Emerging Animal Diseases in the Middle East Region: Potential Threats to Agriculture and Public Health

Workshop Report
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Introduction

The purpose of this workshop was to identify strategic research initiatives that will enable the development of scientific information and veterinary medical countermeasures to protect animals and people. The intent was to identify gaps in knowledge associated with priority emerging and re-emerging diseases that pose a threat to animal agriculture, food security, and public health. Emphasis was given to zoonotic diseases but diseases that impact food security and therefore the livelihood, nutrition, and health of people of the Middle East Region was included. The goal of the workshop was to identify high level research priorities that, if implemented, will provide scientific information that will benefit the health of animals and people. This information captured in this report will be used to identify strategic areas of research for maintaining a healthy and safe food supply for the agricultural communities and consumers of agricultural products in the Middle East Region.
Animal Health Situation and Priorities Overview

North Africa

Summary from OIE of major animal diseases present in North African region

In the field of animal health, the significant sanitary event in the North African region in the recent past was the continuous circulation of foot and mouth disease (FMD) virus [serotype O (O/ME-SA/Ind-2001)] in the region as demonstrated by the notification of the disease in Algeria in March 2015 and the re-occurrence of the disease in Morocco in October 2015 (after 15 years of absence). This re-occurrence highlighted the fragility of the movement control measures and traceability across the countries in the North African region as well as non-harmonized control measures applied by the countries to fight against the disease (e.g. vaccination strategy). The FMD serotype SAT 2 was also notified by Mauritania in 2015. The last notification of FMD in Mauritania was in 2006. In July 2015, Peste des Petits Ruminants (PPR) also re-occurred in Morocco after 7 years of absence with a total of 10 outbreaks. Other countries in the North African region regularly reported PPR over the years. Four outbreaks of Rift Valley Fever (RVF) re-occurred in Mauritania by the end of September 2015 with a total of 19 confirmed cases out of the 2,022 susceptible (sheep and goats). Human cases (25) and deaths (8) were also reported in the country in September 2015. Among the vector-borne disease, it is also worth mentioning continued circulation of Bluetongue. Avian Influenza (H5N1) is currently present in Libya causing outbreaks and serious losses to poultry farmers mainly in the Eastern part of the country bordering with Egypt where Avian Influenza is endemic. In January 2016, Morocco confirmed the presence of Low Pathogenic Avian Influenza (H9N2) which caused mortality and drop in egg production. Rabies is endemic in North Africa (e.g. Tunisia reported in 2015 a total of 380 domestic cases and 6 human cases) as well as other diseases such as Bovine Tuberculosis and Brucellosis. Parasitic diseases (e.g. Echinococcosis, Strongyloidiasis) are also present in the region and the estimation of their prevalence and related impact should be further investigated and updated. Nevertheless, due to the highly unstable political situation in Libya, the risk of introducing into North African region – via Libya – diseases currently present in the Middle East is not negligible. The recent introduction of the FMD virus [serotype O (O/ME-SA/Ind-2001)] is a clear example.

Some major countermeasures needed to improve the sanitary situation in the North African region are reported as follows:

- Effectively to control animal movements in the region;
- Improve the investigation procedures in case of outbreak (trace back and forward);
- Improve the design of surveillance activities (e.g. passive, active, risk based);
- Harmonization of the methodology in conducting surveillance and vaccination campaigns between the countries in the North African region;
- Improve early warning systems for some diseases (e.g. Rift Valley Fever, Lumpy skin disease);
Better monitor the efficacy of the vaccination campaigns, when applied;
Research on entomology and related cartography of vectors;
Improve the biosecurity levels in the laboratories.

Jordan

Research Challenges and Gaps on Animal Diseases in Jordan

Jordan has about 3.5 millions sheep and goats and 65,000 heads of cattle and 15-18 thousand of camels. The production of chicken meat and eggs is sufficient for Jordanians and in some times there is surplus. The Ministry of Agriculture (MA) provides free veterinary services, both clinical and laboratory, through its scattered veterinary directorates in different governorates. They provide free vaccinations to Foot and Mouth Disease (FMD), Pest Petit Ruminants (PPR), Sheep and Goat Pox (SP and GP), Brucellosis (Rev-1 vaccine) and Anthrax, and more recently free vaccination for rabies for pet animals. At the same time, more and more private veterinary clinics and pharmacies are being opened and deliver different types of veterinary services and supports. The Ministry of Health (MH) provides the diagnostic services for rabies. In addition, few non-governmental organizations and centers provide veterinary services and support.

No significant research pertaining to animal health is conducted by the MA, and the majority of research is conducted by the Faculty of Veterinary Medicine (FVM) at Jordan University of Science and Technology (JUST), and National Center for Agriculture Research and Extension (NCARE). In few cases, joint externally funded projects are conducted with the MA. Most of the research conducted at the FVM is of epidemiological-surveys types; for examples several research papers were published on FMD, PPR, Brucellosis, Bovine Viral Diarrhea (BVD), Avian Influenza (A1-H5N1), Middle East Respiratory Syndrome-Corona virus (MERS-CoV) and Johne’s disease. The published work was mainly serosurveillance coupled with some molecular biology techniques such as PCR. In few cases, recent research works is focused in improving the diagnostics, as some papers were published regarding the diagnosis of rabies and brucellosis. The published work is scattered in different disciplines and fields, thus does not compose a significant size and produce significant impact.

The main constrains related to research in animal health and diseases are a clear and guided research programs to solving problems although, there are priority issues addressed by the research funding agencies in Jordan updated each year. The research is aimed mainly at serving the researcher for academic promotions and upgrading the institutions at the international domain. The main gaps in animal health research can be summarized as follows; 1- absence of effective surveillance system for early recognition of signs of animal diseases or agent detection or antibody detection, 2-lack of sufficient knowledge regarding diseases recognition by farmers, veterinarians and animal technicians, as early detection of animal diseases is very crucial, 3- lack of workable and effective control strategy in the early manifestation of the disease, and measures of identifying source of infection, 4- lack of cheap, fast and long shelf life field approved diagnostic tests, 5- how effective is the vaccination programs and its compatibility with the emerging field strains using the molecular laboratory data available, 6- how effective are the
biosecurity and biosafety measures, and awareness and education of submitting proper samples for controlling animal diseases, 7- what type of information and measures (compensation-free medication) need to be used to encourage farmers participation in diseases control, 8- capacity development in outbreak preparedness, field and laboratory training, control strategy and resource mobilization, 9-lack of exchange of information at the regional level as the political unrest in the neighboring countries hinder this process, 10- lack of information on the role of wildlife in animal disease transmission and illegal animal movement across borders.

Palestine

Animal diseases situation in Palestine

- 30-35% of Palestinian National income is depending on livestock and its products, which is affected by many obstacles, and mainly by the negative impact of animal diseases such as the endemic diseases like FMD, Brucellosis, or emerged diseases like Avian Influenza.

- Different ways are used to control these diseases, as vaccination to control some diseases like FMD, PPR but other diseases controlled by culling of the infected flocks and compensation like avian influenza.

- Despite the implementation of Palestinian veterinary services for many vaccination campaigns against various diseases, but there are still many cases continue to occur or increase, whether in the animal or human.

- Palestinian Veterinary Services is trying its best to control the livestock yearly against these diseases, but there are many obstacles against us to reach our goal; mainly lack of vaccine supplies, and the cost of the vaccine itself, which lead to decrease vaccination coverage, the efficacy and efficiency of the vaccine. Also there is lack in Epidemiological surveys, scientific studies and tracing which should be improved in the affected areas in Palestine.

- Recently it was clear that these diseases are spread among Palestinian livestock causing a highly economic impact, and increases the worse economic and social situation among Palestinian farmers, and decreases their capability to breed animals which will affect negatively the livestock breeding in Palestine.

- In 2015 we reported 67 outbreaks of Avian Influenza, 45 PPR, 23 cases of FMD, 13 cases of Brucella confirmed by PCR and 266 by RBT, but we don’t have any information about MERS in Palestine, no human cases, and the last reported case of Rabies was in 2010 no studies on wild animals in Palestine).

- These diseases diagnosed in the central veterinary laboratories belonging to the Palestinian Ministry of Agriculture, but there is a gap in the diagnostic process, because the Lab is not accredited and it has limited diagnostic capabilities (like the Positive results for FMD only without serotype), in addition to that some diagnostic possibilities for some diseases are not available in the Palestinian laboratories.

- There is also a scientific gap in diagnosing and dealing with many diseases, where there is a lot of diagnosed diseases in Palestine but we did not make enough studies on it, to develop
appropriate plans for dealing and control of these diseases for example: Brucellosis which is a zoonotic epidemic disease in Palestine and most neighboring countries; Jordan Lebanon, Oman, Saudi Arabia, Qatar, Yemen, and Israel (OIE, multiannual animal disease status), it is caused by a group of Brucella species; In Palestine mostly Brucella melitensis, for sheep and goat, B. Abortus for cattle, unfortunately we have no data about the incidence of the other species, and we need Immune response study of sheep and goat due to Brucellosis Ocular vaccination.

- Also FMD and PPR considered as one of many important and endemic diseases in Palestine; where about 1.2 million sheep and goat considered at high risk of type O FMD and 34000 cattle for type O, Asia 1.

