

# OIE/FAO FMD Reference Laboratory Network

## Annual Report 2010

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Editors comment: This report is the result of continued cooperation between a number of OIE/FAO reference laboratory staff and would not be possible without the support of a number of International agencies including the OIE, FAO, EU, EuFMD and Defra. I would like to extend my personal thanks to those who have contributed information and comment to the network meetings and to this report, and would especially thank those members of WRLFMD who have supported me in the preparation of this document. Jef Hammond March 2011.

# **Introduction to the OIE/FAO FMD Reference Laboratory Network Report**

The Network of OIE/FAO FMD Reference Laboratories has been established with two principal goals:

(1) To understand global virus distribution and patterns and provide vaccine recommendations

and

(2) To Improve the quality of laboratory testing carried out by international and national reference laboratories.

This requires sharing and joint evaluation of surveillance information from laboratory diagnosis, serotyping, genetic characterisation and vaccine matching tests and harmonisation of standards for diagnostic procedures.

This report is divided into two parts providing an update on progress towards each of these goals.

Additional information about the Network can be found at: <http://www.foot-and-mouth.org/>

# PART 1

Genetic and antigenic diversity and global distribution of foot-and-mouth disease viruses. Information gaps, threats and vaccine recommendations

## 1.1 Executive Summary:

Foot-and-mouth disease (FMD) is highly contagious, infects a wide variety of domestic and wildlife hosts and occurs as multiple non-cross-protective virus serotypes. Its presence restricts trade opportunities for endemic countries and poses a constant threat to those countries free of the disease. FMD viruses are not randomly dispersed throughout the world but are associated with particular ecological niches. The distribution is affected by cyclical upsurges in the prevalence of particular strains that may be associated with viral evolution, waning population immunity and/or opportunities presented by movements of animals and their products. This can give rise to pandemic spread affecting new regions. Global surveillance for FMD aims to identify the current hazards and to predict heightened risk so that appropriate diagnostics and vaccines are available for their detection and control. This requires sustained effort directed towards the monitoring of FMD outbreaks and ideally also of FMDV circulation and persistence, along with collection and characterisation of FMD viruses and integration of findings with associated epidemiological intelligence. Such an extensive effort requires a team approach encompassing national and international disease control services and their laboratories along with commercial vaccine and diagnostic providers. The OIE/FAO FMD Reference Laboratory Network is a vital contributor to the global control of FMD and provides opportunities and expertise for developing and sustaining laboratory capacity and capability, exchange of materials and technologies, harmonising approaches to diagnosis and supporting complementary research. Laboratories within the network regularly receive samples for FMD diagnosis from many parts of the world. The *in-vitro* antigenic properties of selected isolates are assessed for vaccine matching and nucleotide sequencing allows precise characterisation of new isolates and tracing of their origin by comparison with viruses held in virus collections. This analysis assists the monitoring of the 'real time' emergence and spread of FMD virus globally. The clustering of FMD viruses into 7 virus pools, with 3 pools covering Europe, the Middle-East and Asia, 3 pools covering Africa and 1 pool covering the Americas, is now enabling a targeted approach to progressive FMD control through the combined activities of OIE and FAO and the regional authorities. The worldwide distribution of the different serotypes and variants of FMD virus as compiled in 2010 and the associated activities of the network laboratories are presented in this document.

## 1.2 Introduction

Global surveillance for foot-and-mouth disease (FMD) aims to identify the current hazards and to predict heightened risk so that appropriate diagnostic tests and vaccines are available for their detection and control. This requires sustained effort directed towards the monitoring of FMD outbreaks and ideally also of FMD virus (FMDV) circulation and persistence, along with collection and characterisation of FMD viruses and integration of findings with associated epidemiological intelligence. Such extensive efforts require a sustained team approach encompassing national and international disease control services and their laboratories along with commercial vaccine and diagnostic providers.

The work of international FMD reference laboratories in collecting and characterising FMDV isolates has been reviewed (Ferris and Donaldson, 1992; Kitching 2000) and more recently with emphasis on the requirements and methodologies for vaccine selection (Paton et al., 2005). FMDV is unevenly distributed throughout the world reflecting factors such as livestock density and species mix, patterns of husbandry, animal movement and trade, wildlife reservoirs and incentives and capacities for disease control. The virus exists as multiple serotypes and subtypes with absent or incomplete cross-immunity, likely differences in species predilections and modes of persistence and transmission, and with distributions that are partly based on historical and chance events. The situation is dynamic and affected by viral evolution, waxing and waning host immunity and changing ecosystems and trading patterns. Despite the propensity and opportunities for spread of FMDV into new regions, comparisons of VP1 gene sequences of viruses submitted over many years do show a tendency for similar viruses to recur in the same parts of the world (Knowles and Samuel, 2003; Rweyemamu et al., 2008) and this presumably reflects some degree of either ecological isolation or adaptation. On this basis, the global pool of FMD viruses can be subdivided into seven ‘regional pools’ in which genetically and antigenically distinctive virus strains tend to occur within a defined region.

The seven ‘Regional Pools’ referred to throughout this report are shown in Figure 3 and represent:

- Pool 1 – Eastern Asia**
- Pool 2 – Southern Asia**
- Pool 4 – Eastern Africa**
- Pool 5 – Western Africa**
- Pool 6 – Southern Africa**
- Pool 7 – South America**

Virus circulation and evolution within regional virus pools results in changing priorities for appropriately adapted vaccines. Periodically, viruses spread between pools and to free regions.

Ferris NP, Donaldson AI. (1992) *Rev Sci Tech*.11(3):657-84.

Kitching RP. (2000) *Ann N Y Acad Sci*. 916:139-46.

Paton DJ, Valarcher JF, Bergmann I, Matlho OG, Zakharov VM, Palma EL, Thomson

GR. (2005) *Rev Sci Tech*. 24(3):981-93.

Knowles NJ, Samuel AR. (2003) *Virus Res*.91(1):65-80.

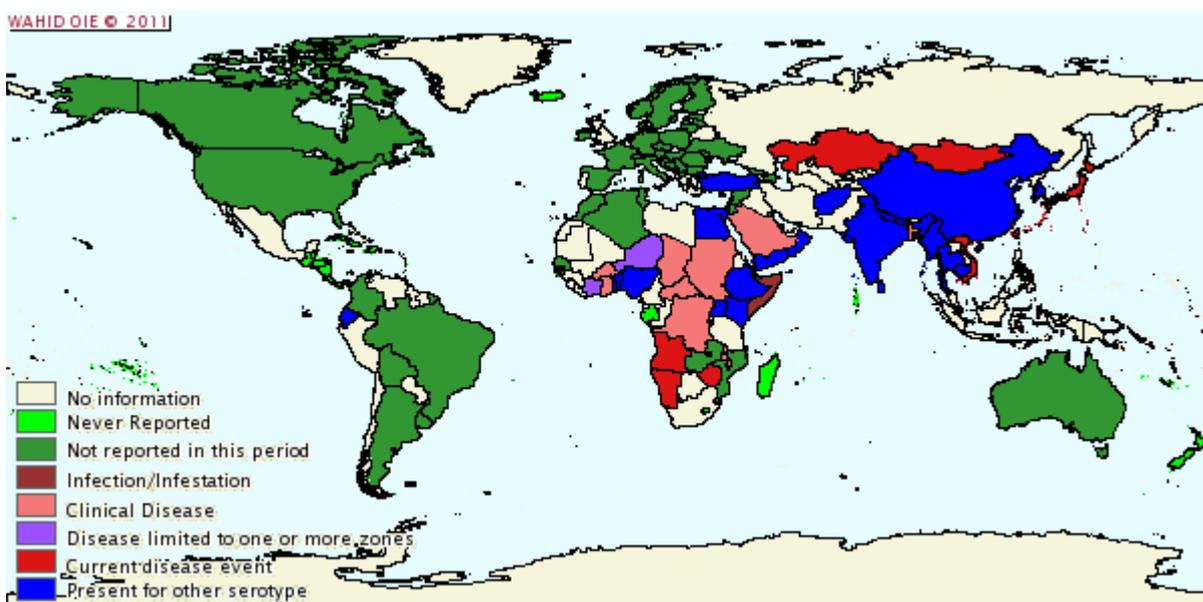
Rweyemamu M, Roeder P, Mackay D, Sumption K, Brownlie J, Leforban Y, Valarcher JF, Knowles NJ, Saraiva V. (2008) *Transbound Emerg Dis*. 55(1):57-72.

### 1.3 Overview of the Global FMD situation in 2010

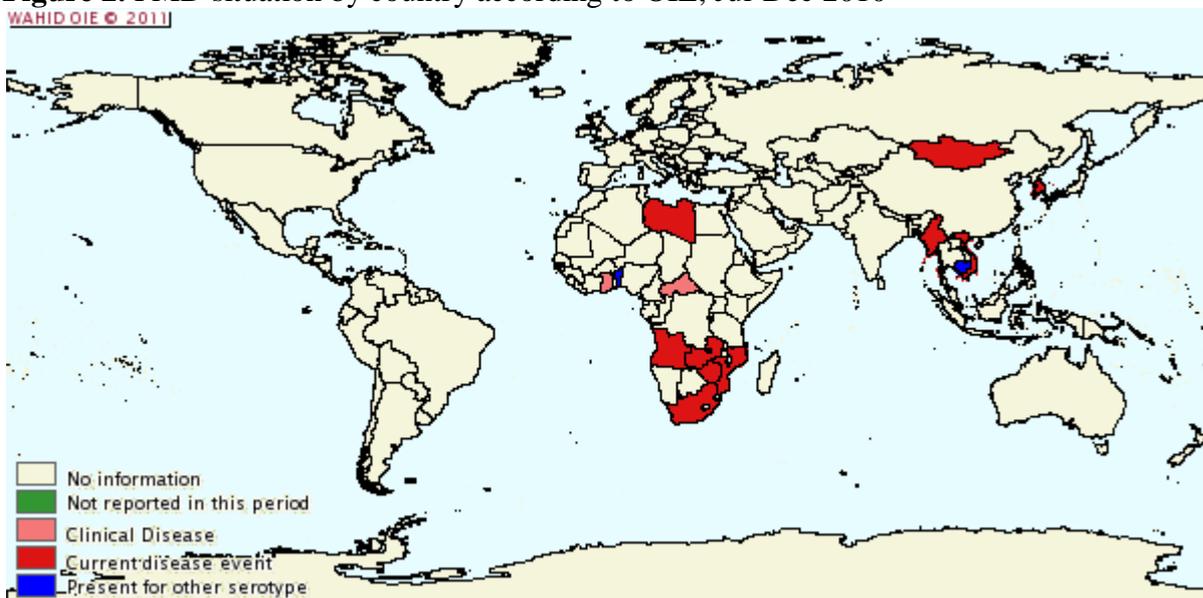
Both Japan and Republic of Korea reported FMD outbreaks in 2010 losing their status as countries listed by OIE as FMD-free without vaccination.

