BIOSAFETY AND DIAGNOSTICS OF BRUCELLOSIS AT LMA

Beradze I., Nikolaishvili M., Donduashvili M., Zakareishvili M.

Laboratory of the Ministry of Agriculture (LMA), Tbilisi, Georgia

Brucellosis is one of the most common livestock zoonosis in Georgia, resulting in significant economic losses. Many species of animalsand humans become ill with Brucellosis. The laboratoryof the Ministry of Agriculture (LMA) investigatessuspect-Brucella samples each year. Over the last three years, over 7,000 samples were tested. From this, 753 were positive. During the CBR, GG-17 project, suspect samples were collected from three regions:Kakheti, KvemoKartli, Imereti. Samples included blood, serum and milk from cattle, sheep, and goats. Samples were tested using serology, bacteriology, and molecular diagnostics. In total, 32 bacterial isolates were recovered and identified: 11 samples contained *B. melitensis*, 22 contained *B. abortus* and were confirmed by AMOS PCR. Samples were also tested using serology, bacteriology, and molecular diagnostics. GIS was also used to map positive cases and suspect isolates to construct maps based on strain location and epidemiological data from the livestock.

БІОБЕЗПЕКА ТА ДІАГНОСТИКА БРУЦЕЛЬОЗУ В ЛАБОРАТОРІЇ МІНІСТЕРСТВА СІЛЬСЬКОГО ГОСПОДАРСТВА ГРУЗІЇ

Берадзе І., Ніколаішвілі М., Дондуашвілі М., Закареішвілі М.

Лабораторія Міністерства сільського господарства, м. Тбілісі, Грузія

RABIES IN GEORGIA

NinidzeL., MenteshashviliS.

National Food Agency of the Ministry of Agriculture, Tbilisi, Georgia (NFA)

Rabies is a zoonotic and endemic disease in Georgia. The disease is and demonstrates a stable epizootic and unfavorable situation. There are two types of epizootic Rabies. The first one is a forest type. In such case the infection used to be spread via wild animals and a city type when domestic animals are infected and the infection is spread via homeless dogs and cats. Some preventive and elimination measures have been carried out against Rabies though the infection still remains an issue. 1,335 cases of Rabies were registared during 2000–2012. Among them are *Mammalias* 86.6 %, domestic animals 12 % and wild mammals 1.2 %.Epidemiologic reseraches of Rabies proves that wild animals play a great role in spreading the infection. The incidence of attack of wild animals on domestic animals and humans has become more frequent, especiallyduring seasonal pasturing in the summer. The territory of Georgia includes a vast mountainous area (>50 %)and approximately 36 % of the country is woody where more than 100 species of mammals are the inhabitants. Pathogensamples of 36 different species of wild animls were investigated at the lab from 2006 to 2012. 17 positive cases wereregistered and revealed.Epidemilogical research proves that cases of Rabies in domestic mammalsare revealed in the villages directly bordered to the woody areas. (The risk of get infected by Rabies in such vicinitiesis especially high).The migration process of wild animals directly impacts the increase in Rabies cases of domesticated animals. There is an active planned/scheduled campaign of oral vaccination twice a year at the regional level.Avaccination risk assesment shall be taken into consideration while the oral vaccination campaign is initiated. The vaccination process shall be kept under surveillance and monitored. In order to estimate the effectivness of the oral vaccination campaign, the lab research of wild animals will be evaluated.

СКАЗ У ГРУЗІЇ

Нінідзе Л., Ментешашвілі С.

Національне управління продовольством Міністерства сільського господарства, м. Тбілісі, Грузія

UDC 636.09:614.4

SURVEILLANCE OF FOOT AND MOUTH DISEASE: A STUDY OF 2011-2012 OUTBREAKS IN KAZAKHSTAN

Sytnik I., Karibayev T., Tyulegenov S., Abenova A., Tashkenbayev A., Yerimbetov S.

