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ACCESSION NO: 0412556 **SUBFILE:** CRIS**PROJ NO:** 1940-32000-057-02S **AGENCY:** ARS 1940**PROJ TYPE:** USDA COOPERATIVE AGREEMENT **PROJ STATUS:** TERMINATED**START:** 01 SEP 2007 **TERM:** 31 AUG 2012**INVESTIGATOR:** RODRIGUEZ L L; TUSNEEM M E**PERFORMING INSTITUTION:**

NATIONAL AGRIC RESEARCH CENTER

ISLAMABAD, PAKISTAN

CHARACTERIZATION OF LOCAL ISOLATES OF FOOT-AND-MOUTH DISEASE VIRUSES AND DEVELOPMENT OF VECTOR BASED VACCINES

OBJECTIVES: Carry out genomic evaluation of field isolates of Foot-and-Mouth Disease Virus (FMDV). Establish and employ improved methods for FMDV detection and typing.

APPROACH: Almost every year, new outbreaks of Foot-and-Mouth Disease (FMD) occur in different parts of Pakistan. At present, serotypes A, O and Asia-1 are prevalent in the country, however, no institute is carrying out routine subtyping of new isolates. On the other hand, FMD-vaccine is also being used in the field; however, the vaccine produced within the country at public institutes is a bivalent product, not including any new sub-type prevalent in the country. This vaccine only covers 5% of the population and another 5% of the population is vaccinated by using imported vaccines from different sources. The above information indicates that there is a need to undertake proper typing and characterization of FMDV on a regular basis. This will lead to the development of type-specific vaccine locally. To undertake the proposed study, field isolates of FMDV will be collected, propagated and characterized on the basis of RT-PCR, sequencing and phylogenetic analysis. The sequence data of the previous isolates for selecting variants to be used for vaccine development and evaluation.

PROGRESS: 2007/09 TO 2012/08

Progress Report Objectives (from AD-416): Carry out genomic evaluation of field isolates of Foot-and-Mouth Disease Virus (FMDV). Establish and employ improved methods for FMDV detection and typing. Approach (from AD-416): Almost every year, new outbreaks of Foot-and-Mouth Disease (FMD) occur in different parts of Pakistan. At present, serotypes A, O and Asia-1 are prevalent in the country, however, no institute is carrying out routine subtyping of new isolates. On the other hand, FMD-vaccine is also being used in the field; however, the vaccine produced within the country at public institutes is a bivalent product, not including any new sub-type prevalent in the country. This vaccine only covers 5% of the population and another 5% of the population is vaccinated by using imported vaccines from different sources. The above information indicates that there is a need to undertake proper typing and characterization of FMDV on a regular basis. This will lead to the development of type-specific vaccine locally. To undertake the proposed study, field isolates of FMDV will be collected, propagated and characterized on the basis of RT-PCR, sequencing and phylogenetic analysis. The sequence data of the previous isolates for selecting variants to be used for vaccine development and evaluation. The goal of this collaborative

research project is to further the understanding of pathogenesis of Foot-and-Mouth Disease Virus (FMDV) variants and to provide knowledge of FMDV isolate serotypes A, O and Asia- 1 characterization to enhance future vaccine development efforts in Pakistan. Activities for FY 2012 included travel by a team of US scientists from ARS, PIADC and the University of California, Davis to Pakistan's National Agricultural Research Center (NARC) and the National Veterinary Laboratory (NVL) where two training courses were conducted, one on sample collection (probangs) & laboratory diagnostics of FMDV using tissue culture techniques and the other on epidemiology basic concepts and FMD Biportal tools applied to the FMD control programs. On-going training for scientists from NARC and partnering laboratories in Pakistan was conducted in FY 2012 inclusive of a two week training course at NVL on the technology transfer of a new more susceptible cell line for detection of FMDV and epidemiology workshops. In this regard, a training manual was prepared and a copy of the same, along with an electronic copy on a CD were provided to each of the participant of the course. ARS, PIADC provided NARC with LF Bovine Kidney (LFBK) cell lines which were successfully propagated at NARC and NVL and used in FMDV isolation and detection studies. ARS, PIADC also provided media and instruction for cell culture. A comprehensive research plan was developed and protocols standardized between the labs for vaccine strain analysis and filed isolate identification. Molecular characterization of local isolates included the examination of forty two samples which were process and 40/42 samples were tested positive with FMDV by viral isolation and rRT-PCR and these positive isolates were sequenced for confirmation of FMDV serotypes and phylogeny. Six samples were positive for serotype A, 14 for serotype O and 13 for serotype Asia 1 and 7 with mixed infection respectively. The nucleotide sequence of the complete capsid coding sequence was used to infer the genetic relationships of 40 recent field isolates from Pakistan. Samples were collected in October and November and processed using LFBK cells. These samples will be propagated and hyper immune serum raised against FMDV vaccines available in Pakistan for vaccine matching studies at ARS, PIADC. The role of the Asian buffalo on FMDV transmission was also examined. 52 livestock farms were identified registered with the project. They will be monitored over the next year. Sample collection inclusive of serum, probangs and swabs will be conducted. A total of 300 animals were ear tagged and blood samples, swabs and probang samples were collected from these animals. These serum samples were analyzed using NSP ELISA and out of the 300 samples, 233 were sero-positive for FMDV non-structural proteins. Twenty four probang samples were inoculated in LFBK cells and CPE's were observed in four samples which will be confirmed. These samples are undergoing serotype identification. In FY 2013, virus isolation and Immuno Histo Chemistry (IHC) on the tissues received from persistent animals is planned. Vaccine matching studies included vaccine trails conducted on 13 animals using two commercially available vaccines in Pakistan in an effort to develop hyper immune serum at NARC, Pakistan. These sera will be shipped to PIADC to be used for vaccine matching studies with the FMDV strains circulating in Pakistan to determine efficacy. These sera will be kept at ARS, PIADC for further testing in FY 2013 with new strains of FMDV isolates circulating in Pakistan. This will help the policy makers in Pakistan to decide which FMDV vaccine to be used in the field to protect any further outbreaks. Epidemiological analysis was conducted during FY 2012 inclusive of time, space and host species information as contributed to accumulated nucleotide and amino acid variation of serotypes. A total of 83 FMDV serotype O VP1 sequences were collected from 2005 through 2011. Results suggested that the lineage-specific genetic variation observed in FMDV O in Pakistan is species-time dependent, but is not specific for species. This information will be used in the implementation of vaccination campaigns in the region. This collaborative agreement will expire in FY 2012 and a new non-funded cooperative agreement has been established to all further our joint research efforts. No publications were produced during the reporting period. Technologies transferred in FY 2012 include LFBK cell line to NARC for use in virus isolation at National Veterinary Laboratories and Animal Health Program, NARC, Pakistan for

the detection and diagnosis of FMDV.

PUBLICATIONS (not previously reported): 2007/09 TO 2012/08

No publications reported this period.