Within endemically and sporadically infected parts of the world there have been upsurges of cases, sometimes leading to the submission of samples to reference laboratories and indicating an enhanced risk of collateral spread. The majority of viruses have been isolated from samples submitted from Africa and Asia which remain the major reservoirs for the FMD virus. In Southern America, FMDV circulation has mainly been detected in Ecuador and Venezuela. There has been a much welcomed increase in information from P.R. China for this report.

**Figure 1.** FMD situation by country according to OIE, Jan-Jun 2010<sup>1</sup>

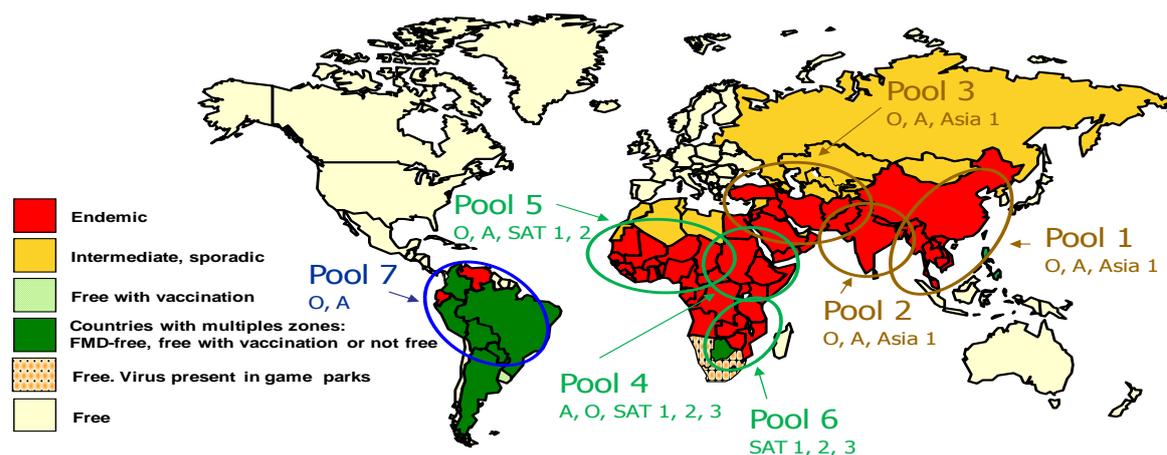


**Figure 2.** FMD situation by country according to OIE, Jul-Dec 2010



<sup>1</sup> The WAHID Interface provides access to all data held within OIE's new World Animal Health Information System (WAHIS): [http://www.oie.int/wahis/public.php?page=disease\\_status\\_map&disease\\_type=Terrestrial&disease\\_id=1&empty=999999&sta\\_method=semesterly&selected\\_start\\_year=2008&selected\\_report\\_period=1&selected\\_start\\_month=1&page=disease\\_status\\_map](http://www.oie.int/wahis/public.php?page=disease_status_map&disease_type=Terrestrial&disease_id=1&empty=999999&sta_method=semesterly&selected_start_year=2008&selected_report_period=1&selected_start_month=1&page=disease_status_map)  
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**Figure 3a.** The conjectured status of FMD in 2010 showing approximate distribution of regional virus pools.



Pool positions are approximate and colours indicate that there are three principal pools, two of which can be subdivided into overlapping areas

**Note on Pools 4-6:** In Africa there are currently three FMD virus pools loosely defined as covering East Africa (pool 4), West Africa (pool 5) and Southern Africa (pool 6). There is some overlap between pools 4 and 5. It has been suggested to extend pool 4 southwards to include Tanzania and to contract pool 6 to exclude that country. Thus pool 6 contains only the SAT serotypes.

### 1.3.1 Summary Information from the WRLFMD Quarterly Reports for 2010

**Pools 1-3:** Pool 1 – Eastern Asia, Pool 2 – Southern Asia, Pool 3 – Eur-Asia

**Afghanistan, Iran, Pakistan and Turkey:** The O-PanAsia-2 and A-Iran-05 lineages continue to dominate in these countries. The PanAsia-2 lineage has been subdivided into six sub-lineages named BAL-09, YAZ-09, FAR-09, SAN-09, ANT-10 and PUN-10. During 2010, the ANT-10 sub-lineage appeared to have become the dominant type O sub-lineage in Afghanistan, Iran, Pakistan and Turkey.

**Bhutan:** The O-Ind-2001 lineage (ME-SA toptype) was detected in Bhutan in samples collected in December 2009. This lineage was also found in Bangladesh and Nepal.

**Japan:** In early April 2010, FMDV type O was reported in Miyazaki Prefecture on the island of Kyūshū. Subsequently, a further 291 outbreaks were reported. The virus was identified as Southeast Asia (SEA) toptype (Mya-98 lineage) and found to be closely related to viruses occurring recently in the P.R. China, Hong Kong SAR, Republic of Korea, Myanmar and Thailand. This was the first outbreak of FMD in Japan since 2000. It appears that although disease was first identified on 9<sup>th</sup> April, FMD was not confirmed by laboratory testing until 20<sup>th</sup> April. The last (292<sup>nd</sup>) outbreak of FMD occurred on 04/07/2010 at Miyazaki city 3, Miyazaki.

**Kazakhstan:** An outbreak of FMD type O occurred on 09/06/2010 in cattle (n=140/639) at Novodolinka, Ereymentau, Aqmola, Kokchetav. A sequence was submitted to the WRLFMD from ARRIAH and found to belong to the ME-SA toptype, PanAsia-2 strain.

**Mongolia:** An outbreak of FMD type O was reported on 21/04/2010 in cattle (n=269/849) at Tashgai Bayanburd, Khalkh gol soum, Dornod. Subsequently, two further outbreaks were reported: on 14/05/2010 in cattle (n=37/375) at Zuun Shorvog, Buyanondor, Matad Soum, Dornod; and on 14/06/2010 in cattle (n=17) at Bayasgalant, Bulgan soum, Dornod.

A VP1 sequence was submitted to the WRLFMD from ARRIAH and shown to belong to the SEA topotype, Mya-98 lineage; Importantly, it appeared to be a different introduction to outbreaks in the P.R. China, Hong Kong SAR, Republic of Korea and Japan, as it was most closely related to viruses from Thailand and Malaysia from 2009. Two new outbreaks of FMDV type O occurred on 26/08/2010 at Sukhbaatar soum, Baga-3 Gun jalga, Sukhbaatar in sheep and goats and on 02/09/2010 at Teeliin bulag, Tsagaanchuluut baga, Chuluunhoroot soum, Dornod in cattle. All outbreaks (n=5) were in the east of the country. Three VP1 sequences were received from FGI-ARRIAH on the 16/11/2010. Analysis of these sequences showed them to be closely related to sequences determined from viruses previously isolated at the WRLFMD, i.e. FMDV O SEA topotype, Mya-98 lineage.

**Myanmar:** On the 10 September 2010, a single outbreak of FMD was detected in cattle at Kun Thee Pin, Maungdaw, Maungdaw, Rakhine State very close to the border with Bangladesh. It was typed as FMDV A, the first occurrence of this serotype in Myanmar since 1978. A VP1 sequence was generated from cDNA amplified from RNA submitted by the Thailand Regional Reference Laboratory (Pakchong), but no virus was isolated in cell culture. Phylogenetic analysis revealed that the virus was not related to type A viruses from Southeast Asia but was most closely related to viruses occurring in India in 2000. The sequence clustered with viruses occurring exclusively in India between 1997 and 2008.

**P.R. China:** FMDV O was detected in pigs in Guangdong province, P.R. China in February and March 2010. Further occurrences occurred in Gansu, Shanxi and Jiangxi provinces in March 2010. In February 2010 an outbreak due to FMDV O occurred in pigs on Penghu Island, Taiwan POC. The animals had been imported for slaughter from the Taiwan mainland. FMDV O was isolated from samples received from pigs in Hong Kong SAR. These viruses belonged to the SEA topotype (Mya-98 lineage) which is currently widely circulating in Southeast Asia.

FMDV type A (ASIA topotype) was found in Lao PDR (2008), Thailand (2009) and Vietnam (2008-2009). The viruses from these countries were closely related to each other and to viruses isolated in the P.R. China (2009) suggesting widespread circulation of this strain (which has yet to be named). Two outbreaks of FMDV A occurred in January 2010 (Xinjiang & Beijing provinces). Nine further outbreaks of FMDV type O occurred between April and June 2010: 07/04/2010 Nanyuan, GANSU. 17/04/2010 Tianfeng, Wushan, Tianshui, GANSU. 13/04/2010 Guding, Shibing, Qiandongnanzhou, GUIZHOU. 23/04/2010 Xuanhe Town, Shapotou District, Zhongwei, NINGXIA. 20/04/2010 Yecheng, Yecheng, Kashi prefecture, XINJIANG. 17/05/2010 Shigatse, TIBET. 10/06/2010 Shayidong, Kuerle, Bayingolin Mongolia Autonomous Prefecture, XINJIANG. 19/06/2010 The Aksu Western Suburb pig farming area, Aksu, XINJIANG. 22/06/2010 Haomen Town Qunawan Road Farm, Menyuan, Haibei Zhou, QINGHAI. Three further outbreaks of FMDV type O occurred in July and August 2010, bringing the total to 17: 27/07/2010: Chengdong village, Shan Dan, Zhang Ye, Gansu (pigs); 23/08/2010: Huangzangsi, Qilian, Habei Prefecture, Qinghai (cattle); 23/08/2010: Animal health inspection station of Er Ba Tai, Ku Che, Akesu, Xinjiang (cattle). A single outbreak of FMDV type O occurred in cattle on 04/10/2010 at Lage village, Dengqen, Qamdo, Tibet, bringing the total number of outbreaks to 18.

**Republic of Korea (South Korea):** FMDV type A (ASIA topotype) was detected in the Republic of Korea where seven outbreaks occurred in cattle and farmed deer in the Kyonggi-Do region between January and March. This virus was closely related to viruses isolated in the P.R. China (2009), Lao PDR (2008), Thailand (2009) and Vietnam (2008-2009). Seven outbreaks of FMDV type O (SEA topotype, Mya-98 lineage) were reported in April 2010, with no further reports in May or June. After the completion of stamping-

out and disinfection in the restricted zones, clinical surveillance and serological testing in the restricted zones were completed by the government veterinary authorities. All results were negative. Therefore, on 7 June 2010, the Ministry for Food, Agriculture, Forestry and Fisheries (MIFAFF) lifted the protection and surveillance zones as well as the risk zone which had been established within Kangwha-gun and Kimpo city on 8 April and 19 April, respectively. In late November 2010 South Korea reported new outbreaks of FMD type O. Analysis of four VP1 sequences received from the National Veterinary Research and Quarantine Service (NVRQS) has shown that these are viruses of the SEA toptotype, Mya-98 lineage, similar to those previously isolated earlier in 2010. At time of writing this outbreak is still ongoing (*Feb 2011*).