- Despite the implementation of vaccination campaign to control the disease, but there is increasing in the infected cases of both human and animals. So this is the time to make a serious studies on how the scientific and professionals dealing to limit and control the spread of this disease, all zoonotic and epidemiological animal diseases.

- According to that, a comprehensive studies, control strategies and prevention programs will surely decrease the incidence of such diseases and control its impacts, taking into account the need for many studies for:
  - Assessment of biosecurity levels along the value chain.
  - Conduct socio-economic assessment for these diseases.
  - Develop contingency plans.
  - Develop a risk based communication strategies including KAP study.
  - Assess proficiently data base which will be used by decision maker for any further disease control strategy.
  - Review, and improve animal health regulations and laws to be adapted for Palestinian situation.

**Israel**

**An overview about animal diseases in Israel**

The special epidemiological situation of Israel and adjacent countries, bring us to cope with a large number of animal diseases, some of them being emerging, which turns the area to be a "paradise" for veterinary epidemiologists.

The reasons for the multiple animal diseases in Israel can be explained by some of the following:

- Central location (Europe Asia & Africa)
- Various microclimates
- Various ethnic populations and modes of agriculture
Outbreaks in neighboring countries
Smuggling and illegal import of animals
Animal passage through borders (import)
Birds Migratory major route (twice a year)
High density of farms
Close distance among farm animals, wildlife and population.
Long summer – optimal conditions for arthropods.
Very sophisticated dairy industry – Every milk drop is recorded (very high sensibility).

Outbreaks of several endemic and epidemic animal diseases in Israel have been recorded, including **highly pathogenic avian influenza (HPAI)**, **Foot-and-mouth disease (FMD)** (in cattle, sheep and wild animals such as wild boars and gazelles), **Lumpy skin disease (LSD)**, **Brucellosis** (e.g. *Brucella melitensis* in small ruminants and seldom in cattle), **Sheep pox, peste des petits ruminants, bluetongue** (outbreaks of different serotypes in different ruminants including wild ones), **Newcastle disease (NDV)**, **classical swine fever**, **rabies** and more others.

Such diseases have tremendous negative impacts on the total output and price of live animals as well as all related industries, ultimately having a negative influence on domestic consumption and export opportunities. Therefore, a large campaign to reduce brucellosis in small ruminant herds in the Negev has been started.

The Israeli Veterinary Services and Animal Health (IVSAH) undertake the identification of ruminants and a variety of compulsory vaccination programmes for FMD, *Brucella melitensis*, Newcastle and rabies. Vaccination against PPR, sheep & goat pox and LSD have stopped to be compulsory recently. The vaccination of cattle against *Brucella abortus* ceased on 01/01/2014.

All other vaccination of ruminants is done as needed or by request of the farmer. Some vaccinations formerly performed by Field Veterinary Services have been handed over to private veterinarians (such as enterotoxaemia and rabies in ruminants). Anthrax vaccination is mandatory in a herd where an outbreak has been confirmed and 10 years forward.
**Morocco**

**Disease situation in Morocco and identified scientific and veterinary countermeasures gaps**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Statut</th>
<th>Current situation</th>
<th>Major Gap in scientific information</th>
<th>Major Gap in Veterinary medical countermeasures</th>
</tr>
</thead>
</table>
| Low pathogenic Avian Influenza   | 1st Emergence in Morocco in 2016           | Epizootic         | Wildlife reservoirs in Morocco  
Surveillance of wildlife  
Mechanism of first emergence in Morocco                                                                                                                                                                                           | Efficacy of vaccine presently marketed in Morocco  
Limited capacity of laboratory diagnosis for LPAI virus typing                                                                           |
| High pathogenic Avian Influenza  | Free                                       | Free              | Surveillance and risk assessment                                                                                                                                                                                                      |                                                                                                                                            |
| FMD                              | Emerging                                   | Controlled in cattle | Transhumance practices and drivers of small ruminants in North Africa  
Surveillance in wildlife                                                                                                                                                                                                            | Efficacy of used vaccine                                                                                                                                                                          |
| Brucellosis (B.abortus)          | Widespread (serological)                   | Enzootic with medium prevalence | Strain typing and epidemiological investigations  
Zoonotic significance in human                                                                                                                                                                                                     | Vaccination control programs implemented in voluntary basis                                                                            |
<p>| Brucellosis (B.melitensis)       | Geographically Limited (serological)       | Enzootic with low prevalence | Strain typing and epidemiological investigations                                                                                                                                                                                    | Lack of vaccination programs                                                                                                                                                                      |</p>
<table>
<thead>
<tr>
<th>Zoonotic significance in human</th>
<th>Limited capacity to Laboratory screening or diagnosis of the disease.</th>
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<tbody>
<tr>
<td>MERS-CoV</td>
<td>Serological evidence only Prevalent in camel Evidence of transmission to humans, virus reservoirs?</td>
</tr>
<tr>
<td>Rabies</td>
<td>Enzootic prevalent Dynamic and methods of control of stray dog population Safety and Efficacy of oral vaccine to control the disease in stray dogs</td>
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</tbody>
</table>

**Tunisia**

**Health situation in Tunisia**

Avian Influenza, FMD, Brucellosis and rabies are included in the list of regulated diseases subjects to specific control measures and control (decree 2200/2009 of 14 July 2009)

1. **Avian Influenza (AI)**

   AI is a disease that has a specific control plan in Tunisia. The network targets two myxoviridae (Avian Influenza and Newcastle Disease). An annual program is applied to determine the immunity level and the circulation of these viruses. Vaccination is used to prevent the ND.

   The choice of samples is crucial to ensure effective diagnostic laboratory. Samples include oropharyngeal swabs (swab) and sera.

   H9N2 low pathogenic AI has been identified in Tunisia by PCR and sequencing technics.

   The monitoring of AI in Tunisia is based on monitoring system of the industrial and traditional breeding and wild birds. The active surveillance looks for the AI virus in industrial breeding operations. The monitoring system of wild birds concerns migratory birds and wild birds, including some autochthonous species

   Diagnostic method used include rapid tests, Ag ELISA, PCR, isolation, IHA, and sequencing.

   Sampling: 460 breeders were targeted in 2015, 40 samples from each breeder were taken (sera (20), trachea (10) and cloacal (10) swabs).

2. **Foot-and-Mouth Disease (FMD)**

   History of the disease is important to understand the evolution and mode of transmission in the region,
An important new approach is to consider all the countries of the region, as the same epidemiology unit.

An FMD control plan was validated by the OIE in 2012. This control plan aimed to identify and determine the FMD viruses circulating in the region. The plan includes research of viruses circulating in susceptible animals in markets, slaughter houses, fattening units, wild life, sentinel breeding operations, and trans-human modes of transmission.

In Tunisia, FMD outbreaks occurred in April 2014. A total of 150 outbreaks were reported, with most of the outbreaks in cattle. Vaccination was the first efficient controlled measure. Stamping out, control of animal movement, zoning, market restriction were not completely applied despite the efforts by Veterinary Services to implement these measures in the field. The absence of a compensation system was determined to be the first weak point in implementing the control measures.

The diagnosis of FMD occurred in the national laboratory of Tunisia (diagnostic and sequencing) and was confirmed by the World Reference Laboratory at the Pirbright Institute.

A program to strengthen Veterinary Services capacity in Tunisia has since been implemented. Some activities have been realized (immunity level, viral circulation, training, animal movement study and cost benefits studies).

A molecular diagnostic study has been realized to determine the chronology of infection of FMD in Tunisia during the epizootic of 2014 (Full genome sequencing of selected Tunisian isolates by NGS and antigenic characterization by monoclonal antibodies (MAbs) profiling). This study informs the movement of the virus, and subsequently the movement of animals.

3. Rabies

This zoonosis is endemic in the whole region. A monitoring program is based on the passive surveillance of cases reported to Veterinary Services. The competent authorities manage the notice of disease outbreaks. Ring vaccination is implemented when the disease is confirmed by the national laboratory. The diagnostic technique used is the IFI (IPT).

The first challenge in the rabies control program is access to dogs that are hard to cash in rural zones, the management of dog populations. The size of the dog population is not updated regularly; therefore, the estimation of the population is dependent on vaccine coverage.

A strategic control program on either side of the country’s borders can be implemented, but dogs can move freely across borders. So the coordination of Veterinary Services across bordering countries is recommended in order to harmonize the surveillance and the control of the disease.

4. Brucellosis

Brucellosis passive surveillance is the main surveillance system. This disease is officially reported by the Tunisian Veterinary Services. Cattle, sheep and goats are the susceptible and affected species. The first control measure is vaccination. The cost of the vaccination is very high.
The clinical diagnosis of clinical cases is very difficult and includes the differential diagnosis for other diseases, such as Chlamydiosis, Coxiella burnetti (Q fever), Border disease, and Rift Valley Fever (RVF). Laboratory diagnosis is based on EAT and serology. Molecular methods must be developed in order to increase the rapidity, specificity and sensitivity of diagnostic tests.

5. **Middle Eastern Respiratory Disease Syndrome, Coronavirus (MERS-CoV)**

The disease was identified three times in humans in Tunisia. Serological evidence in camels has been reported, with 48 percent (99/204) reported seropositive camels by ELISA (IgG). The origin of these positive reactions are unknown. There is no surveillance program specific for MERS-CoV in camels. A guide for a national response plan to the human MERS-CoV epidemic has been established.
Gap Analysis by Disease

Rabies

Disease situation in the Middle East and North Africa

Egypt

Rabies was the cause of 55 reported deaths in Egypt in 2015. According to the last updated report of the National Services Scotland (NHS), Egypt was considered as one of the high risk African countries for Rabies.

