYSTEM

Which of the following elements are currently available in your NRL laboratory? If not available, how long will it take to implement it? Indicate an answer to every question:

QUESTIONS	It is currently available	1-2 weeks	1 month	3 months	6 months	>1 year

Periodic internal verification of all laboratory procedures	X					
Periodic external quality control (international interlaboratory comparison test)	X					
Accreditation status for ASFV molecular diagnosis test (national or international bodies)	X					
Accreditation status for ASF antibody detection test, ELISA (national or international bodies)	X					
Accreditation status for ASF antibody detection test, confirmatory test (national or international bodies)	X					

Comments:

We have accreditation by ELISA method -in German accreditation body DAAKS of 30.04.2013r 20.04.2015-; the National Accreditation Agency of Ukraine (NAAU) have accreditation from 21.03.2014

Using PCR - in NAAU from 09.02.2015

12.-REGIONAL LABORATORIES

- a)Describe how many Regional Laboratories are currently working in the ASF control programme? Name, and location.

Diagnosis at the regional level is carried out by conducting Fluorescent antibody method - all regional veterinary laboratories and branches of the State Research Institute of Laboratory Diagnostics and Veterinary Expertise

If ELISA diagnostic kits for the detection of antibodies are available such kind of tests can be conducted by all regional labs and branches of the State Research Institute of Laboratory Diagnostics and Veterinary Expertise.

Vinnytsia region	**Вінницька регіональна** ***державна лабораторія ветеринарної медицини***	м. Вінниця,вул. Максимовича, 19, 21036
Volyn region	***Державна установа*** **«Волинська регіональна** ***державна лабораторія ветеринарноїмедицини»,***	м. Луцьк, вул. Поліська Січ, 12, 43020
Dnipro region	**Дніпропетровська регіональна** ***державна лабораторія ветеринарної медицини,***	м. Дніпропетровськ, пр. Кірова, 48, 49054
Donetsk region	**Маріупольська міська** ***державна лікарня ветеринарної медицини***	м.Маріуполь Донецька обл. вул. Гризодубової , 3 87518

Zhytomyr region	**Житомирська регіональна *державна лабораторія ветеринарної медицини*,**	м. Житомир, вул.Коростишівська,54 10007
Zakarpattia region	**Закарпатська регіональна *державна лабораторія ветеринарної медицини***	м.Ужгород вул.Минайська,39 88015
Zaporizhzhia region	**Запорізька регіональна *державна лабораторія ветеринарної медицини*,**	м. Запоріжжя, вул. Іванова, 93, 69068
Ivano-Frankivsk region	**Івано-Франківська регіональна державна лабораторія ветеринарної медицини,**	м.Івано-Франківськ, вул.Берегова,24 76019
Kyiv region	**Регіональна *державна лабораторія ветеринарної медицини в* Київській області**	м. Вишневе, К.-Святошинр-н, вул. Балукова,26, 0 8133
Kirovohrad region	**Кіровоградська регіональна *державна лабораторія ветеринарної медицини,***	м. Кіровоград, вул. Велика Пермська, 58/1, 25001
Luhansk region	**Лисичанська міжрайонна *державна лабораторія ветеринарної медицини*,**	м. Лисичанськ, вул. Карла Маркса 135
Lviv region	**Львівська регіональна *державна лабораторія ветеринарної медицини*,**	м.Львів, вул .Промислова 7 79024
Mykolaiv region	**Миколаївська регіональна *державна лабораторія ветеринарної медицини*,**	м.Миколаїв, вул.Луначарського, 2-а 54003
Odesa region	**Одеській філіал ДНДЛДВСЕ,**	Одеська обл. , Біляївський р-н, смт. Хліободарське, вул. Маяцька дорога,27 67667

Poltava region	**Регіональна *державна лабораторія ветеринарної медицини в* Полтавській області,** **.**	Полтавська обл. Полтавський р-н. с.Горбанівка вул. Миру, 2, 38751
Rivne region	**Рівненська регіональна *державна лабораторія ветеринарної медицини*,**	м. Рівне, вул. Макарова, 12, 33010
Sumy region	**Сумській філіал ДНДІЛДВСЕ**	м. Суми, вул. Якіра,17, 40009
Ternopil region	**Тернопільська регіональна державна лабораторія ветеринарної медицини,**	м. Тернопіль, вул. Князя Острозького,68 46006
Kharkiv region	**Харківський філіал ДНДІЛДВСЕ**	м. Харків, вул. Жовтневої революції, 148, 61157
Kherson region	**Херсонська регіональна *державна лабораторія ветеринарної медицини ім. Л.С. Ценковського,***	м. Херсон, Бериславське шоссе, с. Жовтневе, 73484
Khmelnytsk region	**Хмельницька регіональна *державна лабораторія ветеринарної медицини***	м. Хмельницький, вул.Чорновола,176/1 29006
Cherkasy region	**Черкаська регіональна *державна лабораторія ветеринарної медицини,***	м.Черкаси, вул. Смілянська,120, 18000
Chernivtsi region	**Регіональна *державна лабораторія ветеринарної медицини***	м. Чернівці, вул. Сторожинецька,113, 58004
Chernihiv область	**Чернігівська регіональна *державна лабораторія ветеринарної медицини*,**	м. Чернігів, вул. 1-го Травня, 180, 14034

Currently, for the test RT-PCR - the necessary equipment and trained specialists are in:
- Lviv Regional State Laboratory of Veterinary Medicine, Lviv city;
- Dnipropetrovsk Regional State Laboratory of Veterinary Medicine, Dnipro city

- **b) Which of the ASF diagnostic tests are in use in each of the Regional Laboratories?**

Tests are done only Fluorescent antibodies method.

- **c) What is the maximum <u>number of samples that can be analysed per week</u>, by PCR detection and/ or antibody detection tests?**
  - PCR method is not using (labs equipped with necessary equipment, staff that have passed training, in case of necessity they can carry out tests).
  - Fluorescent antibodies method up to 100 samples.