**Russian Federation:** Two outbreaks of FMDV type O occurred on 05/07/2010 at Abagaytuy, Zabajkal'skij Kray and on 26/08/2010 at Village Makarovo, Shilkinsky, Zabajkal'skij Kray. Both outbreaks involved cattle and pigs. Complete VP1 sequences of viruses from both outbreaks were submitted to the WRLFMD by ARRIAH. The sequences indicated that both viruses belonged to the SEA toptotype, Mya-98 lineage, however, the virus from the July 2010 outbreak was most closely related to viruses from Hong Kong SAR, Republic of Korea and Japan, while the virus from the August outbreak was closely related to a virus sequence from recent outbreaks in Mongolia, indicating different origins for the two outbreaks.

**Taiwan POC/Chinese Taipei:** Six pigs, which had been previously shipped to Penghu Island for slaughter from Taiwan Island, were found with vesicular lesions in a detention pen of the slaughterhouse. The sick pigs were immediately destroyed on 12 February 2010 after sampling. Positive results were obtained by the national laboratory by virus isolation, RT-PCR and antigen detection ELISA tests confirming that those pigs were infected with serotype O of foot and mouth disease virus. An outbreak occurred on 22/06/2010 in pigs at Baozhong Township, YUN-LIN. During routine active surveillance in the livestock markets, a pig was found to be NSP antibody positive. The prefecture animal disease control competent authority traced back to the farm of origin. The clinical investigation showed that 112 pigs on the farm had healed vesicular lesions. The prefecture animal disease control competent authority immediately destroyed a total of 163 pigs apparently all in the same pen. Cleaning and disinfection was then carried out on the index farm. The serotype of this outbreak was confirmed as O type on 29 June 2010. As the result of a routine serological survey using an NSP Elisa, 8 pigs in a herd of 1428, at Sinwu Township, T'ao-Yuan (10/08/2010) were found to be positive for FMDV antibodies. The pigs were all clinically healthy and attempts at virus isolation and RT-PCR were all unsuccessful. Further routine serological surveillance using an NSP ELISA, 10 pigs in a herd of 161, at South District, T'ai-Nan Shih (17/12/2010) were found to be positive for FMDV antibodies. The pigs were all clinically healthy and attempts at virus isolation and RT-PCR were all unsuccessful. these outbreaks have all been reported as serotype O.

**United Arab Emirates:** An outbreak of FMDV type O occurred in gazelle the Al Ain Wildlife Park & Resort, Abu Dhabi in March-April 2010. Analysis of VP1 sequences showed the virus to belong to the ME-SA toptotype, PanAsia-2 strain and to be closely related to viruses from Iran and Pakistan.

#### **Pools 4-6:** Pool 4 – Eastern Africa, Pool 5 – Western Africa, Pool 6 – Southern Africa

**Botswana:** An outbreak of FMD caused by the SAT 2 serotype occurred in 200/349 cattle at Lesoma, Chobe, Kasane between 26/07/2010 and 20/09/2010. Lesoma is a village located in the north-east of Botswana close to the borders with Zimbabwe and Namibia. In

conjunction with the Botswana Vaccine Institute (BVI), VP1 sequence analysis was performed on viruses isolated from an outbreak on 26/07/2010 at Lesoma (Kasane) close to the borders with Namibia, Zambia and Zimbabwe. The viruses were SAT 2 topotype III and most closely related to SAT 2 viruses from Botswana (Kasane) in 2005.

**Ethiopia:** FMDV types O (EA-3 topotype) and SAT 2 (XIII topotype) were detected in samples collected in February 2010.

**Kenya:** FMDV types O, SAT 1 and SAT 2 were isolated from samples collected in 2009 and 2010. A single type O virus belonged to the EA-1 topotype and was closely related to the vaccine strain K77/78. Nineteen SAT 1 viruses all belonged to topotype I (NWZ), but clustered with previously isolated viruses in four different sub-lineages. The single SAT 2 virus belonged to topotype IV and was closely related to two viruses previously isolated in 2009.

**Mozambique:** Four outbreaks of FMD were reported in cattle in the south of the country (Gaza province) on 24/09/2010. It is thought that FMD originated from infected cattle moved from Chicualacuala District (close to the Zimbabwe border) to Bilene and Chokwe Districts (which are located further south in Mozambique).

Two further outbreaks of FMD type SAT 2 were reported in cattle in the south of the country (Motaze, Magude, Maputo) on 17/12/2010. In conjunction with the BVI the VP1 sequences of seven virus isolates from the earlier outbreaks (September 2010 at Bilene, Gaza) showed they belonged to SAT 2 topotype I and were most closely related to viruses from South Africa (Kruger National Park).

**Namibia:** An outbreak of FMD SAT1 was reported on 09/04/2010 in cattle (n=88/1066) on Impalila island, Kabbe constituency, Caprivi. It was suspected that transmission of FMDV occurred from wild African buffalo (*Syncerus caffer*) resident in the area.

**Nigeria:** FMDV types O and A were isolated from samples collected in 2009. A single type O virus belonged to the EA-3 topotype and was most closely related to viruses from Nigeria (2007) and Sudan (2004-2008). Eight type A viruses belonged to the AFRICA topotype (lineage G-I) but fell into two distinct sub-lineages, one of which clustered closely with Kenyan viruses. The single SAT 2 virus belonged to topotype IV, but was not closely related to other SAT 2's. Four type A viruses all belonged to the AFRICA topotype, G-IV lineage; but fell into two distinct sub-lineages which correlated with their place of collection.

**South Africa:** An outbreak of FMD occurred in 19/80 cattle on 06/08/2010 at Malati, Ba-Phalaborwa, Limpopo in the FMD Protection Zone adjacent to the Kruger National Park.

**Zambia:** In conjunction with the BVI, the VP1 sequence of viruses isolated from an outbreak on 22/09/2010 at Mbala, Northern Province were determined. These belonged to the type O EA-2 topotype and were most closely related to viruses from the Democratic Republic of the Congo (2006), Uganda (2004-2006) and Tanzania (2009).

**Zimbabwe:** An outbreak of FMD type SAT 2 was reported in cattle (n=163/1377) at Kitwe dip tank, Plumtree, Mangwe, Matabeleland South on 28/05/2010. In conjunction with the BVI, the VP1 sequences of viruses isolated from this outbreak were determined. They belonged to SAT 2 topotype II and were most closely related to viruses isolated from African buffalo and cattle in Botswana, Namibia and western Zimbabwe, although none were very closely related (see below). Further outbreaks were reported: on 04/06/2010 at

Inswingo Dip Tank, Mangwe, Matabeleland South; and on 01/06/2010 at Kwhite, Mangwe, Matabeleland South and Ingwizi Dip Tank, Mangwe, Matabeleland South. Animals were suspected to have been in contact with previously infected animals during a previous outbreak that occurred in September 2009 at a feedlot in the area.

### **Pool 7: South America**

**Ecuador:** Nine FMD type O viruses were isolated from samples sent from various locations in Ecuador. These were the first samples WRLFMD had received from this region. With the gratefully appreciated assistance of Panaftosa we were able to compare the VP1 sequences to those from six virus isolates from Ecuador in 2009. These all belonged to the EURO-SA toptotype and viruses from each year clustered together and the clusters were most closely related to each other.

**Venezuela:** Panaftosa reported the presence of FMD type A in Venezuela in July 2010.

### **1.3.2 Information gaps**

Submission of samples from endemic regions has continued to be mainly in response to perceptions of increased number or severity of outbreaks, although in some cases there are proactive projects promoting sample submission. Reactive sampling provides an incomplete survey of the global virus pool and often lacks context in the form of information on the history accompanying the samples. Nevertheless, the bias towards things that are out of the ordinary may be helpful in providing early warning for new epidemics. It is hoped that there will be growing uptake of regional FMD control schemes following the continuation of the OIE/FAO Progressive Control Pathway FMD initiative under the Global Framework for eradication of transboundary animal diseases. The starting point for countries that are currently endemically infected with FMDV will be surveillance to identify the types of virus present and the weight of infection.

The main gaps in knowledge about the global distribution of FMDV come from countries without control schemes, especially in sub-Saharan Africa and in southern and central Asia.

### **1.3.3 Threats**

The greatest diversity of FMD viruses are in Africa and there are relatively few vaccines available that have been developed to protect against current African strains. Vaccines used in Africa may also lack stability and potency contributing to poor protection and increasing the threat of spread of outbreaks in the region and beyond. Historically, FMD viruses have rarely spread out of Africa, apart from sporadic incursions into the Middle East. However, changing patterns of global travel may alter this risk.

Despite growing efforts to control FMD in India and China and the attendant prospect of a reducing incidence of infection within their very large livestock populations, FMD viruses continue to circulate both in these countries and regionally. Therefore, Asia remains an important reservoir for serotypes O, A and Asia 1. FMD viruses have traditionally spread from Southern Asia, threatening FMD-free regions to the north and west in Central Asia and Europe. In fact, Asia has been the main source of outbreaks affecting the Middle East and Europe in the last twenty years (Valarcher et al., 2008). There is also a continued possibility of spread of FMDV through countries of the former Soviet Union into Europe and China and from Indo-China into northern and eastern neighbours. Vaccine strains

developed locally to control FMD within Asia are not maintained within European vaccine banks.

The incursions into the Middle East and North Africa of the O PanAsia 2 and A Iran 05 strains has continued and these still present a significant threat for further spread including to the west into Europe, and northwards into countries of the former Soviet Union. Serotype Asia 1 has apparently not been present in the Middle East since 2004, apart from a single incursion into Bahrain, early in 2009 that did not spread. Natural immunity against this serotype will therefore be low in Middle Eastern countries and based on the episodic incursions of the past, a reappearance may be due. Therefore, the risk of a new westward spreading epidemic should be borne in mind. A reservoir of the virus is certainly still present to the east in Southern Asian countries but following the reported single isolate from Pakistan which showed an unusually poor match to the Asia 1 Shamir vaccine held in many European vaccine antigen reserves no further isolates with these antigenic differences were identified in 2010.

In South America, Ecuador and Venezuela are the two countries which remain endemic, representing a threat to the cattle population in the areas free with or without vaccination in South America. Virus O<sub>1</sub> is still circulating in Ecuador and Venezuela as well as virus A<sub>24</sub> in Venezuela. The recent identification towards the end of 2010, of a variant type O virus in Ecuador, has necessitated the development of a revised strategy for FMD control in the region which is further discussed later in the report. Virus C<sub>3</sub> has not been identified since 2004 in the Amazon region from Brazil. In the early 2000, reintroduction of virus O occurred in the common border regions of Paraguay, Bolivia, Argentina and Brazil. As a consequence, a High Surveillance Zone was defined in the area and extensive serosampling for viral activity studies are being implemented in a joint programme between the four countries. The generally improving situation in South America may give rise to a reassessment of strain priorities for vaccine banks held by FMD-free countries elsewhere.

#### **1.4 Vaccine recommendations**

These take two forms. Regional recommendations are given in section 1.5, whilst the WRLFMD recommendations for FMD free countries are given in section 1.7.