The percentage of rabid cases in accordance to species at the period from 1997 to 2001 were 66.6, 25, 16.6, 58.3, 8.3, 8.3, 8.3, and 8.3 percent in dogs, jackals, cats, bovine, donkeys, foxes, and hyenas, respectively. The number of cases due to animal bites was the highest in 2007. The number of animal bite cases per governorate in 2007 was the highest in Beheira (17,958 cases) and the least at Sinai (69 cases).

The percentage of species of animals biting people at El-Wadi El-Gedid governorate from hospital records in the period from January 2012 to April 2013 were 49.6, 7.2, 12.4, 26.2, 4.3 percent in dogs, cats, fox, donkeys, and rats respectively.

The number of animal bite cases in 2015 was 324,986 cases.

Rabies laboratories in Egypt
1. Animal Health Research Institute Rabies lab (Dokki – Giza)
   - One of the accredited labs by the EU for the titration of rabies antibodies in pets (FAVN and ELISA)
   - Diagnosis of rabies virus (isolation, Identification and molecular studies)
2. Veterinary Serum and Vaccines Research Institute for vaccine and sera production:
   - Preparation of vaccines and antisera for animal use
3. VACCERA:
   - Preparation of vaccines and antisera for human use.

Rabies control
1. Vaccination of dogs owned by people.(25000 cases at 2015)
2. Reduction of stray dog’s population (413,720 cases at 2015)
3. Coordination between Ministry of Health and General Organization for Veterinary Service (sharing information and control measures)

Molecular studies
Studies on rabies isolates with phylogenetic analysis have been conducted.
Israel

Rabies is a notifiable disease in Israel, in accordance with the Animal Disease Ordinance (new version) of 1985, and the Rabies Ordinance of 1934. The ordinance of 1985 obligates any person who diagnoses or suspects a case of the disease to inform an official veterinarian. The District Veterinary Offices and Municipality Veterinarians are both empowered and responsible for implementing all the legislation relevant to rabies including prevention and control.

Since 1956, all domestic dogs in Israel must be vaccinated against rabies by law, first at age three months and subsequently revaccinated annually. These regulations contributed to a substantial shift in the dominant rabies reservoir in Israel. Dogs were the most commonly affected animals until the late 1950s. Starting in 1956, red foxes (*Vulpes vulpes*) and to a lesser extent golden jackals (*Canis aureus*) were the primary rabies reservoirs. During the mid-1970s, a major transition from urban dog rabies variant to sylvatic fox rabies variant occurred. Since 1998 wildlife rabies has been controlled through the use of oral rabies vaccines. The implementation of the fox-targeted oral vaccination program led to a dramatic decrease in the number of confirmed rabies cases. The disease practically disappeared from almost all of Israel. However, a new outbreak started in the north-east region of Israel in 2005, with a few cases sporadically appearing along the Syrian border. During 2005-2015, stray dogs emerged as the main animal reservoir in northern Israel while lower numbers of rabies cases were confirmed in other animals. Biological and molecular characterization of rabies isolates from infected foxes and dogs showed that they differed, suggesting 2 distinct and separate lineages. The analysis of the G-L intergenic region sequences of Turkish, Jordanian, southern Lebanese and north-eastern Israeli rabies virus isolates indicated that dog-mediated rabies showed a close relationship to Turkish dog rabies. It therefore appears that the canine rabies outbreaks in Israel resulted from north to south movement of infected animals in the eastern Mediterranean area. The last 3 human cases in Israel were confirmed in 1996 -1997. The transition from fox-mediated rabies to dog-mediated rabies in the northern Israel area is of great concern to public health officials because of the close contact between dogs and man. Measures undertaken by the Israeli Veterinary Authorities include intensification of supervision of compulsory pet dog vaccination, increased frequency and density of oral vaccine bait distribution in affected areas and initiation of cooperation with Jordan in a joint wildlife oral rabies vaccination project in the Jordan Valley. A new public awareness campaign has also emphasized the importance of annual dog vaccination and reporting to the proper medical authorities of any close physical contact with suspect animals, particularly wildlife.

The Israeli rabies surveillance and monitoring system is divided into two parts: a) the obligation of the owner of an animal that has bitten either a human or an animal is to present this animal to an official quarantine station within 24 hours of the insult. Any animal that has bitten is required to remain under observation within a quarantine facility under the supervision of an official veterinary officer for a period of 10 days. If the animal does not show any clinical signs of disease during this isolation period it is returned to its owner. Any animal that dies during quarantine or shows clinical signs that could be attributed to rabies is sent for diagnosis to the Rabies Laboratory at the Kimron Veterinary Institute; b) sylvatic rabies control – The State of Israel runs a passive rabies sylvatic motoring program. Mammals found dead that might be suspected of harboring rabies are sent to the laboratory for diagnosis and tested free of charge. The National Rabies Laboratory of the Kimron Veterinary Institute is practicing the OIE
standards for rabies identification as follow: The rabies virus diagnosis is undertaken using the fluorescent antibody test (FAT); All positive results are confirmed by virus isolation in tissue culture and by the polymerase chain reaction (PCR); PCR is also applied on all autolysed specimens; In all cases of human exposure, further tests (cell culture or PCR) on the same sample are applied, in addition to FAT. The identification of the agent is supplemented by using specific nucleic acid probes, or the polymerase chain reaction followed by DNA sequencing of genomic areas, in order to identify the geographical area of virus origin.

**Research Gaps**

**Diagnostics**
- Lack of implementation of OIE prescribed and WHO recommended standard diagnostic techniques throughout the Middle East Region.
- Nonexistence of intra-regional proficiency testing to ensure reliable data on diagnoses, e.g. FAT, and the incidence of rabies under the auspices of a recognized laboratory.
- Absence of standards to ensure proper evaluation of molecular techniques in local laboratories in rabies-endemic countries using more lineage specific & universal primers for RT-PCR assays, and development of improved sequencing protocols.

**Epidemiology**
- Absence of decentralized regional surveillance methods including laboratory confirmation based on validated protocols and specimens.
- Insufficient epidemiological analysis of data to accurately estimate the incidence of rabies and disease burden.

**Biological medical products**
- Absence in the region of standard laboratory potency tests for rabies immunoglobulin and vaccines to guarantee quality and safety (Kimron can provide quality testing).

**Host ecology**
- Lack of means for identifying different host species in relation to disease transmission and population dynamics in relation to disease persistence
- Absence of data on dog population for development of regional approaches for the implementation of OIE standards on dog population management.

**Research priorities**

- Generation of information on the (i) epidemiology and population dynamics of rabies in regional reservoir host and (ii) patterns and extent of movement of infected animals to predict the spread of rabies.
- Generation of a supraregional rabies database for rabies surveillance data (use of the [WHO European rabies database](https:// european-rabies-database.who.int) can be considered).
- Enhanced and continuous characterization of lyssavirus species and variants in the Middle East region to identify determinants of rabies spread and predicting and controlling the spatial spread of rabies.
- Development of coordinated research on new modes of delivery of inactivated vaccines to find more efficient and easier means for controlling rabies in the animal reservoir in resource-poor areas.
- Research on the occurrence of lyssaviruses in indigenous bat species.
- Evaluation of commercial lateral flow devices for use in rabies diagnosis in the Middle East region.
- Initiation of pilot projects on oral vaccination of stray dogs in the Middle East region under one the One Health approach as a complementary measure for dog rabies elimination programs taking into account the ecology of dogs.
- Development of innovative attractive bait casings for local dog populations to improve vaccination coverage and efficiency of ORV of stray dogs.

**Potential research collaborations**

Countries that have interest and willing to collaborate in the following five areas of research:

<table>
<thead>
<tr>
<th>Country</th>
<th>Ecology</th>
<th>Epidemiology</th>
<th>Pathogenesis</th>
<th>Diagnostic Discovery</th>
<th>Vaccine Discovery</th>
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<td>Egypt</td>
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<td>Tunisia</td>
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Proposed contributions to potential research collaboration by Country:

**Egypt**
- Collection of rabies virus isolates for regional epidemiological research.
- Building capacities for rabies post vaccination immunity in humans and animal.
Israel
- Accredited laboratory and experience staff members can share experience with other countries in the Middle East region.
- Molecular analysis of rabies virus isolates for regional epidemiological research.
- Initiation of pilot projects on oral vaccination of stray dogs in the Middle East region under one the One Health approach as a complementary measure for dog rabies elimination programs taking into account the ecology of dogs.

Jordan
- Development of a continuous regional training program for better rabies diagnosis and epidemiological data collection.
- Collection of rabies virus isolates for regional epidemiological research.
- Application of the Immunohistochemistry test in rabies diagnosis.
- Development of coordinated research on new modes of delivery of inactivated vaccines to find more efficient and easier means for controlling rabies in the animal reservoir in resource-poor areas.

Morocco
- Evaluation of commercial lateral flow devices for use in rabies diagnosis in the Middle East region.
- Collection of rabies virus isolates for regional epidemiological research.

Palestine
- Establishment of rabies diagnosis laboratory and disease surveillance.