National Reference Veterinary Center (NRVC), Astana, Republic of Kazakhstan

Foot and mouth disease (FMD) is a highly contagious viral disease which affects cloven-hoofed animals. Its economic impact on the livestock industry in Kazakhstan and neighboring regions is of great importance. In order to assess the epizootic situation of FMD in Kazakhstan, sera samples were collected from cattle, goats, and sheep in 2011-2012, according to our standard disease surveillance plan, which covered three designated areas: FMD-free, vaccinated, and outbreak. A total of 14 areas were covered, including 10 to 20 districts in each region, resulting in a total amount of 76,851 sera from cattle and small ruminants. In the outbreak areas, sera samples were collected and tested by ELISA to detect antibodies to nonstructural proteins of FMD virus (FMDV). In addition, tissue samples from suspect animals were tested for viral antigen by ELISA and viral genes by quantitative PCR (qPCR). To characterize the virus currently circulating in Kazakhstan, tissue samples were sent to the FAO/OIE Regional Reference Lab for FMD (FGI-ARRIAH) in Vladimir, Russia for direct sequencing of the viral capsid protein 1 (VP1) gene. Sequence comparison and phylogenetic analysis of the complete VP1 coding region revealed virus was type O PanAsia strain. It was this same FMD serotype which was responsible for the 2001 pandemic in United Kingdom and outbreaks in other regions including Kazakhstan where it was previously unseen. This study represents the first thorough analysis of the epidemiological situation of Kazakhstan for FMD, and should be of great help for further efforts in the implementation of prevention measures and control of FMD.

Introduction. Foot and mouth disease virus (FMDV) is a member of the genus Aphthovirus within the family Picornaviridae. It is a single-stranded, positive-sense, un-enveloped RNA virus. FMDV is typically transmitted by direct contact among infected and susceptible

animals and can be aerosolized in the environment. Due to its highly contagious nature, FMDV is a significant transboundary disease. It affects various mammals, including livestock, pigs, sheep, goats and buffalo. There are 7 immunological distinct serotypes of FMDV: A; O (most common in world); C; Asia1; and South African Territories1, 2, and 3 (SAT1, SAT2, and SAT3); which limits vaccine effectiveness. It is endemic in Central Asia, including Kazakhstan, and outbreaks have severe economic effects. In 2011 and 2012, numerous outbreaks of this disease were registered in various regions of Kazakhstan. The resultant efforts to control the virus and the economic impact of culling animals, as well as impact on trade, make this virus an important agriculture pathogen in the country.

Due to its highly contagious nature, FMDV is a significant transboundary disease. FMD vaccines are developed by inactivating virus, and the subsequent antibody response to the proteins making up the structure of the inactivated virus provides immunity. FMD vaccines should not contain non-structural proteins (NSP), which are produced during replication of live virus in a natural FMD infection. This study seeks to take advantage of this differential antibody response to detect the prevalence of animals exposed to natural FMD infections without the data being skewed from the antibody response of vaccinated animals. For this study, ELISA tests that detect antibodies to NSP in animals that have been infected with FMD and mounted an immune response were used. Since ELISA tests that detect NSP are not serotype specific, they will detect antibodies produced by all FMDV strains. The monitoring area where samples were collected was divided into zones: FMD-free, vaccinated and epizootic zones. During the monitoring studies selected blood serum samples were tested for detection of antibodies to NSP. The purpose of this research was to determine the epizootic situation by region, as well as testing the effectiveness of ELISA for detection of antibodies to NSP 3ABC to differentiate vaccinated animals from infected.

Serological monitoring studies did not include PCR tests. Real-time PCR was conducted only in cases where clinical symptoms were displayed for express diagnosis during outbreaks, since antibodies to NSP are not always detected in this case. Samples of saliva, aphthae, or organs from dead animals were tested. Samples taken from animals with a clinical presentation of FMD were also tested for FMD antigen by ELISA.

Data and samples obtained from areas of recent outbreaks in 2012 were used in this study. Also to better understand the spatial and temporal dynamics of the disease, we used geographic information system (GIS) to map and analyze historic and contemporary outbreak data from 1955 to 2012 for each major livestock group in Kazakhstan. The spatial temporal analyses and categorical exploratory mapping of outbreaks against transport networks were used to investigate spatial patterns and develop hypotheses for further modeling of this disease.