Continuous molecular and antigenic characterisation of field viruses remains of utmost importance to generate intelligence and to inform rapid development of new vaccines that will provide coverage for specific regions. Regional vaccine selection does not always investigate whether vaccines produced elsewhere would be suitable, or conversely whether locally produced vaccines would have a wider application. This underscores the need for greater cooperation between the work of different regional reference laboratories. Commercial and national restrictions can prevent exchange of vaccine strains between reference laboratories and this lessens opportunities to evaluate the applicability of different vaccines to different regions. Harmonisation of local vaccine selection procedures is a priority so that results obtained in one laboratory can be extrapolated to other situations. Different manufacturing and licensing standards for vaccines also hinders the possibility for sharing of vaccines between regions.

Matching tests to check the antigenic suitability of vaccines to protect against circulating strains continue to reveal gaps in cover against SAT 2, and particularly important progressively towards the end of 2010 against serotype O PanAsia 2 isolates. There is still an urgent need for new SAT vaccine strains with good immunogenicity, adaptation to suspension cultures of BHK-21 cells and post-inactivation stability. Very late in 2010, a

PanAsia 2 vaccine was introduced which shows early promise in vaccine matching tests carried out at Pirbright but its efficacy in the field remains to be determined.

As well as improving the efficacy, stability and safety of production, research on FMD vaccines is still urgently required to establish a better understanding of the vaccine coverage required for protection under different livestock systems and to improve alternatives for potency testing of vaccine batches. Further research is also needed to improve vaccine selection methods.

## 1.5 Regional situation

### 1.5.1 Pool 1: EASTERN ASIA

Network labs receiving samples in 2010

Laboratory	Sample Nos.	Countries of origin
WRLFMD	108	Cambodia, Hong Kong, Japan*, Laos, Myanmar, South Korea, Thailand, Vietnam
RRLSEA	98	Cambodia, Myanmar, Vietnam, Thailand
LVRI	34	China

\* *sequence only*

**Indonesia, Singapore, Brunei** and the island states of **Malaysia** remained FMD-free without vaccination.

No outbreaks of FMD have been reported from the **Philippines** since 2005 and the country expects to be recognised as officially free of FMD in May 2011.

**China:** In **China**, 20 outbreaks have been reported in 2010 by LVRI (Figure 5 and Table1). No outbreaks of Asia 1 serotype were reported this year.. Three outbreaks of serotype A and 17 outbreaks of serotype O were confirmed by LVRI. Type A FMD occurred at the beginning of 2009 with 3 new outbreaks of A serotype confirmed between January and February 2010. VP1 sequencing showed that these viruses have a common source. A comparison with WRLFMD sequence data revealed a strong similarity to A/Tai/08 virus. The main concern has been the spread of the O MYA-98. This strain mainly has mainly affected pigs, although cattle and goats/sheep can also show clinical signs in some cases. Epidemiological analysis indicates that animal movements associated with trade are the main factors for the spread of this type of FMD outbreak and for transmission between provinces. From February to May 2010, there was rapid spread over a wide spatial distribution with high numbers of infections frequencies and strong clustering of outbreaks. From June 2010, the number of new cases has declined.

–For FMDV type A, according to the epidemic situation, vaccination is carried out on a small-scale on dairy cattle. The main control measures have been based on the culling of infected and suspected infected animals, controlling animal movements, epidemiological surveillance and quarantine measures.

–For the Asia 1 serotype, monovalent and bivalent vaccines which combined with type O (China99) are available for large-scale vaccination of ruminants.

–For serotype O, a Cathay-like virus vaccine (Os99) is also used in pigs.

–Previously, no vaccines have been produced against O- Myanmar 98 in China. In 2010 an O- Myanmar 98 vaccine strain was selected and developed as a vaccine by LVRI. Animal experiments show that the protection rate is 81.3% ( 13/16) between O-Mya98 and the vaccines with Os99. The new vaccine (O-Mya98) is used at a PD50 of 10.81 in pigs and

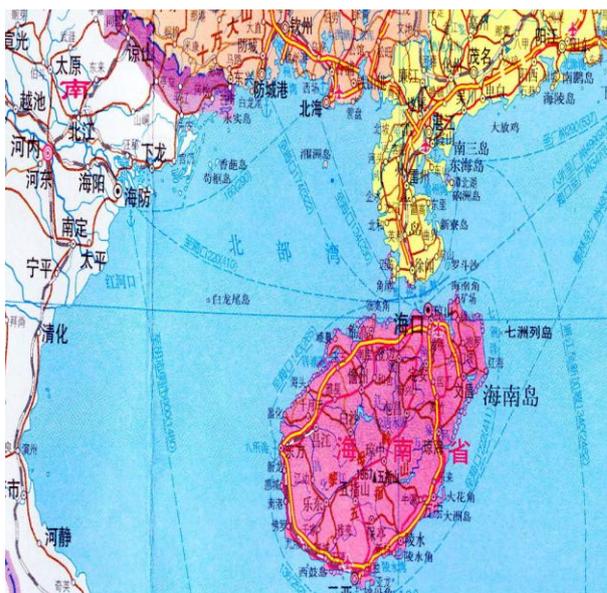
7.08 in cattle. In addition, a new synthetic peptide vaccine against O-Mya98 has been developed and animal protection experiments with this vaccine are ongoing.

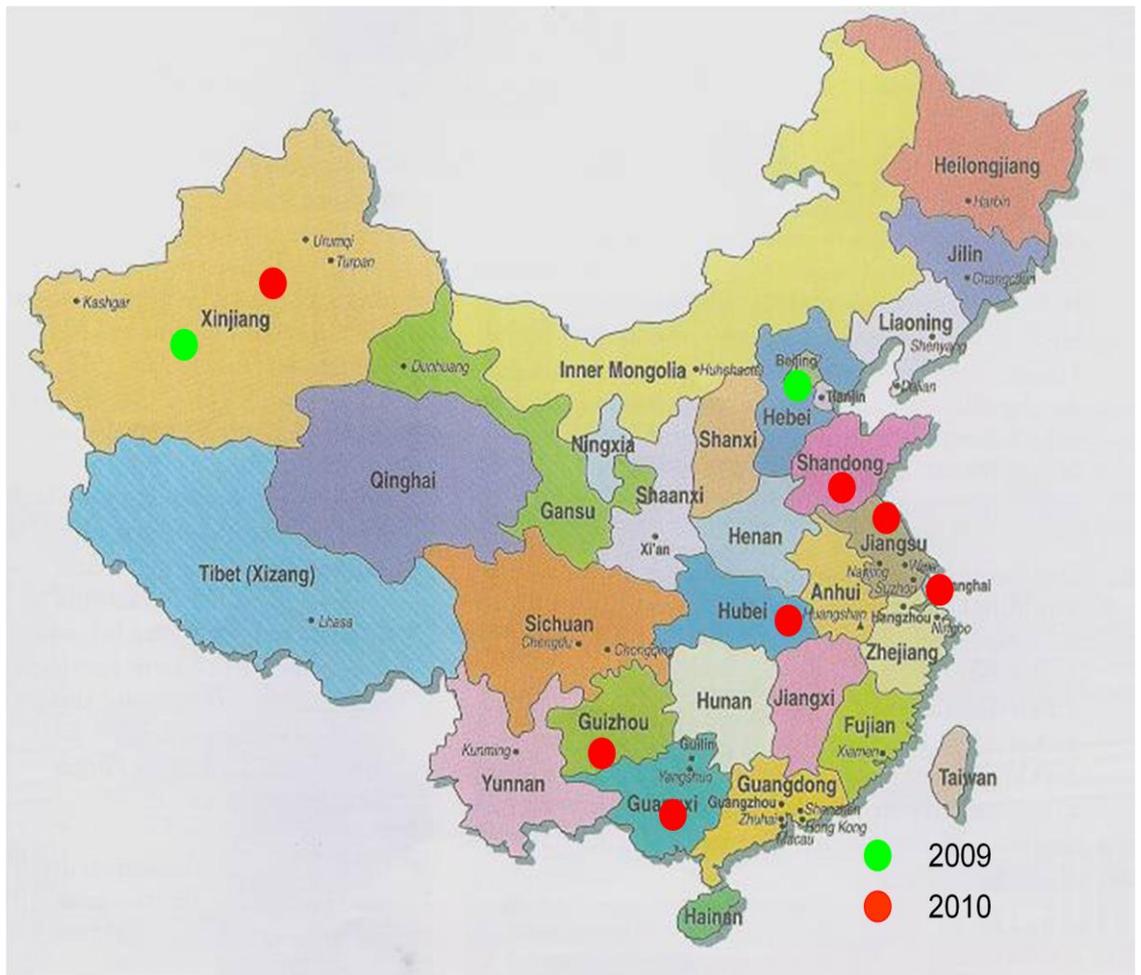
–Hainan province is being established as an FMD free zone with vaccination and is monitored 4 times a year by NFMDRL see Figure 4.

–Vaccination occurs 2 times a year in spring and autumn. More than 700 million doses are used at each time implying over 1.4 billion doses are produced and administered in China per year.

Details associated with the cases confirmed by LVRI are shown in the Figures 4 and 5 and Table 1 below: This information has been submitted directly by LVRI.

**Figure 4.** Hainan Province





**Figure 5.** Map of China showing regions with reported outbreaks of FMDV.

**Table 1. FMD Information from LVRI, China**

No.	Report Date	Location	Serotype	Species	Susceptible	Cases	Deaths	Destroyed
1	22/01/2010	Longtou village, Lixian town, Daxing district, Beijing, BEIJING	A	Cattle	575	23	0	575
2	02/02/2010	Beicheng county, XINJIANG	A	Cattle	44	26	0	44
				Sheep / goats	125	0	0	125
3	01/03/2010	Huangjingwei, Baiyun District, Guangzhou, GUANGDONG	O	Swine	8383	1474	0	8383
4	12/03/2010	Longcheng, Longgang, Shenzheng, GUANGDONG	O	Swine	1108	69	0	1108
5	30/03/2010	Heping town, Yuzhong, Lanzhou, GANSU	O	Swine	1096	206	7	1089
6	01/04/2010	Xinfu, Xinzhou, SHANXI	O	Cattle	98	18	0	98
7	08/04/2010	Jingye, Guanzhou development district, Guanzhou, JIANGXI	O	Swine	3479	223	0	3479
8	12/04/2010	Jiangjiazai village, Nanyuan, GANSU	O	Sheep / goats	389	58		389
				Swine	831	640		831
9	21/04/2010	Tianfeng, Wushan, Tianshui, GANSU	O	Swine	403	47	0	403
10	23/04/2010	Guding, Shibing, Qiandongnanzhou, GUIZHOU	O	Cattle	73	47		73

				Swine	41	25	2	39
11	30/04/2010	Xuanhe Town, Shapotou District, Zhongwei, NINGXIA	O	Swine	778	152	1	777
12	02/05/2010	Yecheng, Yecheng , Kashi prefecture, XINJIANG	O	Swine	771	771	15	756+8328
13	31/05/2010	Shigatse, TIBET	O	Swine	701	12	0	701
14	21/06/2010	Shayidong, Kuerle, Bayingolin Mongolia Autonomous Prefecture, XINJIANG	O	Swine	1280	83	0	1280
15	28/06/2010	The Aksu western suburb pig farming area, Aksu, XINJIANG	O	Swine	355	24	0	355
16	28/06/2010	Haomen town Qunawan road farm_, Menyuan, Haibei Zhou , QINGHAI	O	Swine	153	17	0	153
17	02/08/2010	Chengdong village, Shan Dan, Zhang Ye, GANSU	O	Cattle	32	0	0	32
				Sheep / goats	248	0	0	248
				Swine	184	28	0	184
18	31/08/2010	Animal health inspection station of Er Ba Tai, Ku Che, akesu, XINJIANG	O	Cattle	22	8	0	22
				Sheep / goats	180	0	0	180
19	31/08/2010	Huangzangsi, Qilian, Habei Prefecture, QINGHAI	O	Cattle	163	39	0	163

**Taiwan (Chinese Taipei)** reported 2 outbreaks of type O in 2010, no samples were received by a reference laboratory

In 2010, 98 samples were sent to the RRLSEA in Pakchong from **Myanmar, Cambodia, Vietnam and Thailand** and serotyping revealed types O and A.