Saudi Arabia
- Collection of rabies virus isolates for regional epidemiological research.
- Estimation of dog population and development of regional approaches for the implementation of OIE standards on dog population management.

Tunisia
- Collaboration on pilot projects on oral vaccination of stray dogs.
Brucellosis

Disease situation in the Middle East and North Africa

Israel

Brucellosis caused by *Brucella*, Gram negative bacteria of the alphaproteobacteria class, is a world-wide distributed zoonosis associated with domesticated animals. In the definitive host, *Brucella* species cause early third semester abortion storms disseminating massive numbers of free bacteria through placentae, fetal membranes and fluids thus expediting the transmission of the disease into the herd, in addition to surrounding animals and humans. Consumption of raw milk and unpasteurized dairy products is a major route of transmission into the population. *B. melitensis* and *B. abortus* cause small ruminant and cattle brucellosis, respectively, the former being a primary cause of human brucellosis and widely distributed in Israel, Jordan and regional countries including Egypt, Saudi Arabia, Turkey and Gulf countries. *B. abortus* was declared eradicated in Israel in 1985 but is still endemic in Turkey and Egypt. Camel brucellosis due to *B. melitensis* has been reported in Saudi Arabia and sporadically in Israel but the prevalence of *B. abortus* cases in Sudan may indicate a more global concern. *B. suis* has also been reported in Egypt.

Control of brucellosis is generally conducted through test and cull policies combined with vaccination, often based on compensation to farmers. Because countries lacking the financial resources to implement such an approach, a carpet vaccination program of the whole population has been proposed as a more feasible program, to initially be carried out for several years in order to reduce disease prevalence so as to permit a test and cull policy. However, estimation of the disease prevalence and determining which *Brucella* species exist in the epidemiological units are perquisites that must be fulfilled prior to conducting full programs.

Isolation and typing of *Brucella* from individual animals is the gold standard necessary to confirm the disease. Mass testing of whole populations using such serological approaches as complement fixation (CFT), microplate agglutination (MAT) and rapid card tests has been practiced in the past. To date, new robust tests such as c-ELISA, i-ELISA and Fluorescence Polarization Assay (FPA) have been shown superior to the old ones, underlying the necessity for their accreditation and ISO approvals.

A single dose of live attenuated vaccine strains has been shown to be sufficient in conferring lifelong protection in cattle and small ruminants. *B. melitensis* Rev.1 and *B. abortus* S19 are the vaccines of choice for these species, respectively. Due to the fact that the vaccine may cause abortion when administered to pregnant animals and that strain persistence and a persistent serological response may be elicited when adult animals are inoculated, there exists controversy as to vaccination methodology concerning the time, dose and route of administration. Interference with diagnosis due to DIVA questions further hampers vaccine application in control programs.

Our laboratory is a National, OIE and FAO reference Laboratory for human and animal brucellosis with involvement in the field both locally and abroad, and participating in
competitive international research grants. Dr. Banai is recognized OIE expert on *B. melitensis* and *B. abortus* and has published in peer reviewed journals and books.

**Egypt and Middle East**

Brucellosis is still a national as well a regional problem that threatens both animal resources and public health. In Egypt, the first scientific paper reporting brucellosis in farm animals was published in 1939. Since then, the disease has been detected at high levels among ruminants, particularly in large intensive breeding farms. Consequently, a control program including serological surveys and voluntary vaccination of ruminants was established in the early 1980s with the implementation of the open door policy and establishment of many intensive animal farms for producing milk, dairy products, and meat. The incidence of the disease in intensive dairy farms are higher than smaller animal farms.

In Egypt and most Middle Eastern countries, the predominant *Brucella* strain isolated from different domestic animal species is *B. melitensis*, particularly biovar (3), although various *B. melitensis* strains are isolated from sheep and goats (original host), cattle, buffalos and camels. Among different species of the genus *Brucella, B. melitensis* is the most virulent and pathogenic species for animals and man.

Brucellosis is a classical zoonotic disease, where humans contract the disease only from animals or animal products. Human Brucellosis is underestimated in many developing countries, as the disease is mostly misdiagnosed. The World Health Organization (WHO) estimates that half a million cases of human brucellosis are reported worldwide every year, and that for every case diagnosed there are four cases that go undetected (WHO, 1997). The control of the disease in animals has a direct impact on the public health and minimizes the risk of human brucellosis.

**Major Gaps in Brucellosis Control Programs**

Although Egypt and Middle East countries have the potential and the resources for implementing successful brucellosis control and eradication programs, most of these programs often end without achieving the broad objectives. The reasons for the failure of most control programs for brucellosis are due to the following gaps:

1. **The lack of standardization of Brucellosis serological tests for most animal species (except cattle), particularly for camels.**
2. **The absence of accredited institutes that produce standard and reproducible diagnostic antigens and reagents for diagnoses of brucellosis to ensure quality and sustainability of diagnostic materials.**
3. **The disorganized application of vaccination programs, including the selection of proper vaccines applicable to the current epidemiological situation in the region and sustainability of these programs.**
4. **The lack of active veterinary and public health extension programs.**
5. **The improper veterinary quarantine measures applied on imported animals on country border sites.**
6. The lack of proper epidemiological survey for wild animals transmitting the disease.

**Research Gaps**

Do cattle abort due to *B. melitensis*? Is disease in cattle similar to the pathogenesis of *B. abortus* in cattle?

Does S19 cattle vaccination confer protection against *B. melitensis*?

Does *B. melitensis* in cattle transmit to small ruminants and humans?

How do you quality assure the unstable attenuated Rev. 1 strain?

*B. melitensis* strain Rev. 1 Elberg, passage 101, 1970, has been established as a standard strain deposited in the European Pharmacopoeia. Different manufacturers provide home established seed-stocks of different Rev. 1 variants. How can a unified seed stock be accredited to secure the biological properties of the vaccine in the field? (Our laboratory has established a lyophilized seed stock bank of this strain, awaiting the development of a universal standardized vaccine. Moreover, novel recombinant super vaccine strains can be developed based on this seed strain).

Can DIVA tests be developed that differentiate vaccinated from infected populations?

Considering MLVA, whole genome sequencing, and proteomics, how should diagnosis of the disease be conducted in the future?

Which control policy is preferential and under which conditions should eradication be pursued?

What is a realistic timetable that is feasible for control and/or eradication of *B. melitensis* in a country or regionally?

Could eradication be achieved in a single country without regional collaboration?

For regional collaboration, could different approaches be implemented in various locations?

Intriguingly, in recent years several aberrant strains have been included in genus *Brucella*. Traditionally, Brucella have been considered to be human pathogens whereas the zoonotic status of newly identified species is currently unknown.

**Research priorities:**

- Whole genome sequencing with analysis may be beneficial in the Middle East. Use of sequencing data in North America has been very beneficial in understanding the epidemiology of some outbreaks.
- Need studies to understand the epidemiology/pathogenesis of *Brucella* species and host range
- Need for more efficacious and safer vaccines, particularly for *B. melitensis*
  - Non zoonotic
  - Efficacious in relevant livestock species (cattle, sheep, goats, camels)
  - DIVA vaccines
- Need data on cross protection other than natural hosts (*B. melitensis* in cattle).
- Vaccines that are efficacious and safe in young and adult animals.
- Vaccines that are safe in pregnant animals
- Need for vaccines that do not require a cold-chain

- Small targeted vaccination studies/programs in very limited areas may be beneficial to explore adaptive management approaches where the authorities in a country may try some approaches on a small scale and assess their impact before broadening the scale of the program.
- Genotypic and phenotypic characterization of the original clone of the Elberg REV-1 vaccine strain as a standard for quality control testing of current manufacturers of REV-1 vaccines.
- Pathogenicity studies to understand mechanisms of abortions to develop in vitro models that can be used in countries where live animal studies are not allowed due to safety concerns.
- Improve diagnostics
  - Diagnostics that can differentiate *Brucella* species in different animal species
  - DIVA diagnostic test
  - Need pen-side test for diagnosis in the field
  - Multiplex diagnostic test for pathogens that are the cause of abortions
- Research on best approach to implement control programs in the Middle East

**Potential research collaborations**

Countries that have interest and willing to collaborate in the following five areas of research:

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Proposed contributions to potential research collaboration by Country:

**Egypt:**
- Accredited Laboratory and experience staff members can share experience with other countries in the ME
- Standardization of serological tests in animal species other than cattle i.e in sheep, goats, buffalos and camels.
- To provide the field local *Brucella melitensis* strain that proposed to be the origin of the new vaccine candidate.
- Apply the Detection of Brucella DNA in Serum of Different Animal species as a diagnostic tool.

**Israel:**
- To provide International community with Elberg (Rev.1) strain
- Diagnostic cooperation with detection of Brucella DNA in serum of animals

**Jordan:**
- Assess in application of the new proposed vaccine in the field.
- Application of the Immunohistochemical tech. in Diagnoses of Brucellosis
- Share in serological diagnoses in the field.

**Morocco:**
- Design epidemiological plan and assess the role of wildlife in transmitting Brucellosis.

**Palestine:**
- Study the transmission of the disease through borders with neighboring countries.
- Enhancing the ability our lab staff to culture and biotyping of Brucella organisms.