Materials and Methods. During 2011-2012, ELISA tests were carried out at the NRVC to identify antibodies to nonstructural proteins of foot and mouth disease. The ELISA tests for antibody were performed using the following test kits: SVANOVIR® 3ABC-Ab, CHEKIT FMD IDEXX, and PrioCHEK FMDV NS. In 2011-2012, the total blood serum samples examined for antibodies to NSP were 49,682 and 76,851, respectively. Samples tested at NRVC included stored animal sera from recent outbreaks. In cases when clinical symptoms allowed for express diagnosis of FMD during outbreaks, stored samples of animal saliva, aphthae, or organs from animals were tested by real-time quantitative PCR (qPCR).

For qPCR, RNA extraction was conducted from clinical material using the Qiagen RNeasy Mini kit and detection of RNA was performed using the Tetracore FMDV qPCR kit according to the manufacturer's instructions and run on Roche LightCycler® 2.0 instrument. To conduct research to identify the FMD virus antigen (Ag FMD) by ELISA and serotype (type A22, type O or type Asia-1) testing was carried out using kits from the manufacturer in the OIE reference laboratory (Vladimir, Russia). Sequencing of the FMD virus viral capsid protein 1 (VP1) gene was conducted in the OIE reference laboratory (Vladimir, Russia).

The National Veterinary Reference Center (NRVC) in Astana, Kazakhstan maintains a database containing information on FMD outbreaks across several decades, including 2000-2012. For the FMD data-set development, these data were used to develop a series of GIS data layers to explore the spatial patterns of the disease by virus type and geography. We also used these data to construct density surfaces and space-time models of the disease. Kernel density analysis was used for the kernel density estimation technique in the ArcGIS Spatial Analyst extension to map areas of outbreak concentration by each livestock group. These outputs were visualized using the standard deviation color ramp to identify areas with significant concentrations (clusters) of outbreaks on the Kazakh landscape.

Results. Quantitative and Qualitative Analysis-Eight percent of the 76,851 serum samples collected from cattle, sheep and goats in 2012 tested positive for antibodies to non-structural FMD proteins using ELISA (Table 1). Typing of stored samples of saliva, aphthae, or organs from infected animals with a clinical presentation of FMD according to the OIE reference laboratory (Vladimir, Russia), revealed Type O virus to be associated with cases in all regions. In addition, one region, Zhambyl, was found to have antigen of FMD virus type A22.

Table 1 – Positive samples

Samples positive for NSP FMDV antibodies by region					
No	Region	Animal species	Samples Received	Samples Tested	Samples Positive
1	Aqmola	Cattle, sheep, goats	1670	1670	25 (1.5 %)
2	Almaty	Cattle, sheep, goats	130	130	110 (84.6 %)
3	Aktobe	Cattle, sheep, goats	3215	3215	24 (0.7 %)
4	Atyrau	Cattle, sheep, goats	2476	2476	34 (1.4 %)
5	Eastern Kazakhstan	Cattle, sheep, goats	17105	17105	2721 (16 %)
6	Zhambyl	Cattle, sheep, goats	36856	36856	3215 (8.7 %)
7	Western Kazakhstan	Cattle, sheep, goats	3300	3300	146 (4.4 %)
8	Karaganda	Cattle, sheep, goats	1257	1257	46 (3.7 %)
9	Kyzylorda	Cattle, sheep, goats			
10	Pavlodar	Cattle, sheep, goats	2427	2427	14 (0.6 %)
11	Southern Kazakhstan	Cattle, sheep, goats			
12	Kostanay	Cattle, sheep, goats	5570	5570	15 (0.3 %)
13	Mangistau	Cattle, sheep, goats	504	504	7 (0.1 %)
14	Northern Kazakhstan	Cattle, sheep, goats	2341	2341	4 (0.2 %)
Total			**76851**	**76851**	**6361 (8.3 %)**