**Cambodia:** 103 outbreaks in 18 provinces affecting 52,000 cattle and 10,500 buffalo. Twenty samples were sent to RRLSEA with 12 confirmed as type O.

**Malaysia:** 51 outbreaks of mainly type O and some A; no samples submitted to RRLSEA. Malaysia has recently been identified by the SEACFMD campaign as a 'hotspot' for disease spread in the SEA region with high levels of animal movements into other parts of SEA.

**Myanmar:** 11 outbreaks in 2010. Three samples were sent to RRLSEA with 2 confirmed as type A.

A VP1 sequence was generated by WRLFMD from cDNA amplified from RNA submitted by the RRL Pakchong, but no virus was isolated in cell culture (see below for details).

**Philippines:** no outbreaks.

**Indonesia:** no outbreaks.

**Laos:** 10 outbreaks in 10 provinces those typed were O. Affected cattle and buffalo with deaths in young animals.

**Vietnam:** 329 outbreaks in 98 districts; increased number of outbreaks towards the end of the year. Serotypes O and A were reported. There was mortality in pigs and this is possibly due to the presence of highly virulent PRRS circulation at the same time.

**Thailand:** 35 outbreaks predominantly in cattle.

Forty eight samples were submitted to RRLSEA for serotype identification, which confirmed 19 type O and 3 type A outbreaks.

The antigenic and genetic variation of viruses in the Southeast Asia region continues to be monitored at RRLSEA.

The  $r_1$ -values for type O isolates from Thailand showed a match to the Thai vaccine strain O189/87. Type A isolates from Thailand showed a match to the Thai vaccine strain A118/87.

Genetic analysis of type O viruses from Thailand revealed that they were of the SEA toptype, whilst genetic analysis of type A revealed them to be of the Asia toptype.

FMD viruses of serotypes O and A were isolated from samples sent to **WRLFMD** in 2010 from **Cambodia** (5xO), **Myanmar** (3xO), **Thailand** (6xO) and **Vietnam** (12xO & 1xA).

Viruses from **Cambodia** were characterised as ME-SA toptype, strain PanAsia

Viruses isolated from samples from **Myanmar** were characterised as SEA toptype, strain Mya-98.

**Importantly**, On the 10 September 2010, a single outbreak of FMD was detected in cattle at Kun Thee Pin, Maungdaw, Maungdaw, Rakhine State very close to the border with Bangladesh. It was typed as FMDV A, the first occurrence of this serotype in Myanmar since 1978. A VP1 sequence was generated from cDNA amplified from RNA submitted by the RRL Pakchong, but no virus was isolated in cell culture. Phylogenetic analysis revealed that the virus was not related to type A viruses from Southeast Asia but was most closely related to viruses occurring in India in 2000. The sequence clustered with viruses occurring exclusively in India between 1997 and 2008.

The genetic typing of viruses from **Thailand** confirmed the findings from RRLSEA that type O viruses were mainly of the indigenous SEA toptype Mya-98.

Ten of the type O viruses from **Vietnam** were identified as ME-SA toptype, PanAsia strain with the remaining 2 viruses characterised as SEA toptype, Mya-98 strain. The single A type was of the Asia toptype which clustered with viruses from Thailand isolated in 2009.

The type O viruses from South East Asia that were tested for vaccine matching at WRLFMD showed a generally poor to moderate match to O Manisa and in many cases a better match to O IND R2/75.

**Republic of Korea (South Korea):** FMDV type A (ASIA toptype) was detected in cattle and farmed deer in the Kyonggi-Do region between January and March. This virus was closely related to viruses isolated in the P.R. China (2009), Lao PDR (2008), Thailand (2009) and Vietnam (2008-2009). Seven outbreaks of FMDV type O (SEA toptype, Mya-98 lineage) were reported in April 2010. Vaccine matching studies carried out at WRLFMD demonstrated a good match with O Manisa and O IND R2/75 for the O SKR and A May-97 for the A SKR isolates respectively.

In late November 2010 South Korea reported new outbreaks of FMD type O. Analysis of four VP1 sequences by WRLFMD received from the National Veterinary Research and Quarantine Service (NVRQS) demonstrated that these viruses are of the SEA toptype, Mya-98 lineage, similar to those previously isolated earlier in 2010. At time of writing this outbreak is still ongoing (*Feb 2011*).

**Japan:** In early April 2010, FMDV type O was reported in Miyazaki Prefecture on the island of Kyūshū. Subsequently, a further 291 outbreaks were reported. VP1 sequence was received by WRLFMD from the National Institute for Animal Health, Tokyo, Japan, on 27/04/2010. The virus was identified as Southeast Asia (SEA) toptype (Mya-98 lineage) and found to be closely related to viruses occurring recently in the P.R. China, Hong Kong SAR, Republic of Korea, Myanmar and Thailand. This was the first outbreak of FMD in Japan since 2000. The last (292<sup>nd</sup>) outbreak of FMD occurred on 04/07/2010 at Miyazaki city 3, Miyazaki. Throughout this period and up to time of writing no field samples have been received by WRLFMD for characterization or vaccine matching and no reports on vaccine matching appear to have been reported elsewhere.

Vaccine strains that may be suitable for use in the region include:

Serotype	Internationally available	Locally produced
O	O <sub>1</sub> Manisa, O 3039	China 1999, Thailand 189/87, O-Mya 98(*China)
A	Malaysia 97	China 1972, Thailand 118/87
Asia 1	Shamir	China 2005, Thailand /85

Serotype O Cathay-like virus vaccines (e.g. O Taiwan 97, O Philippine 97, or O 1685 Russia 95) could also be useful where viruses of this topotype affect pigs.

\* recent development for use in China

### 1.5.2 Pool 2: SOUTHERN ASIA.

Network labs receiving samples in 2010:

Laboratory	Sample Nos.	Countries of origin
WRLFMD	303	Afghanistan, Nepal and Pakistan
PDFMD	244	India

**India, Pakistan, Sri Lanka, Bangladesh, Bhutan and Nepal** remain endemically infected with FMDV.

#### **India**

During 2010, 187 outbreaks were recorded from which 244 samples were submitted for laboratory investigation.

The predominant serotype isolated was type O (82% of those typed) and it was found in all regions of India. India reported 187 outbreaks upto end September 2010. 161 type O, 16 type A and 10 Asia 1.

In India, serosurveillance continues to be carried out on randomly sampled bovines with 29,763 sera collected in 2009 at a rate of 100 per district from 335 districts in 21 states. Tests revealed a 27.9% seroprevalence for antibodies to non-structural FMDV proteins with a range from 5.1% in Himachel Pradesh to 56% in Rajastan.

Representative candidate vaccine strains were tested with recent field isolates and matching studies revealed that IND/R2/75 is still the most suitable vaccine strain for type O.

#### **Serotype O virus lineages circulating in India during 2008-2010.**

The viruses of the Ind2001 lineage predominated after a gap of 8 years and outcompeted the PanAsia II lineage. Reemergence of this lineage has been tracked back to 2008, during which it caused sporadic outbreaks in Northern India. It then spread to the majority of states in the Eastern and Central regions and reached the Southern state of Kerala by the end of 2009, travelling through Andhra Pradesh and Karnataka. Fourteen out of 18 states where disease due to type O was experienced were traced back to this lineage. These viruses diverged (genetically) from Ind2001 viruses isolated in 2001 by 7.3% and 13.1% compared to the in-use vaccine virus (O/IND/R2/1975). In addition, Pan Asia II was responsible for outbreak in Karnataka and Gujarat and there were outbreaks of FMD in Mithun in Arunachal Pradesh due to PanAsia I; this lineage also caused outbreaks in Bihar.

#### **Type A FMD virus isolates**

During 2009-2010, all the isolates were found to cluster within genotype VII non-deletion and the VP3<sup>59</sup> deletion lineages. Viruses from Punjab and Uttarakhand shared ancestry and clustered in the same VP3<sup>59</sup> deletion lineage, VIIIf, where as viruses from two different outbreaks from Orissa and those from Andhra Pradesh clustered together and revealed no deletion in the VP3 coding region. The viruses from Andhra Pradesh and Orissa as well as those from Punjab and Uttarakhand appear to be epidemiologically related strains as they show less than 2% nucleotide divergence among themselves

### Type Asia 1 FMD virus isolates

Of the three lineages (B, C and D) present in the country, Lineage C has been responsible for all Asia1 outbreaks in the country since 2005. Outbreaks were recorded in Gujarat, Madhya Pradesh, Maharashtra, Uttar Pradesh and West Bengal.

The FMDV Asia1 isolates from Gujarat and Uttar Pradesh were sequenced for molecular epidemiological studies. These Asia1 field isolates were grouped with lineage C highlighting the dominance of this lineage.

Representative candidate vaccine strains were tested with recent field isolates of A and Asia 1 and studies demonstrated that vaccine strains IND/40/2000 for type A and IND/63/72 for Asia 1 remained the most suitable for use in the region.

**Bhutan:** The O-Ind-2001 lineage (ME-SA toptotype) was detected in Bhutan in samples collected in December 2009. This lineage was also found in Bangladesh and Nepal.

Samples received at WRLFMD from **Afghanistan** (176) and **Pakistan** (76): Samples from Afghanistan (176) were all received in RNA later and therefore were only able to be characterised by RT-PCR and sequencing where the majority were identified as type O with some type A also. The O-PanAsia-2 and A-Iran-05 lineages continue to dominate in these countries. The PanAsia-2 lineage has been subdivided into six sub-lineages named BAL-09, YAZ-09, FAR-09, SAN-09, ANT-10 and PUN-10. During 2010, the ANT-10 sub-lineage appeared to have become the dominant type O sub-lineage in Afghanistan and Pakistan and also in Iran and Turkey.

Serological tests on serotype O viruses from Pakistan showed the best match against the O Ind R2/75 vaccine strain. For serotype A viruses from Pakistan, the best match was against the A Iran 05 strain vaccine. No serotype Asia 1 viruses were recovered from the samples from Pakistan in 2010.