**Tunisia:**
- Improve the capacity of national Lab in Diagnoses .and apply interlab comparison with Reference Laboratory.
- Developing imunohistochemistry technique to differentiate between Brucella, Toxoplasma, Neospora and chlamydia.
- DIVA vaccine in the field.
- Study the behavior of the disease in different animal species particular at border points.
- Need to study the level of immunity for protection of animals.
Middle East Respiratory Syndrome-Coronavirus Virus (MERS-CoV)

Disease situation in the Middle East and North Africa

Dromedary camels are distributed throughout the MENA region and have been MERS-CoV seropositive, virus positive (by PCR or virus isolation). However, human cases who obtained the virus have been primarily observed in Saudi Arabia and Qatar. Travel associated cases have been observed throughout MENA, Europe, North America and Asia.

There is a lack in epidemiological information for the circulation of MERS-CoV in dromedary camels in MENA and the link between circulation in dromedary camels and the epidemiology of MERS-CoV in humans.

Egypt

In view of the current MERS-CoV global situation, the potential threat to Egypt due to close similarity of camel-associated and human-associated MERS-CoV sequences, there was a hypothesis that camels are the source of infection for human and might constitute a zoonotic animal reservoir.

Camel population in Egypt

- Local resident camels in villages estimated to be 66,228 heads.
- Imported camels estimated as average of 1,760,000 heads/year (>85%) are originated from East African Countries (Sudan Ethiopia, Somalia and Djibouti).

Surveillance Plan:

- The first national surveillance plan conducted by GOVS was in 2014 to understand the situation of MERS-CoV in Egypt through collecting samples from camels and contact persons in cooperation with AHRI-MOH-NRC.

Targeted areas were camel markets, slaughter houses, farms and small house holders. Samples collected from five governorates (Cairo – Giza - Sharkia – Qualubiab – Matrou. Total Samples actually tested were 158, total RNA positive by PCR 22 (13.9%) and total Serum positive by micro neutralization assay 112 (70.9%).

A cross sectional surveillance study was conducted in 2015 in collaboration between GOVS, FAO and NRC to detect MERS-CoV and closely related corona viruses in camels, other domestic animals and bats. The conducted study demonstrated that the MERS-CoV neutralizing antibody prevalence in camels from Egypt is very high (84.5%), both in village and imported camels and the presence of active viral infection circulating in the country.
Adult camels had higher sero-prevalence (87.3%) compared to young camels (51.8%) and this was found to be statistically significant. In addition to presence of MERS-CoV neutralizing antibodies in donkeys and sheep and complete absence of the virus in tested bats.

A longitudinal surveillance study will be conducted in 2016 to estimate the duration of MERS-CoV virus shedding in camels and the possibility/evidence of intermittent shedding and/or reinfection during fattening period in different animal secretions. Conduct full-genome sequencing in order to understand the genetic and antigenic characteristics of isolated MERS-CoV and other Corona viruses.

**Saudi Arabia**

The MERS-CoV our team in Saudi Arabia was one of the first teams to address the issue in wild and domesticated animals, succeeded in producing a prevalence map of the virus in camels in the Kingdom of Saudi Arabia early 2014. Efforts continued to screen several wild animals including some bats, carnivores, ungulate, and rodent species. In addition, studies were conducted to assess possible routes of transmission into human populations, resulting in some interesting observations that will be published in the near future. Additionally, a study was conducted to assess the effect of seasonality on virus shedding in dromedary camels throughout the year.

There are many studies taking place in 2016 and hopefully additional information may shed light on the epidemiology of the virus, which can be communicated to the national Saudi authorities.

**Research Gaps**

**Gap in available scientific information**
- Camel movement within and between countries
- Epidemiological designed surveillance studies
- Occurrence of human disease throughout MENA
- Genetic information on the currently circulation of MERS-CoV
- Strain characterization
- Transmission from camel to human
- Risk factors associated with zoonotic transmission (slaughterhouses)
- Cultural connection between human and camels in different countries (Anthropology)
- Role of wildlife (bats)
- Role of other livestock (sheep, goats)
- Differences between different camels (e.g. racing, beauty & livestock, breeds)

**Gaps in veterinary medical countermeasures**
- There are currently no available veterinary medical countermeasures. Studies into the feasibility of dromedary camel vaccination should focus on protective efficacy in young dromedary camels.
**Research priorities**

- Establishment of a research framework throughout the MENA network for surveillance and epidemiological studies into MERS-CoV. Sampling should occur in standardized fashion. MENA MERS-CoV consortium to conduct studies in an organized fashion throughout the region with veterinary and academic stakeholders. Inform Chief Veterinary Officers and public health officials of the findings.
- Human serological studies to identify risk factors for zoonotic transmission. E.g. specific risk groups (camel herders, slaughterhouse employees).
- Research framework should include stakeholders from the public health side. Human cases should be followed-up with ecological studies into the origin of the virus (camel or travel associated).
- A joint genomic project linked into the epidemiological field studies to identify strain diversity and association with human disease. Isolated MERS-CoV strains will be available for further strain phenotypic characterization.
- Host response (innate, humoral and cellular) against MERS-CoV in dromedary camels

**Potential research collaborations**

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Proposed contributions to potential research collaboration by Country:

The World Organisation for Animal Health (OIE) provides advice and guidance to implement the OIE research recommendations on MERS-CoV and any other relevant OIE standards and guidelines. Potential research collaborations throughout the MENA Region:
• Utilize the FAO and OIE research recommendations to study into the role of dromedary camels (and other animals) in the epidemiology of MERS-CoV in MENA and to include all the partners throughout the MENA region. This should also include studies into camel movements across the region and assess implications for animal and human health.
• It will be essential to include primary stakeholders for from the veterinary, agricultural, academia and public health sides throughout the MENA region.
• The framework should include wildlife professionals to prepare for novel cross-species and zoonotic introductions of emerging viruses.
• Continuous regional meetings to exchange results / information and foster research collaborations.

**Egypt**
Genetic characterization of archived positive samples, epidemiologic/ surveillance studies/ ecology of virus and host range.
**Israel**
The Kimron Veterinary Institute of Israel is interested in cooperation in research concerning the ecology and epidemiology of MERS-CoV. Point of contact is Dr. Dan David: Dand@moag.gov.il

**Tunisia**
Genetic characterization of archived positive samples, epidemiologic/ surveillance studies/ ecology of virus and host range.
Avian Influenza

Disease situation in the Middle East and North Africa

Summary of avian influenza (H9N2) in Jordan and the Middle East

Avian influenza (H9N2) continues to circulate and cause disease in various types of poultry in the Middle East. This subtype causes mainly respiratory disease in chickens (Gharaibeh, 2008). The most common disease affecting poultry in the Middle East is respiratory disease. Among the most commonly diagnosed respiratory disease in these flocks with respiratory disease is avian influenza H9N2 infection.

There are no national or regional plans to control or at least monitor the spread of this infection. Local farmers and companies depend on biosecurity and vaccines to limit the economical effects of its infection in their poultry flocks.

Although the circulating virus continues to evolve and change its nucleic acid sequence, vaccination is fairly effective in sparing the older chicken flocks (layers and breeders) from mortality and decrease in productions numbers. However, the effectiveness of the vaccine in protecting broiler (meat-type) chickens is far less prominent. This is due to many reasons including the less effective immune system of these types of chickens, the presence of maternal antibodies which interfere with response to the vaccine, the shorter life span, and relatedness of the vaccine strain to the field strain (Gharaibeh and Amareen, 2015).

Furthermore, avian influenza H5N1 continue to cause severe losses in the Egyptian poultry industry with occasional human infections and fatalities. Also, other countries (Iraq and Lebanon) have reported new infections with this virus in 2016.

Diagnostic capabilities should be enhanced in Jordan and many of the Middle East countries. There is a need to pursue national and regional collaboration to put down strategies to monitor the spread of this infection and hence better control it.

Egypt

Avian Influenza virus (H5N1) was first detected in domestic poultry in Egypt in February 2006. Since then, more than 3687 confirmed foci have been reported from different poultry species. Cases were confirmed in farms (1143), backyard flocks (2226), live bird markets (294), check points (13), zoos (5) and slaughter houses (6). Accordingly, over 40 million birds have been culled, leading to serious damage to the poultry industry. In addition to 1609 H9N2 cases were confirmed from 2011 till 2016 in farms (1287), backyards (115), LBM (206) and slaughterhouse (1).

From 2006-2016, there were 350 laboratory-confirmed cases of H5N1 human infections have been reported in Egypt, of these, 116 cases (33.1%) died.
In 2016 there were 101 confirmed poultry cases in farms (12), backyard (56) and live bird markets (33), on the other hand there were 4 H5N1 human infections have been reported.

The genetic analysis of avian influenza viruses indicate that the current circulating HPAI H5N1 viruses were related to the new dominant clade 2.2.1.2 cluster, recorded since late 2014. On the other hand, the LPAI H9N2 viruses were related to G1-lineage (Asian viruses) which spread in Middle East since 2006.