Spatial patterns. The spatial-temporal analysis (Figure 1) demonstrates division of the territory of the Republic of Kazakhstan into infection zones. Regions of the Republic of Kazakhstan that vaccinate animals with a trivalent vaccine (subtypes A, O, Asia-1) are indicated in Figure 2. In addition, several FMD outbreaks among cattle and small ruminants were registered during the reporting period in different regions of the country (Figure 3). Zones with high rates of infection and outbreaks were observed in most parts of Zhambyl region, western and south-western areas of the Almaty region, and central and southern areas of the East Kazakhstan region. Zones of moderate rates of infection were observed in central, eastern and northern areas of the South-Kazakhstan region; central, northern, eastern and southern areas of the Almaty region; northern and western territory of East Kazakhstan region; and central and southern areas of the Karaganda region. In the area of low prevalence of FMD are Kyzylorda Northwest Territories; western, northern and eastern territories of Karaganda region; western territory of Aktobe and East Kazakhstan regions; and eastern and central areas of Atyrau and West Kazakhstan regions.

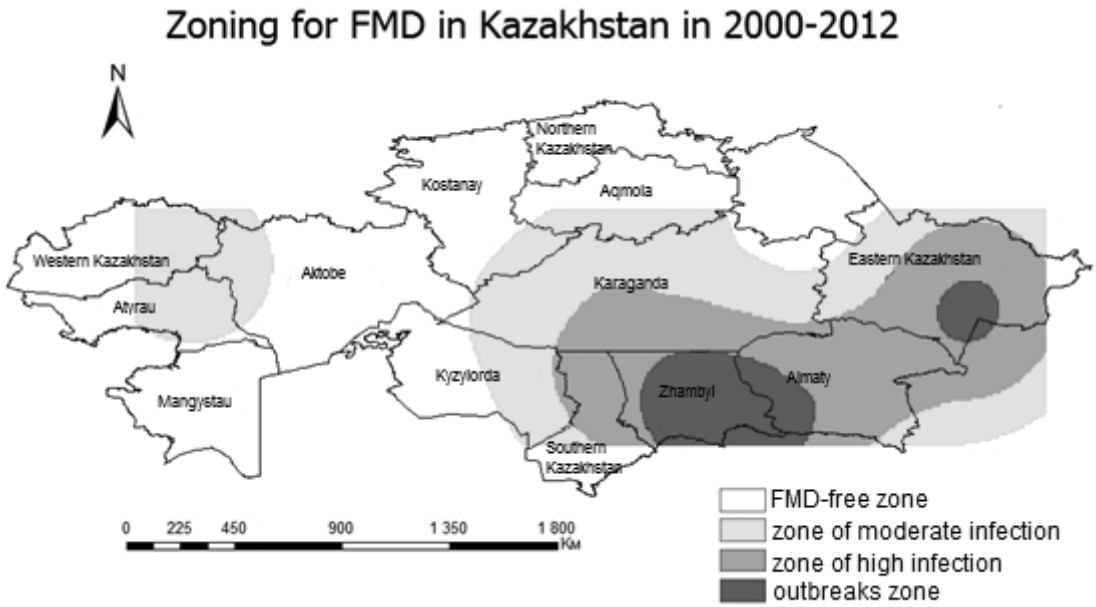

Figure 1. The map shows division of the territory of the Republic of Kazakhstan into infection zones according to the number of FMD affected areas for the period between 1991 and 2012, prepared in ArcGIS using the kernel density estimation technique. The zone of high infection is located in central and south-eastern regions of the Republic where outbreaks frequently occur and presence of seropositive animals is high. The outbreaks zones are more localized and represent areas of actual disease occurrence.

Conclusion. The study data presented here is very important to demonstrate and analyze the epizootic situation in the country overall. This knowledge will enable better allocation of available resources for future FMD outbreak, prevention and control. Overall, it is evident that outbreaks are registered primarily in southern regions of the country, which is attributed to a higher population of livestock compared to other regions as well as to a possible complex epizootic situation with FMD in neighboring countries that have also experienced outbreaks. In the future, a comparative study of antigen detection rate in vaccinated and non-vaccinated animals is planned to be conducted to continue the project.