Vaccine strains that may be suitable for use in the region include:

Serotype	Internationally available	Locally produced
O	O <sub>1</sub> Manisa	IND R2/75*
A	A Iran 05, A <sub>22</sub> Iraq	IND 40/2000*, Turkey 1/2006 (A Iran 05 lineage)
Asia 1	Shamir	IND 63/72*

\* Trivalent vaccine comprising these three strains is nationally mandated in India

### 1.5.3 Pool 3: EUR-ASIA

Network labs receiving samples in 2010:

Laboratory	Sample Nos.	Countries of origin
WRLFMD	323	Iran, Qatar, Turkey, United Arab Emirates
FGI-ARRIAH	15	Lebanon, Mongolia, Kazakhstan, Russia

FMD viruses continue to circulate in many Middle-Eastern countries, the prevailing serotypes in 2010, being as in 2009, O (PanAsia-2 lineage) and A (Iran 05 lineage). There were no outbreaks of serotype Asia 1 reported in 2010.

Fewer countries reported outbreaks in the Middle East in 2010, however there was increased activity in **Turkey** and **Iran** with both countries reporting very high numbers of outbreaks throughout the year of both serotype O (PanAsia-2) and A (Iran 05).

Turkey reported upwards of 1500 outbreaks in 2010 and also suggested that the O Manisa vaccine was failing to provide protection in the field against the circulating O type viruses. In response to these observations, Turkey began producing a new variant O vaccine based on a local field strain for use in that year. No details are available on its efficacy however.

The PanAsia-2 lineage has been subdivided into six sub-lineages named BAL-09, YAZ-09, FAR-09, SAN-09, ANT-10 and PUN-10. During 2010, the ANT-10 sub-lineage appears to have become the dominant type O sub-lineage in Turkey and Iran.

It seems probable that antigenic changes may have conferred an advantage for the spread of the A Iran 05 strain, but this is less clear for O PanAsia-2, however there is increasing evidence that O Manisa vaccine is failing to provide protection in the field and that more field isolates are failing the laboratory vaccine matching tests carried out with O Manisa at WRLFMD Pirbright and this will need careful monitoring in the coming year.

The priority vaccines still remain O Manisa (or similar strains), A Iran 05 strain and Asia 1 Shamir (or similar strains). Importantly vaccine strain O4625 from Merial matched a number of O isolates from the region where O Manisa did not and Intervet towards the end of 2010 introduced a new PanAsia 2 vaccine which gave a match with O viruses from Turkey in a limited study. The matching of these vaccines needs close monitoring in the coming year.

The major gaps in submissions are still considered to be from some central Asian Republics, the Caucasus and some Middle East countries concerned about the impact of transparency on trade. The main problems for vaccine selection are an inability to compare vaccine matching results between centres due to the use of different vaccine strains, non-standardised methods and field isolates that are not shared. There is still a lack of information on the cross-reactivity of the new A Iran-05 vaccine strains against other circulating A viruses. There were far fewer reports of A outbreaks in the region in 2010 and based on previous experience, it may be expected that serotype O but not A will be sustained within the region. In India, the O India 2001 strain has replaced O PanAsia-2 and it will be interesting to see if this also occurs in neighbouring regions. Initial studies suggest that the Indian vaccine strain O IND R2/75 could be a useful alternative to Manisa in other Asian and EurAsian countries.

**Notably**, an Asia 1 epidemic may be due in EurAsia, since cases are occurring in Pakistan, whilst other countries including Iran and Turkey with high levels of circulating O and A viruses will by now have a low population immunity to this serotype (last seen in 2004). Increased imports and movements of live cattle and small ruminants into and through the Middle East from Africa and through new trade routes may also increase the risk of African strains being introduced.

Vaccine strains that may be suitable for use in the region include:

Serotype	Internationally available	Locally produced*
O	O <sub>1</sub> Manisa, O4625, O Panasia 2	Russian O <sub>1</sub> PanAsia
A	A Iran 05, A <sub>22</sub> Iraq	Turkey 1/2006 (A Iran 05 lineage)
Asia 1	Shamir	Georgia 2000

\* Vaccines are also produced locally in Iran, Turkey, Egypt and Jordan

The main differences between vaccine requirements of pools 1-3 relate to serotype A. The serological match between A<sub>22</sub> Iraq and many A Iran 05 lineage field isolates is poor.

#### 1.5.4 Pool 4: EASTERN AFRICA

Network labs receiving samples in 2010:

Laboratory	Sample Nos.	Countries of origin
WRLFMD	354	Eritrea, Ethiopia, Kenya, Tanzania

For this section the laboratory information supplied from Embakasi and WRLFMD has been complemented by epidemiological insights and local diagnostic findings supplied by the participants at the Second EARLN meeting held in Addis Ababa, February 13-14, 2011.

#### Summary report 2010 Eastern African Regional Laboratory Network (EARLN)

**Table 2.** Summary report 2010 Eastern African Regional Laboratory Network (EARLN)

Country	Reported and confirmed FMD outbreaks (n)	Samples investigated (n)	Regional typing results	WRLFMD molecular typing
Burundi	3	No data available	No data available	No data available
DR Congo	No data available	No data available	No data available	No data available
Eritrea	No data available	No data available	No data available	A G-IV (7) (2006-2009)*
Ethiopia	117	ET: 21	SAT 2 O	SAT 2: VIII O EA-3
Kenya	249	ET: 211 PB: 70 Sera: 6330	O (44) A (4) SAT 1 (110) SAT 2 (2)	O: EA-1* O: EA-4** SAT1: NWZ (19) SAT2: IV (1)
Rwanda	No data available	No data available	No data available	No data available
Somalia	No data available	No data available	No data available	No data available
Sudan	2	2	O and SAT 2	No samples submitted
Tanzania	52	1 75	O (11) SAT 2 (2)	A Africa G-I (8)* O EA-2 (3)* SAT 2 IV (1)*
Uganda	13	Unclear	O	

\*: Samples collected during previous years, e.g. 2006 – 2009 in Eritrea, 2008-2009 in Tanzania, but shipped in 2010 to WRLFMD

\*\* : First time that FMDV serotype O topotype EA-4 was detected in Kenya.

Serotypes O, A, SAT 1 and SAT 2 have all been reported from this area in recent years and all countries are thought to be endemically infected with FMD virus. Type C was last isolated in 2004 in Kenya. In 2010 type O seems to have become dominant in the region.

In 2010 samples from **Kenya** have been typed as SAT 1 and O with SAT 1 predominating. In January and February 2011 many outbreaks have been confirmed, almost all type O until now. Field training of outbreak investigation teams has considerably contributed to the quality of samples, resulting in a much lower percentage of samples with “no virus recovered”. The samples were also referred to the WRLFMD

for genetic and antigenic characterisation, type O was characterised as toptype EA-4, and type SAT-1 was characterised as toptype NWZ. Most samples originated from the Rift valley, the Central region and the Eastern region.

All vaccines used were locally produced (KEVEVAPI). In 2009-2010 type A, O, SAT 1 and SAT 2, were used in respectively 303,000, 918,000, 755,000, and 841,000 doses of vaccine (either in monovalent, bivalent, trivalent or quadrivalent vaccines). There is a need for training and protocols for vaccine matching, since there seems to be a poor coverage of the currently available vaccines for serotypes O and SAT 1. There is also need for establishment and training on molecular virological techniques such as Real-time PCR.

In **Uganda**, 12 outbreaks were reported in 2009 and 13 outbreaks were reported in 2010 up to February 2011. From some outbreaks samples were collected, but only few samples were investigated and typed due to the lack of diagnostic kits and reagents. Also the funds to send samples to reference labs were very limited. In 2010 and 2011 the outbreaks have been concentrated in the North-East region (close to the border with Kenya), the South region (close to the border with Tanzania), and the Central region. Main measures have been quarantine and vaccination. A trivalent vaccine (O, SAT 1, SAT 2) supplied by KEVEVAPI has been used; generally vaccination is effective.

In **Tanzania**, 26 and 52 outbreaks were reported in 2009 and 2010, respectively. However, samples were only received from 3 reported outbreaks. Many more samples were received without prior notification (spontaneous submissions): 69 tissue samples in 2009 and 75 tissue samples in 2010. In 2009 serotypes A, O, and SAT 2 have been found, whereas in 2010 only SAT 2 and – predominantly O have been found. In 2009 the samples were also referred to the WRLFMD for genetic and antigenic characterisation, type O was characterised as toptype EA-2, type A was characterised as toptype G-I and type SAT 2 was characterised as toptype IV. In 2010 only 2 samples were submitted to WRLFMD. In three consecutive years (2009-2011) outbreaks were reported in cattle for export, gathered on the same holding ground.

A sero-monitoring study was carried out, screening 276 sera from unvaccinated cattle by LBPE for antibodies against serotypes A, O, SAT 1, SAT 2, and SAT 3. However, the results were very confusing due to many multiple cross reactions. There are no specific records with respect to the amount of vaccine used, and the specifications of the vaccines used. These data, however, are considered important especially after the vaccine matching results from 2009.

In **Ethiopia**, 119 and 117 outbreaks were reported in 2009 and 2010, respectively. In 2010 the outbreaks concentrated mainly in the regional states Amhara and Oromia (South, Central and West Ethiopia). SAT 2 has migrated from 2007-2008 in the West to the Central region in 2009-2010. In 2009 the samples were also referred to the WRLFMD for genetic and antigenic characterisation, type O was characterised as toptype EA-3, and type SAT 2 was characterised as toptype VIII. In 2010 also samples were submitted to WRLFMD for genetic characterisation with for SAT 2 again toptype VIII and part of the results still pending. More samples are needed for a good epidemiological coverage of FMD circulation in Ethiopia.

A limited amount of vaccine is used in the field (about 300,000 doses), produced by the National Veterinary Institute, and by Indian Immunologicals LTD (O Manisa and A22).

In **Sudan** two outbreaks in central Sudan were reported and investigated in the time period 2009 – 2010. These viruses were serotyped locally as serotype O and SAT 2. Passive/clinical surveillance is hard to achieve due to logistic constraints and difficult

infrastructure in this vast country. A Sudanese isolate (O/SUD/2008), which is antigenically close to O1 Manisa, O BFS 1860, and O Ind R2/75, has been adapted to BHK cells, and has been used as an experimental vaccine strain. Also the economic impact of FMD on dairy farms has been studied in Khartoum State. A survey was carried out in 27 randomly selected dairy farms. A sub selection was made of 8 farms with relatively good data management. The loss of milk production during FMD outbreaks was 9-55%. In one farm the financial losses accumulated to 73%.

In Sudan currently no vaccination program is applied, but in 2010 a quadrivalent FMD vaccine (O, A, SAT1 & SAT2) was imported from KEVEVAPI Lab. (Kenya) and used for vaccinating animals for export and locally in limited farms. If necessary a bivalent (type O and SAT 2) vaccine would be preferred.

In **Burundi** only few outbreaks have been reported. No samples were collected, and there is no FMD laboratory operational. In the past serotypes O, A, SAT 1 and SAT 2 have been confirmed by WRLFMD. Either laboratory capacity should be established or samples should be submitted to a FMD lab in the region. Burundi has already contacts with Embakasi laboratory in Kenya. Some quadrivalent vaccine is imported from Kenya, mainly used for ring vaccinations around outbreaks.