Avian influenza vaccination of poultry has been extensively implemented in Egypt since the reported occurrence of the first HPAI H5N1 outbreak in 2006. AI vaccination has contributed in reducing the number of outbreaks in commercial poultry farms. However, there is no evidence of its effectiveness in the reducing the viral load in the environment and the spread of the disease in the country. HPAI outbreaks in household poultry and human infections due to A/H5N1 continue to regularly occur every year. The efficacy of AI vaccination in commercial poultry farms is undermined by the increase in the mutation rate of the virus drifting away from the vaccine strain and posing new threat for emergence of potential pandemic strain. Changes should be made in the AI vaccination strategy in commercial poultry sectors in order to prevent and monitor the evolution of the virus. The implementation of a revised HPAI control strategy that advocate for increased surveillance and bio-security in commercial poultry sector is necessary in order to ensure better control of the disease in Egypt.

**Major gaps in avian influenza control programs**

- Build and equip BSL III laboratory.
- Equip BSL III laboratory animal facility with isolators.
- Capacity building of laboratory personnel (training on bioinformatics, reverse genetics, analytic epidemiology, and BSL III laboratory animal facility).
- Continuous surveillance activities (reagents and equipment).
- Partnership (Twinning) with international reference lab.

**Avian Influenza and Newcastle in Israel**

Avian Influenza (AI) and velogenic Newcastle disease (vND) are among the most important infectious diseases of poultry, representing a serious challenge and threat for the poultry industry and an emerging disease threat for public health, especially H5 and H7 AI subtypes. For this reason, vND and AI infections caused by type A H5 and H7 are listed by the World Organization for Animal Health (OIE) and their notification is mandatory to better control the infections and limit their trans-boundary spread.

Both AI and ND viruses are genetically and antigenically variable and recent reports have indicated or suggested a sub-optimal performance of commercially available ND and AI vaccines against genetically divergent virulent field viruses. We can share the Israeli experience in both of these diseases including controlling measures, vaccination, monitoring, epidemiology and diagnosis as well as some recent research.
Highly Pathogenic H5N1

The first outbreak of Avian Influenza Virus (AIV) H5N1 occurred in Israel in the spring of 2006, beginning in the southwest part of Israel at the border with the Gaza strip. The affected flock were culled and the adjacent flocks within a risk zone were monitored. The outbreak rapidly spread to several focuses within Israel, and stopped due to the firm efforts of the Veterinary Services. In April 2012 AIV H5N1 was detected in turkeys and in cats, which ate the carcasses. An additional H5N1 outbreak occurred in January 2015, in 10 farms in two waves, the first in January-February and the second in May. The HP-AIV H5N1 isolates in all outbreaks were similar among the various focuses within the same outbreak, and within the three outbreaks in Israel. Genetic similarity was detected with Egyptian isolates from poultry and humans in the years 2013-2015, and with isolates from the West Bank and the Gaza Strip, all belonging to clade 2.2.1. We have conducted a genetic epidemiological study in corporation with Royal Veterinary College investigating the transmission of the virus according to spatial distances and genetic differences of affected farms.

The diagnostic assay for AIV included initial amplification by real-time RT-PCR for the matrix and hemagglutinin genes. In AIV HA H5 positive cases, the neuraminidase gene is tested.

AIV Low Pathogenic H9N2

In Israel AIV H9N2 were isolated and characterized molecularly since the year 2000. All AIV H9N2 isolates belong to the G1 lineage, possess a cleavage site attributed to low pathogenic AIV and were classified into five phylogenetic groups. As no intermediate phylogenetic forms were isolated, it was assumed that the new virus introductions source in Israel had been external. By a complex computation of the dynamic phylogeny, we showed that the Israeli H9N2 viruses indeed share similarity to strains circulating at the Asian continental level, inferring the epidemiology of H9N2 in Israel and within the region. It is interesting to note that both in the case of the recent H9N2 epidemic (caused by group IV) and H5N1 incursions, the strains isolated in Israel are similar to the viruses circulating in Egypt and in other bordering countries, confirming the trans-boundary spread of the infections and the need to increase the efficacy of the control measures at trans-national level.

Although H9 viruses are classified as low photogenic, there pathogenicity in the field can be severe and the clinical signs and mortality can be very high, despite the fact than in experimental studies with specific pathogen free chicks little clinical disease is observed. The high mortality observed in the field in many cases are associated with mixed infections with other pathogens or poor environmental conditions. Because of the economic costs, AIV H9N2 infection is controlled by vaccination. The isolates A/ty/Israel/965/02 and A/ck/Israel/215/07, belonging to the second and fourth genetic groups, respectively, were selected for vaccine strains. Because of the introduction of new phylogenetic groups and the evolution of the virus, surveillance and characterization of new viruses is required to assure that the available vaccines are adequate for disease control.

Although it has been shown by an advanced computation method that vaccination drives the AIV mutation rate, the overall state of AIV H9N2 infection, disease and losses have decreased during the last 2 years since the introduction of the variety of H9N2 vaccines. This has decreased the economic cost of the disease, and viral detections of AIV H9N2 infection have become rare. A
binational USA-Israel study helped to clarify the actual situation, and showed that at low to moderate levels of infectivity a cross-clade protection was afforded. Moreover, we showed that antigenic cartography can be used to predict the most effective vaccine virus in case of avian and human highly pathogenic influenza viruses, for the low pathogenic H9N2 is not feasible, due to limited systemic replication.

Research Gaps

Ecology - A major knowledge gap is the understanding of how the virus persists in the region despite the heat and arid conditions that is generally thought to inactivate these viruses quickly. The role of wild birds remains a major concern as well as peri-domestic birds. However, wild birds are the purview of the Environmental Ministries and not the Agriculture Ministries.

Epidemiology - All countries suffer from lack of resources to do surveillance at the levels they would prefer to be at. The decreased surveillance has been a combination of decreased international research support and the high cost to import reagents. Several of the countries have the capability to generate sequence information and most release the information to public databases.

Pathogenesis - An increased understanding of the important species that may be a reservoir for avian influenza and Newcastle disease virus remain an important goal. This again may involve experimental work with wild birds are peri-domestic birds like pigeons or sparrows. The role of co-infections with other pathogens increasing disease associated with H9N2 avian influenza is a major field observation. However, little is known precisely on how these co-infections increase disease and/or increase susceptibility to infection.

Diagnostic Discovery - Currently available diagnostics, including real-time RT-PCR and hemagglutination inhibition tests are performing adequately for diagnosis and initial characterization. However, as the viruses continue to evolve there are concerns that these tests may result in false positives. Ongoing evaluations of diagnostic tools are needed. In addition, all the countries agreed that having reagents for other common poultry diseases are needed to support the poultry industry to not only provide rule-outs for avian influenza and Newcastle disease, but actually determine a diagnosis in a disease outbreak.

Vaccines - For avian influenza, the goal of having efficacious vaccines is a constantly moving target that requires good epidemiological data to understand what is circulating and appropriate vaccines that are licensed and available. For the H9N2 avian influenza viruses, because they are low pathogenic strains, field strains can be used in adjuvanted vaccines. The field strains do need to be monitored to assure the vaccine match is still good. However, with the highly pathogenic strains, a different approach must be used. A number of different vaccines including reverse genetics and viral vectored vaccines are available. However, as a general rule, the available vaccines only provide partial protection to poultry in the field because of antigenic drift. Commercial companies have not been willing to incur the costs to constantly update vaccines, so there is a need for technology that can be developed locally to meet the local needs. Although not all countries vaccinate for avian influenza, those that do have vaccine development and testing as a higher priority.
Research priorities

All the participants in the avian influenza section have interest in sharing sequence and epidemiology information. All the countries also had interest in improving overall poultry diagnostics, so that the diagnostic laboratories can not only diagnose avian influenza and Newcastle disease, but also test for other common poultry diseases like infectious bursal disease on infectious bursal disease. For avian influenza all the countries are concerned with both highly pathogenic H5 influenza and low pathogenic H9 avian influenza. Current epidemiology suggests that viruses do move between the different Middle Eastern countries, so that there are opportunities to share beneficial research information and potentially vaccines between countries. There is interest in doing wild bird surveillance, but in most of the countries wild bird surveillance is the responsibility of the Environment Ministries and not the Agriculture Ministries. There is some interest and concern about the effect of climate and global warming on the persistence and transmission of the virus.

Potential research collaborations

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Proposed contributions to potential research collaboration by Country:

**Egypt** - The country has decreased capacity for epidemiologic surveillance because of lack of resources, but still retains the equipment and expertise to increase the amount of surveillance that can be performed. Egypt has facilities to safely handle HPAI and can do pathogenesis
experiments as well as ferret work (a model for human infections), which can be used to inform public health. There is interest in doing field and laboratory studies on doves because scientists in Egypt feel this may be a reservoir host.

Post vaccination serologic monitoring is also needed to determine how effective vaccination is in the country. There is interest in developing reverse genetics technology for vaccine development in Egypt and/or testing other new vaccine technologies.

**Israel** - Israel has recently collaborated on H9N2 epidemiology in commercial poultry flocks, and is interested in understanding the role of vaccination and the evolution of the virus. Considerable interest in having a better understanding of how virus is introduced on farms remains a goal, in part because of the anecdotal experience of outbreaks on farms with perceived good biosecurity. Doing a retrospective analysis on the 2015 H5N1 outbreaks was one approach.

**Jordan** - H9N2 continues to be a major issue for the poultry industry, with most surveillance being concentrated in this sector. However, because the funding for the surveillance comes from the poultry industry, the results are not released publicly. Therefore a major goal would be to have surveillance that is publicly funded so that both the level of surveillance can increase and the results can be published and sequences released to GenBank.