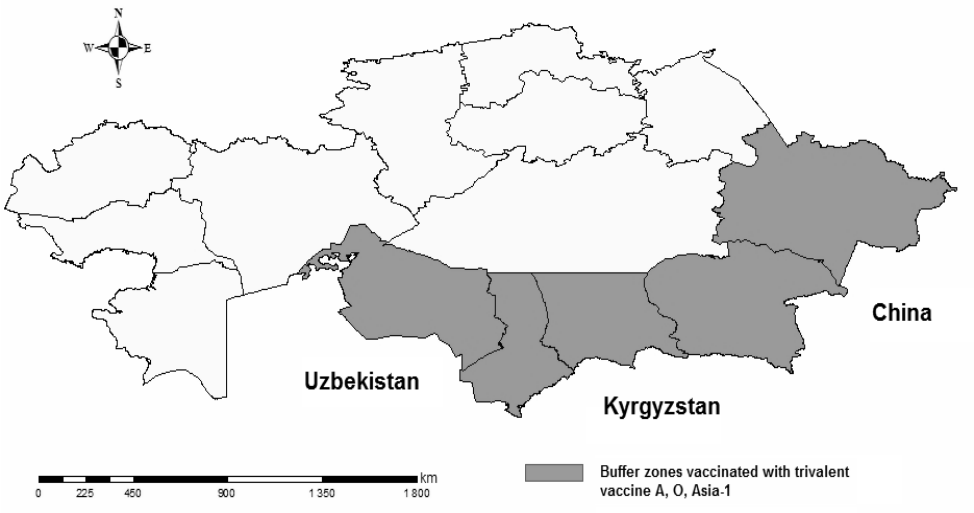

Figure 2. Areas vaccinated in 2011-2013.

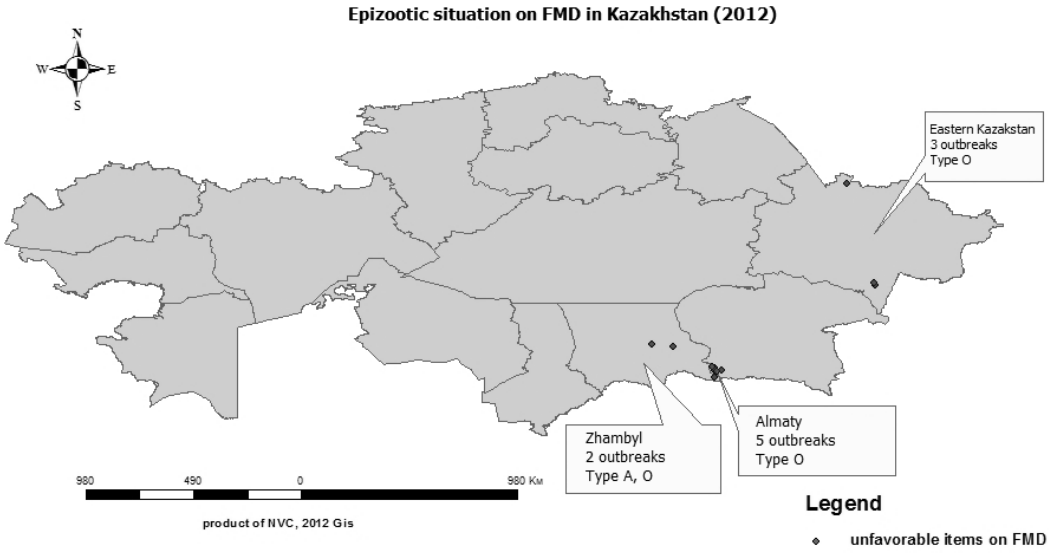

Figure 3. According to the spatial data on the FMD epidemiological situation in 2012 outbreaks were registered in southern regions of the country and the eastern part of Kazakhstan.