In 2010 no information was available with respect to FMD outbreaks and diagnostic results from **Rwanda, Eritrea, DR Congo, and Somalia**. These countries were also not represented in the second Annual EARLN meeting. It is certainly the intention of EARLN to include these countries in the network activities. Eritrea submitted in 2010 archival samples from the time period 2006 – 2009 to WRLFMD. These samples (n=7) were all characterised as serotype A topotype AFRICA genotype G-IV.

Gaps identified as priorities for sampling/submission include: Sudan, Burundi, Rwanda, DR Congo, Eritrea, Somalia. In Tanzania, official outbreak investigations should result also in submission of samples.

FMD vaccination is applied only at a limited scale in 6 countries in the region:

- Kenya (KEVEVAPI vaccine, Kenya)
- Ethiopia (NVI Ethiopia and Indian Immunologicals)
- Uganda (KEVEVAPI vaccine, Kenya)
- Somalia (pre-export at Berbera port, KEVEVAPI vaccine Kenya)
- Burundi (KEVEVAPI vaccine, Kenya)
- Sudan ( KEVEVAPI vaccine, Kenya) Quadri-valent Vaccine

Vaccine strains that may be suitable for use in the region include:

Serotype	Internationally available	Locally produced in 2009/2010
O	O <sub>1</sub> Manisa	Kenya 77/78, Egypt 2/72, Ethiopia O 281
A	Eritrea 98	Kenya 5/80, Egypt 06, Ethiopia A110
SAT 1	See pool 6	Kenya T155/71
SAT 2	Saudi 2000, Eritrea 98, see pool 6	Kenya 52/84

### 1.5.5 Pool 5: WESTERN AFRICA

Network labs receiving samples in 2010:

Laboratory	Sample Nos.	Countries of origin
WRLFMD	83	Nigeria, Senegal

Few samples have been submitted from this region to OIE/FAO Reference Laboratories for investigation of FMD outbreaks, although the disease is known to be present. Samples sent to WRLFMD from **Nigeria** collected in 2008-2009 revealed the presence of serotypes O and A. Local capability for laboratory investigations including serotyping and characterisation of FMD viruses appears to be also very limited.

Nigeria has the highest human population in Africa and more than 100 million FMD susceptible livestock animals, with small ruminants predominating as well as significant numbers of cattle and pigs. As with several other countries in the region there is a mix of sedentary and pastoral livestock farming, the latter contributing to extensive transboundary animal movements.

Five of 33 samples submitted to WRLFMD from **Senegal** collected in 2009 were RT-PCR positive for FMDV, with only 1 virus isolated and identified as SAT 2 by serotyping.

**The following information was collected by the FAO following a meeting in Bamako, Mali in December 2010, and from the preliminary analysis of serosurvey in Chad (2009).**

FMD is endemic in the whole region and epizootic outbreaks are regularly observed, but rarely investigated. In West and Central Africa collection and testing for FMD identification are rare. A few countries submitted samples to the WRLFMD for virus identification and genotyping in 2010.

- **Mali:** Tests performed in 2010 in the national lab confirmed the SAT2 virus, but no further genotyping study has been made.
- **Nigeria:** the WRL received in 2010 samples collected in 2009 in Nigeria. Serotypes A and O were confirmed and the genotyping was as follows:

FMDV type A: Topotype: AFRICA on 4 isolates.

- WRLFMD Ref No: NIG/3/2009 / Date collected: 14/01/2009
- WRLFMD Ref No: NIG/36/2009 / Date collected: 01/01/2009
- WRLFMD Ref No: NIG/38/2009 / Date collected: 01/01/2009
- WRLFMD Ref No: NIG/39/2009 / Date collected: 01/01/2009

FMDV type O / Topotype: EA-3 on one isolate:

- WRLFMD Ref No: NIG/15/2009 / Date collected: 25/08/2009

- **Senegal:** samples collected in late 2009 were submitted to WRL and confirmed the SAT2 virus in one sample (Topotype VII). Date collected: 09/10/2009.
- **Chad:** *Results from a study made by the collaboration of vesicular disease lab of Brescia/Italy with the University of Liege (Belgium) and the National veterinary lab of N'Djamena (Chad)*

A seroprevalence study was performed in 2009 in Chad through a random collection of samples (N= 796 cattle). 3ABC-ELISA and SP ELISA tests revealed an apparent prevalence of 36% (32,7 – 39,5 – 95% CI). The disease is present in all the sampled

provinces, with a higher prevalence in provinces of the South of the country, which are known to be humid and as a major zone of transboundary animal movements. All the serotypes A, O, SAT1 and SAT2 have been detected and were shown to be widely present. Further investigations to assess the efficiency of the clinical and epidemiologic networking are underway.

- **Regional lab networking:**

Under the regional lab network for West and Central Africa (RESOLAB), a specific network on FMD is being built. Labs will be encouraged to collaborate in the area of sample collection, analysis and shipment. In addition laboratories capacities for FMD diagnosis will be enhanced through training focusing on FMDV detection and identification, and serology studies. The information relevant to the region and to the international community will be shared for discussion.

Nigeria, Mali and Senegal proposed to assist the other members countries by performing diagnosis and further assistance by shipping their samples and strains to the WRLFMD for confirmation and genotyping.

Vaccine strains that may be suitable for use in the region include:

Serotype	Internationally available	Locally produced
O	O <sub>1</sub> Manisa	
A	Eritrea 98, A <sub>22</sub> Iraq	
SAT 1	Rhodesia 12/78, Botswana 1/68,	Botswana 1/77, KNP 196/91, Kenya T155/71, SAR 9/81
SAT 2	Saudi 2000, Eritrea 98, see pool 6	Nigeria 6/81*

\* Current availability of this vaccine is not known

### 1.5.6 Pool 6: SOUTHERN AFRICA

Network labs receiving samples in 2010:

Laboratory	Sample Nos.*	Countries of origin
WRLFMD	20	Botswana, Mozambique, Zambia and Zimbabwe
ARC-OVI	398 (13175)	South Africa
	50 (75)	Malawi
	- (901)	Mozambique
	2 (170)	Namibia
	- (14)	Sultanate Of Oman
	- (78)	Swaziland
	- (15)	Kenya
	192 (211)	Zambia
	- (555)	Zimbabwe
	- (4)	Czech republic
RRLSSA	24	Botswana, Namibia and Zimbabwe

\* clinical samples except for those in parenthesis that represent serology samples.

Twenty one samples were sent to WRLFMD from Botswana, Mozambique, Zambia and Zimbabwe in 2010 for characterisation and analysis.

**Botswana:** In conjunction with the Botswana Vaccine Institute (BVI), VP1 sequence analysis was performed on viruses isolated from an outbreak on 26/07/2010 at Lesoma (Kasane) close to the borders with Namibia, Zambia and Zimbabwe. The viruses were SAT 2 topotype III and most closely related to SAT 2 viruses from Botswana (Kasane) in 2005.

**Mozambique:** Two outbreaks of FMD type SAT 2 were reported in cattle in the south of the country (Motaze, Magude, Maputo) on 17/12/2010. In conjunction with the BVI the VP1 sequences of seven virus isolates from earlier outbreaks (September 2010 at Bilene, Gaza) showed they belonged to SAT 2 topotype I and were most closely related to viruses from South Africa (Kruger National Park).

**Zambia:** In conjunction with the BVI, the VP1 sequence of viruses isolated from an outbreak on 22/09/2010 at Mbala, Northern Province were determined. These belonged to the type O EA-2 topotype and were most closely related to viruses from the Democratic Republic of the Congo (2006), Uganda (2004-2006) and Tanzania (2009).

**Zimbabwe:** In conjunction with the BVI, the VP1 sequences of viruses isolated from an outbreak on 28/05/2010 at Kitwe Dip Tank, Plumtree, Magwe (Matabeleland South) were determined. They belonged to SAT 2 topotype II and were most closely related to viruses isolated from African buffalo and cattle in Botswana, Namibia and western Zimbabwe, although none were very closely related.

Twenty four samples were sent to RRLSSA from Botswana, Namibia and Zimbabwe.

**Namibia** April 2010, Impalila island has cattle and buffalo, vaccinated animals.

**Zimbabwe** June 2010, Plumtree very close to border with Botswana and 2002 outbreaks, started in a dairy herd, has not been vaccinating for a while,

**Botswana** , August 2010, Zone designated as buffalo zone - Vaccination was used to successfully control the disease

The priority vaccines are SAT 2 and SAT 1, but there is insufficient information to be more precise about the strains that should be included. For SAT 1, past vaccine matching results have indicated that vaccine strains are relevant. For SAT 2, there is now more concern over vaccine matching which will require much more analysis of field strains by laboratories in the network.

**The following contribution to the report was provided directly by ARC-Onderstepoort Veterinary Institute**

**Tests for FMD conducted from January 2010- December 2010**

**Table 3.** Number of tests performed for FMD

Test	Number of tests
LPBE	73,518
Typing ELISA	56
3ABC ELISA	5,696
VNT	427
^Virus Isolation	593
PCR	623
SEQ	29

#### **Molecular Epidemiology of FMD**

Part of the diagnostic services is to provide molecular epidemiological insights into FMDV strains causing

outbreaks or those circulating in the host species like buffalo. During the reporting period, a SAT 2 outbreak was characterised from buffalo located at Limpopo Safaris. Also, SAT 1 outbreaks were characterised from bovine in Namibia and bovine and buffalo located in the FMD protection zone in South Africa. Also, virus isolations have been completed from probang samples collected from the Kruger National Park and are awaiting molecular characterisation.

## **Namibia**

During 2007 and 2008, SAT 2 outbreaks were characterised from the Caprivi region and Kavango regions of Namibia respectively. Molecular characterisation, based on sequencing the partial ID gene, showed that the SAT 2 outbreak strain from the Caprivi region of Namibia clustered as part of the northern toptype of FMDV viruses and grouped with previously characterised SAT 2 isolates from Namibia, Zimbabwe and Botswana.

Sequencing and phylogenetic analysis of the outbreak strain from Kavango showed that this isolate clustered with previously characterised SAT 2 strains from Namibia (NAM/304/98 and NAM/01/92) as part of the western toptype of viruses. This isolate differed by 8% from the 1998 and 1992 isolates, while the difference at nucleotide level between this isolate and the isolate characterised in December 2007 was 28%.

The SAT 2 outbreak strains from Namibia clustered in two different toptypes, indicating a diversity of SAT 2 strains circulating in Namibia.

In April 2010 a SAT 1 outbreak occurred in the FMD infected zone where there are free-roaming wild African buffaloes, long-term reservoirs of FMD virus. The total cattle population on Impalila island is 1,066 cattle of all age and sex groups farmed under communal farming systems. Impalila island has been designated as a containment zone approximately 30 square kilometres. The island is surrounded by the Zambezi and Chobe rivers. As a control strategy, a buffer zone of 70-80km radius has been established around the island, in which a total of 120,000 cattle will be vaccinated using an inactivated trivalent vaccine against serotypes SAT 1, 2 and 3 of foot and mouth disease. The whole of Caprivi region will be subject to intensified surveillance to determine the extent of the event. The outbreak of FMD is located in the infected zone and has no effect on the status of the FMD free zone where vaccination is not practised of Namibia.