**Morocco** – No information provided.

**Palestine** - Because of the proximity of Israel and Palestine, the research needs for both countries are similar. However, the resources are more limited in Palestine and there is more effort to maintain and improve diagnostics.

**Saudi Arabia** – No information provided.

**Tunisia** - The primary interest is in the rapid diagnosis of outbreaks and doing surveillance to understand where avian influenza outbreaks are present. Interest to share information within the region was expressed.
Foot-and-Mouth Disease (FMD)

Disease situation in the Middle East and North Africa

Regional overview:

Foot-and-mouth disease (FMD) virus affects livestock worldwide. There are seven different serotypes, each with a diversity of topotypes, genetic lineages and strains. Some lineages have different properties that may contribute to sporadic spread beyond their recognized endemic areas. During the recent past (2007-2014) there have been important global events in terms of strain emergence and distribution that have impacted Middle Eastern countries (Brito, Rodriguez et al. 2015). One of the most important events was the re-emergence of lineage O/ME-SA/Ind-2001 in 2008. Notably, this lineage normally restricted to India, Bangladesh, Nepal and Bhutan, was also found in Saudi Arabia and Libya in 2013 and has caused several outbreaks in Tunisia and Algeria in 2014–2015. In 2012, FMDV SAT2 caused outbreaks in Egypt and the Palestinian Autonomous Territories (Ahmed, Salem et al. 2012). Another significant event was the emergence of FMDV Asia1 Sindh-08 in the Middle East. Lessons learned from past events, point out the need for an integrated strategy that comprises coordinated global and regional efforts for FMDV control and surveillance. Specific local characteristics related to host, environment and virus that condition FMD occurrence in the Middle East should be carefully considered in establishing research gaps and priorities for the region.

FMD situation in Egypt

Epidemiological and current situation

The epidemiology of foot-and-mouth disease (FMD) in Egypt is complicated by the circulation of endemic FMD viruses (FMDV), as well as incursions of exotic viral strains from the Middle East and Africa.

- Between 1972 and 2005 only serotype O was reported in Egypt.
- Widespread outbreaks due to serotype FMD type A topotype African lineage G-IIk$^{EN05}$ occurred by importation of infected cattle in 2006.
- FMD Type O Panasia II strain which has circulated from 2007-2011.
- FMD type A Iran-05BAR-08 “Libya 2009” was introduced During the period between 2010–2011.
- Multiple introductions in 2012: FMD type O topotype EA-3 unnamed viruses strain, FMD type A topotype AFRICA, lineage G-IV$^{iSM-12}$, Two lineages of FMD SAT2 topotype VII, Ghb-12 and Alx-12 have introduced at the same time and caused widespread (170/203 confirmed/suspected outbreaks) in Feb-May 2012 and it disappears before SAT2 vaccine practices. The immediate source of the FMDV SAT2 causing these outbreaks is presumably from one or more Sub-Saharan African countries; it was not possible to use sequence data to categorically define the exact origin of these viruses.
FMD type SAT2- Alex Eg/12 lineage was reported again in Jan-Jun 2014 moreover 2 outbreaks in Middle and south county region near the end of March 2016.

- FMD toptype A strain Iran 05 BAR-08 Outbreaks were circulating with no evidence that other FMD virus types were circulating In 2013, but it was very closely related to strain found in Rafah, Gaza Strip, Palestinian Autonomous Territories in April 2013. Limited FMD type A tobtpe.Iran-05 lineage BAR-08 outbreaks were in 2014-2015.
- FMD type O unnamed EA-3 topotype outbreaks were distributed all over the country In 2014 and it was continued circulation in 2015-2016. It was epidemiologically characterized by sustainability all around the year with the occurrence of two peaks winter and a smaller summer peak.
- Generally FMD in Egypt is epidemiologically characterized by a seasonal appearance and alternately displacement each other, one winter peak followed disappearance and or reoccurrences in a next year. 1 to 3 FMD lineages are circulating every year but only one of them is country wide predominant than the others. It is usually one of the FMD type O lineages in most of the years.

These data highlight the ease by which FMDV can cross international boundaries and emphasize the importance of close monitoring of the FMD situation in the region to define risks of future outbreaks, as well as to ensure that control measures and vaccine used are appropriate for these virus strains.

**Diagnostic capacity** The flow chart for testing of virological samples is: 1) testing of samples in the Ag serotyping and virus isolation, followed by 2) pan and type specific FMD qRT PCR for Ag ELISA negative poor quality samples (saliva, swaps) .3) direct sequencing with type-specific VP1 primer sets for Ag ELISA positive samples or TC virus isolates. NSP ELISA and SP ELISA kits have been used for some post vaccination serological studies.

**Control** Risk factors for FMD infection in calves were related mainly to: 1) Livestock owners moving between different premises and animal markets 3) Milk collectors and feed suppliers visiting multiple premises per day. 3) Vaccination teams not applying strict biosecurity measures

The main questions on FMD control in Egypt are Vaccine quality in form of Vaccinal strains, the role of vaccination and the control coast benefits. The available FMD WRL available data about vaccine matching results against the recently circulating FMD type O and A lineages from the African and Asian poles was impressive for the imported vaccine. The locally produced vaccine has not been yet evaluated against the currently circulating strains.

Recently there is agreement between Egypt and Sudan on the steps, time line and actions will be applied spontaneously if there is a suspected clinical disease in the country of origin quarantines.

But further coordination, regular sample shipment to the FMD WRL and immediate publishing the results include antigen matching and sequence data is now urgently required to formally assess the possible risk of transmission between countries in the region and contingency planning.

**Research Gaps**

Gaps in available scientific information
• Specific country detailed vaccine matching information (in addition to WRL reports)
• Gaps in information sharing – on FMD strains circulating
• Gaps in other animal species in addition to domestic livestock – e.g. circulation in wildlife
• Lack of tracking tools for outbreaks due to porous multiple/uncontrolled borders and lack of information sharing

Gaps in veterinary medical countermeasures

• Sharing of reagents and information among countries of strains and animal sera from natural outbreaks
• Development of vaccinated animal sera for in-country vaccine evaluation studies
• Sending samples to reference labs that do not have a shared back program with country of origin - due to regulatory issues
• Causes of lack of protection in vaccinated animals
• Need training for field diagnostic capacities - need training of field personnel in sample collection and submission
• Need for methodologies for vaccine evaluation in the laboratory and under field conditions (e.g. vaccine evaluation)

Research priorities

Priority 1:

• Development of standardized in-country assessment of vaccine quality (efficacy, and matching with circulating strains surveillance)
• Production of reference sera
• Post vaccination monitoring methodology – e.g., duration of immunity
• Serological surveillance using NSP tests to identify hot spots of transmission and educate vaccination programs
• Serotype specific testing of NSP - positives

Priority 2:

• Knowledge attitude practices analysis in farmers and trades in FMD in endemic settings
• Studies on patterns/determinants of animal mobility within countries and between countries

Priority 3:

• Optimization of RT-PCR techniques that will cover more strains
• Molecular characterization of outbreak strains (isolates) for diagnostics and surveillance purposes

Priority 4:

• Role of wild species (e.g., boars, gazelles) on maintenance and transmission of FMD
• Evaluation of pen-side diagnostics (FMD antigen) as tool in the field and laboratory

Potential research collaborations

The Global Foot-and-mouth Disease Research Alliance (GFRA) was introduced to the group as a mechanism to foster international collaborations. Its aim is to build a global alliance of partners to generate and share knowledge - in a virtual FMD laboratory - to develop tools that can better combat the threat of the disease.

GFRA will facilitate the mission of the alliance by supporting strategic objectives that will advance the progressive control and eradication of FMD. One of the main activities of GFRA is the organization of biannual research meetings to be held in host countries.
Countries that have interest and willing to collaborate in the following five areas of research:

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<th>Pathogenesis</th>
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Proposed contributions to potential research collaboration by Country:

**Egypt:**
Has established vaccine manufacturing and laboratory diagnostic capacities. Does not have adequate vaccine matching capacity. In addition has epidemiological capacity.

**Israel**
Has diagnostic discovery capacity and reference material that could be made available to other countries. Has epidemiological capacity.