Reference

1. Samuel, A.R. Foot-and-mouth disease type O viruses exhibit genetically and geographically distinct evolutionary lineages (topotypes) [Text] / A.R. Samuel, N.J. Knowles // J. Gen. Virol. – 2001. – Vol. 82. – P. 609–621. **2.** Evading the host immune response: how foot-and-mouth disease virus has become an effective pathogen [Text] / M.J. Grubman [at al.] // FEMS Immunol. Med. Microbiol. – 2008. – Vol. 53, № 1. – P. 8–17. **3.** Knowles, N.J. Recent Developments in the Epidemiology of Foot-and-mouth disease: Genetic Relationships Determined by Nucleotide Sequence Analysis [Text] / N.J. Knowles, A.R. Samuel // Foot-and-mouth disease Newsletter. – 1994. – № 1(2). – P. 13–19. **4.** Knowles, N.J. Molecular epidemiology of Foot-and-mouth disease virus [Text] / N.J. Knowles, A.R. Samuel // Virus Res. – 2003. – Vol. 91. – P. 65–80.

КОНТРОЛЬ ЯЩУРУ: ДОСЛІДЖЕННЯ СПАЛАХУ 2011-2012 РР. У РЕСПУБЛІЦІ КАЗАХСТАН

Ситник І., Карібаєв Т., Тюлегенов С., Абенова А., Ташкенбаєв А., Єрімбетов С

Національний ветеринарний референс-центр, м. Астана, Республіка Казахстан

Ящур високо контагіозне вірусне захворювання, яке вражає парнокопитних тварин. Для оцінки епізоотичної ситуації щодо ящуру в Казахстані було відібрано 76851 зразок сироватки крові від великої рогатої худоби та дрібної рогатої худоби в 14 областях протягом 2011–2012 років. Проведено виявлення антигенів вірусу ящуру та антитіл до збудника методами ІФА, а також виявлення РНК ящуру за допомогою ПЛР.

SEROLOGICAL RESEARCH METHODS AT THE LABORATORY FOR THE MINISTRY OF AGRICULTURE, TBILISI, GEORGIA

Tigilauri T., Donduashvili M., Goginashvili K.

Laboratory of the Ministry of Agriculture (LMA), Tbilisi, Georgia

The Laboratory of the Ministry of Agriculture is the leading diagnostic laboratory for animal diseases in Georgia. LMA has the capability to identify and study especially Dangerous Pathogens (EDP's), such as Brucellosis, Rinderpest, and ASFV. According to approved DTRA-based diagnostic algorithms, bacteriological, serological and molecular biological test are completed at LMA. The great majority of samples received at LMA are tested serologically. For Brucella disease, in 2009-2012, a total of 13,882 animal blood and serum specimenwere tested by Rose Bengal assays and 1,644 out of them were confirmed by ELISA. Due to the variability andcost of the ELISAassay, a new methodology has been implemented at LMA. The lab frequently receives single samples and the use of ELISAisn't cost or time-efficient. Accordingly, ELISA has been replaced by a new confirmatory using fluorescence polarization. The FPA assay was introduced at the LMA in 2012, under the TAP 1 project. In total, 46 out of 980 RB positive samples were confirmed by FPA. In another study during the period 2009–10, the LMA team investigated 1,041 ASF suspected samples by ELISA-Ab and. ELISA-Ag. Based on the results of LMA serological tests (1,440 samples were tested in 2009), Georgia was recognized as free from Rinderpest by OIE. Serological tests were also completed for FMD, in 2011–2012, 16,061 samples were investigated and 6,776 samples were tested within the scope ofan FAO investigation. LMA has high qualified specialists that also participate in a competitive testing process with various diseases conducted by different reference laboratories world-wide; many of which are confirmed by these laboratories.

СЕРОЛОГІЧНІ МЕТОДИ ДОСЛІДЖЕННЯ ЛАБОРАТОРІЇ МІНІСТЕРСТВА СІЛЬСЬКОГО ГОСПОДАРСТВА, ТБІЛІСІ, ГРУЗІЯ

Тігілаурі Т., Дондуашвілі М., Гогінашвілі К.

Лабораторія Міністерства сільського господарства, м. Тбілісі, Грузія