**Jordan**
Has diagnostic capacity and epidemiological capacity.

**Morocco**
No information was available.

**Palestine**
Has diagnostic and epidemiology capacity.

**Saudi Arabia**
Has diagnostic and epidemiology capacity.

**Tunisia**
Has diagnostic capacity.
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# Report Contributors

<table>
<thead>
<tr>
<th>#</th>
<th>Name</th>
<th>Organization</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mohammad Ghazi Kayali</td>
<td>Human Link</td>
<td><a href="mailto:ghazi@human-link.org">ghazi@human-link.org</a></td>
</tr>
<tr>
<td>2</td>
<td>Morcos Ibrahim Yanni Gerges</td>
<td>Animal Health Research Institute</td>
<td><a href="mailto:morcosyanni@yahoo.com">morcosyanni@yahoo.com</a></td>
</tr>
<tr>
<td>3</td>
<td>Mahmoud Essam Rashad Hamdy</td>
<td>Animal Health Research Institute</td>
<td><a href="mailto:merhamdy@hotmail.com">merhamdy@hotmail.com</a></td>
</tr>
<tr>
<td>4</td>
<td>Abdullah Abdulzaher Mohamed Selim</td>
<td>Animal Health Research Institute</td>
<td><a href="mailto:abdullahselim@yahoo.com">abdullahselim@yahoo.com</a></td>
</tr>
<tr>
<td>5</td>
<td>Ahmed Refaat Ahmed Habashi</td>
<td>Animal Health Research Institute</td>
<td><a href="mailto:a_habashi@yahoo.com">a_habashi@yahoo.com</a></td>
</tr>
<tr>
<td>6</td>
<td>Sherein Galal Mohamed Khoulasy</td>
<td>Animal Health Research Institute</td>
<td><a href="mailto:shereengalal@yahoo.com">shereengalal@yahoo.com</a></td>
</tr>
<tr>
<td>7</td>
<td>Mohamed Atea Ahmed Ezzelden</td>
<td>General Organization Veterinary Services</td>
<td><a href="mailto:ashrafatea@yahoo.com">ashrafatea@yahoo.com</a></td>
</tr>
<tr>
<td>8</td>
<td>Naglaa Radwam Ahmed Hassan</td>
<td>General Organization Veterinary Services</td>
<td><a href="mailto:nagla.hassan6@gmail.com">nagla.hassan6@gmail.com</a></td>
</tr>
<tr>
<td>9</td>
<td>Sofiane Sghaier</td>
<td>L’Institut de Recherche Vétérinaire de Tunisie</td>
<td><a href="mailto:sghaiersoufien@yahoo.fr">sghaiersoufien@yahoo.fr</a></td>
</tr>
<tr>
<td>10</td>
<td>Heni Haj Ammar</td>
<td>Ministry of Agriculture</td>
<td><a href="mailto:heni_hah@yahoo.fr">heni_hah@yahoo.fr</a></td>
</tr>
<tr>
<td>11</td>
<td>Ahmed Rejeb</td>
<td>Ecole Nationale de Médecine Vétérinaire</td>
<td><a href="mailto:rejebahmed2002@yahoo.fr">rejebahmed2002@yahoo.fr</a></td>
</tr>
<tr>
<td>12</td>
<td>Alessandro Ripani</td>
<td>World Organization for Animal Health</td>
<td><a href="mailto:a.ripani@oie.int">a.ripani@oie.int</a></td>
</tr>
<tr>
<td>13</td>
<td>Jaoud Berrada</td>
<td>Institut Agronomique et Vétérinaire Hassan II</td>
<td><a href="mailto:jaouad.berrada@gmail.com">jaouad.berrada@gmail.com</a></td>
</tr>
<tr>
<td>14</td>
<td>Youseff Lhor</td>
<td>Institut Agronomique et Vétérinaire Hassan II</td>
<td><a href="mailto:y.lhor@yahoo.fr">y.lhor@yahoo.fr</a></td>
</tr>
<tr>
<td>15</td>
<td>Abdulaziz Nasser Alagaili</td>
<td>King Saud University</td>
<td><a href="mailto:aziz99@gmail.com">aziz99@gmail.com</a></td>
</tr>
<tr>
<td>16</td>
<td>Iyad Ahmad Mashal</td>
<td>River Company for Fair Development Services</td>
<td><a href="mailto:iyd@bezeqint.net">iyd@bezeqint.net</a></td>
</tr>
<tr>
<td>17</td>
<td>Imad Mukarker</td>
<td>Ministry of Agriculture</td>
<td><a href="mailto:drimad_s@yahoo.com">drimad_s@yahoo.com</a></td>
</tr>
<tr>
<td>18</td>
<td>Iyad A. J. Adra</td>
<td>Ministry of Agriculture</td>
<td><a href="mailto:iyadadra@yahoo.com">iyadadra@yahoo.com</a></td>
</tr>
<tr>
<td>19</td>
<td>Imadeddin Moh’d Ahmed Albaba</td>
<td>Freelance Consultant &amp; Researcher</td>
<td><a href="mailto:imadibaba@gmail.com">imadibaba@gmail.com</a></td>
</tr>
<tr>
<td>20</td>
<td>Issa H. I Shatla</td>
<td>River Company for Fair Development Services</td>
<td><a href="mailto:rc.river.company@gmail.com">rc.river.company@gmail.com</a></td>
</tr>
<tr>
<td>21</td>
<td>Khawala Salem Qasem Alnujoon</td>
<td>AMIDEAST</td>
<td><a href="mailto:Khawala.Alnujoon@fao.org">Khawala.Alnujoon@fao.org</a></td>
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<tr>
<td>22</td>
<td>Mustafa I. M. Tamayza</td>
<td>Animal Health Expert</td>
<td><a href="mailto:mismael@gmail.com">mismael@gmail.com</a></td>
</tr>
<tr>
<td>23</td>
<td>Cyril Gay</td>
<td>USDA Agricultural Research Service</td>
<td><a href="mailto:Cyril.Gay@ARS.USDA.GOV">Cyril.Gay@ARS.USDA.GOV</a></td>
</tr>
<tr>
<td>24</td>
<td>Ibrahim Shaqir</td>
<td>USDA Agricultural Research Service</td>
<td><a href="mailto:Ibrahim.Shaqir@ARS.USDA.GOV">Ibrahim.Shaqir@ARS.USDA.GOV</a></td>
</tr>
<tr>
<td>25</td>
<td>Luis Rodriguez</td>
<td>USDA Agricultural Research Service</td>
<td><a href="mailto:Luis.Rodriguez@ARS.USDA.GOV">Luis.Rodriguez@ARS.USDA.GOV</a></td>
</tr>
<tr>
<td>26</td>
<td>Steve Olsen</td>
<td>USDA Agricultural Research Service</td>
<td><a href="mailto:Steven.Olsen@ARS.USDA.GOV">Steven.Olsen@ARS.USDA.GOV</a></td>
</tr>
<tr>
<td>27</td>
<td>John Shekailo</td>
<td>USDA Agricultural Research Service</td>
<td><a href="mailto:John.Shekailo@ARS.USDA.GOV">John.Shekailo@ARS.USDA.GOV</a></td>
</tr>
<tr>
<td>28</td>
<td>David Suarez</td>
<td>USDA Agricultural Research Service</td>
<td><a href="mailto:David.Suarez@ARS.USDA.GOV">David.Suarez@ARS.USDA.GOV</a></td>
</tr>
<tr>
<td>29</td>
<td>Vincent Munster</td>
<td>National Institute of Allergy &amp; Infectious Diseases</td>
<td><a href="mailto:munsterv@niaid.nih.gov">munsterv@niaid.nih.gov</a></td>
</tr>
<tr>
<td>30</td>
<td>Jane Coury</td>
<td>National Institute of Allergy &amp; Infectious Diseases</td>
<td><a href="mailto:courym@niaid.nih.gov">courym@niaid.nih.gov</a></td>
</tr>
<tr>
<td>31</td>
<td>Boris Yakobson</td>
<td>Ministry of Agriculture</td>
<td><a href="mailto:BorisY@moag.gov.il">BorisY@moag.gov.il</a></td>
</tr>
<tr>
<td>32</td>
<td>Menachem Benai</td>
<td>Ministry of Agriculture</td>
<td><a href="mailto:menachemba@moag.gov.il">menachemba@moag.gov.il</a></td>
</tr>
<tr>
<td>33</td>
<td>Michel Bellaiche</td>
<td>Ministry of Agriculture</td>
<td><a href="mailto:michelb@moag.gov.il">michelb@moag.gov.il</a></td>
</tr>
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<td>34</td>
<td>Shimon Perk</td>
<td>Ministry of Agriculture</td>
<td><a href="mailto:Shimonpr@moag.gov.il">Shimonpr@moag.gov.il</a></td>
</tr>
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<td>Tamir Goshen</td>
<td>Ministry of Agriculture</td>
<td><a href="mailto:tamirgo@moag.gov.il">tamirgo@moag.gov.il</a></td>
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<td>Ministry of Agriculture</td>
<td><a href="mailto:Orane@moag.gov.il">Orane@moag.gov.il</a></td>
</tr>
<tr>
<td>37</td>
<td>Conrad Frueling</td>
<td>Friedrich-Loeffler Institute</td>
<td><a href="mailto:Conrad.Freueling@fli.bund.de">Conrad.Freueling@fli.bund.de</a></td>
</tr>
<tr>
<td>38</td>
<td>Thomas Mueller</td>
<td>Friedrich-Loeffler Institute</td>
<td><a href="mailto:Thomas.Mueller@fli.bund.de">Thomas.Mueller@fli.bund.de</a></td>
</tr>
<tr>
<td>39</td>
<td>Saad Gharaibeh</td>
<td>Jordan University of Science and Technology</td>
<td><a href="mailto:saadgh@just.edu.jo">saadgh@just.edu.jo</a></td>
</tr>
<tr>
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<td>Jafar Obekhat</td>
<td>Ministry of Agriculture</td>
<td><a href="mailto:jobeitat12@yahoo.com">jobeitat12@yahoo.com</a></td>
</tr>
<tr>
<td>41</td>
<td>Nabil Haiat</td>
<td>Jordan University of Science and Technology</td>
<td><a href="mailto:hailatn@just.edu.jo">hailatn@just.edu.jo</a></td>
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<tr>
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<td>Zaidoun Kalanzi</td>
<td>UN Food and Agriculture Organization</td>
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