Molecular characterisation of the SAT 1 outbreak strain showed that the current Namibian SAT 1 strain clusters with SAT 1 strains characterized from Botswana in 1998 and 2006 with a nucleotide difference of 11% and 12 %, respectively. They also cluster as part of the western toptype of viruses.

## **South Africa: within buffer zone**

On 21 April 2010, 19 buffalo sera were submitted by Capricorn Veterinary Laboratories, Limpopo Province as part of a buffalo test package. These buffalo originated from Limpopo Safaris buffalo breeding project. Routine serological testing using the LPBE for the SAT serotypes showed high titers for SAT 2 antibodies for 5 of the 19 animals. Upon verification of these positive serological results, the state vet of Limpopo Province was informed of the high SAT 2 antibody titers and it was suggested that the herd be re-examined and probang samples taken. On 20 May 2010, 9 sera, EDTA blood and probang samples were received from the herd. The 5 positive buffalo were re-bled and

probang samples taken from an additional 4 buffalo. Serological testing showed that the 5 sera remained positive for SAT 2 antibodies on the second sampling. Positive virus culture was obtained from only one probang sample and was serotyped as SAT 2. Molecular characterisation of the virus showed that the outbreak strain clustered with a buffalo isolate characterised from Punda Maria, KNP in 2003 with a 15% nucleotide difference (Fig 2).

On 13 August 2010, 9 bovine epithelium samples were received from a suspect FMD outbreak in Gravelotte, Limpopo province. Diagnostic PCR results were positive on the initial epithelium samples submitted, however, virus isolation proved to be a challenge. Blind passage of the culture material yielded positive culture on pig kidney cells at PK1 (passage 1). The positive culture harvest was typed as SAT 1 and the virus sequenced. This outbreak was within the FMD protection zone of South Africa and thus did not affect the export status of the country.

Subsequently, 14 sera and probang samples were received from buffalo at the Grietjie Farm 55, Phalaborwa. Twelve of the 14 sera tested positive for the SAT serotypes of FMD with the LPBE, however the serum titers for SAT 2 were lower than SAT 1 and 3. Diagnostic PCR and virus isolation was positive for most of the probang samples taken, which was subsequently typed as SAT 1. The positive culture harvest was subsequently sequenced.

On 22 August 2010, the laboratory received 15 sera and probang samples from the same cattle herd at Gravelotte. Some of the animals sampled in the initial investigation were re-sampled. The sera were positive for SAT 1 and 3 by the LPBE. Three of the probang samples were positive for virus isolation and typed as SAT 1. This virus was sequenced.

As a follow up, on 26 August 2010, a further 10 animals were sampled within the herd at Gravelotte. Samples were taken from cattle that were not sampled before and submitted to the lab for FMD diagnosis. All clinical material resulted in positive FMDV culture and PCR. These samples were also typed as SAT 1 and sequenced.

Partial sequencing of the 1D gene showed that all virus strains sequenced from the bovine herd and the sequence obtained from the buffalo herd were identical to each other, indicating that the same strain of FMDV was responsible for both outbreaks (Fig 3). These isolates also clustered with buffalo isolates from the KNP, indicative of the role that buffalo from the KNP play in FMD outbreaks in the areas adjacent to the park.

### **Pharyngeal scrapings collected from KNP**

During September 2009, 13 buffalo probang samples were received from Satara region in the KNP. These probang samples were processed for FMD culture, firstly on primary pig kidney cells and then passaged on IBRS<sub>2</sub> cells. No virus could be isolated from these samples, neither were they positive on PCR.

During November 2009, 7 buffalo probangs were received from Skukuza boma. The testing for FMD virus was required as these buffalo were been moved to Gorongosa National park. No virus could be isolated from these buffalo using both pig kidney and IBRS cells. All samples were negative by PCR.

As part of a Transfrontier Conservation Area (TFCA) buffalo project 26 buffalo probangs were received from various regions within the Greater Limpopo TFCA. FMD

virus could be isolated from 3 samples from the Pafuri region of the park, with 2 typed as SAT 2 and one typed as SAT 3. These viruses are in the process of been sequenced for molecular characterisation and addition to the KNP database of sequences.

## **Serological subtyping and vaccine matching**

### **Genetic and antigenic characterisation of recent SAT-type FMDV isolates**

The ARC-OVI, TADP are currently conducting research funded by the FAO to characterise the genetic and antigenic relatedness of FMD isolates originating from southern Africa, with specific emphasis on the most recent SAT1 outbreak isolates from South Africa, Zimbabwe, Namibia, Botswana, Malawi and Zambia. SAT1 isolates, selected based on the 1D phylogeny, were further characterised to determine the genetic diversity within the capsid-coding region of the genome (1B/C/D). The deduced amino acid sequence is central in determining the antigenic determinants of the virus and will be used in combination with structural data and virus neutralisation data to provide the best prediction of cross-reactivity between vaccine strains and outbreak viruses.

To generate the reagents required to perform the virus neutralisation test (VNT), bovine convalescent antisera was prepared against 4 reference FMDV isolates, representative of topotypes I, II and III. Five animals were infected with each of the viruses and antisera collected at 28 days post-infection. In addition, rabbit and guinea pig antisera were prepared to be used in ELISAs for the determination of  $r_1$ -values.

Antigenic relatedness of the FMD isolates were determined by one-way antigenic relationships ( $r_1$ -values) using the VNT. The antibody titres against the homologous and heterologous viruses were determined by following two approaches. The VNT was performed for each outbreak and reference virus against each of the 5 individual sera per strain. In addition, the titres were also determined using a pool of the five sera. The use of pooled sera has not been established for the SAT types and was therefore included in this study. The data has been compiled and upon statistical analysis should give a good indication if pools can also be used in the southern African context. This will be an advantage as it will lead to great reduction in time and cost for the test.

### **Genetic and antigenic characterisation of FMDV isolates from the 2009 FMD outbreak in South Africa**

In 2009 South Africa experienced an outbreak of FMD in domestic cattle within the disease control zone of the Mpumalanga Province. Several viruses isolated during the course of the outbreak were fully characterised. Genetic characterisation revealed only four amino acid changes within the structural proteins, 1 change in 1A and 3 variable positions located on the 1D, of which one was surface exposed. Most of the outbreak viruses had a  $r_1$ -value of 1.0 against the topotype 1 virus, SAR/9/81. In general, the 2009 SAT outbreak isolates was most cross-reactive against the topotype 1 viruses and to a lesser extent to viruses from topotype 2.

Vaccine strains that may be suitable for use in the region include:

Serotype	Internationally available	Locally produced
O	O <sub>1</sub> Manisa	Kenya 77/78, Egypt 2/72
A	Eritrea 98	Kenya 5/80, Egypt 06
SAT 1	Rhodesia 12/78, Botswana 1/68,	Botswana 1/77, KNP 196/91, Kenya T155/71, SAR 9/81

SAT 2	Zimbabwe 7/83, Eritrea 98, Saudi 2000	Zimbabwe 11/89, Zimbabwe 5/81, Zambia 3/81, KNP 19/89, Kenya 52/84, Kenya 65/82
SAT 3	Zimbabwe 9/81, Zimbabwe 2/83	KNP 10/90

Not all of the above-mentioned vaccine strains are in production and there are major problems in finding new strains suitable for vaccine production. This is not only due to the lack of availability of field isolates and sera for use in vaccine matching tests, but also the fact that prospective vaccine strain adaptation for production purposes is a cumbersome process and that commercial returns are uncertain on investment to generate new vaccine strains.

### 1.5.7 Pool 7: SOUTH AMERICA

Network labs receiving samples in 2010:

Laboratory	Sample Nos.	Countries of origin
WRLFMD	9	Ecuador
PIADC-FADDL	5	Ecuador
PANAFTOSA	10	Ecuador
LFADLCT (SENASA)	22	Ecuador

**Ecuador:** More than 40 FMD type O outbreaks were reported in cattle in 2010. According to the Agricultural Health Agency (Agrocalidad) cases were reported in Bolivar, Los Rios, Napo, Santa Elena, Sucumbios, Orellana, Cotopaxi, Imbaburam Santo Domingo, Manabi, Pichincha.

In addition Panaftosa reported the presence of FMD type A in **Venezuela** in July 2010.

**Ecuador: WRLFMD isolated** 9 FMD type O viruses from samples sent from various locations in Ecuador. These were the first samples WRLFMD had received from this region. With the gratefully appreciated assistance of PANAFTOSA we were able to compare the VP1 sequences to those from six virus isolates from Ecuador in 2009. These all belonged to the EURO-SA topotype and viruses from each year clustered together and the clusters were most closely related to each other.

Vaccine matching studies carried out at WRLFMD demonstrated no match with current vaccine strain for the region O1 Campos by VNT. Other laboratories including SENASA and PIADC-FADDL reported the same results. SENASA also reported that the current O1 Campos vaccine also failed to protect vaccinated animals in a challenge study using a recent field isolate from Ecuador. In contrast PANAFTOSA reported that the results from their studies using complement fixation test together with Lp-ELISA and VNT test to determine the expectancy of protection (EPP) indicated that a systematic revaccination campaign would be effective in protecting the national herd against the field strains

Based upon the discrepancy in these findings a technical meeting of interested parties and reference laboratories was organised for these results to be discussed.

#### **Report of technical meeting:**

On Monday December 20<sup>th</sup> a technical meeting was held in Quito, convened by AGROCALIDAD - Ecuador, with the purpose of discussing the results of several tests carried out on virus isolated from the FMD outbreaks that occurred during 2010. Main attendees were representatives of FMD OIE Reference Laboratories of SENASA – Argentina and PANAFTOSA PAHO/WHO. Representatives of FAO, IICA, the President of OIE for the Americas and the Director of Animal Health of Peru were also invited.

During the meeting, both laboratories provided a full report of the tests carried out on the isolates received from Ecuador and discussed the results. However, no common understanding/conclusion regarding the results was achieved. Each laboratory came to different conclusions and provided different recommendations for vaccination in Ecuador. This was considered an unsatisfactory conclusion given the current status of the eradication program. Following the meeting and given that no consensus was reached, it was decided that each laboratory would provide written recommendations to Ecuador, which would be based on their own results.

A further meeting was then organised for 14<sup>th</sup> and 15th March 2011 in the Headquarters of CAN in Lima Peru, to develop an action plan and way forward for this situation.

**Stop Press: Outcome from Lima meeting- note added during editing.**

*The determinations made by the reference laboratories of the OIE of FMD viruses prevalent sampled in Ecuador have detected a virus that has genetically evolved and is now antigenically different from the vaccine virus O1 Campos. (Vaccine matching)*

*From the determinations carried out by the OIE Reference Laboratories, the vaccine in current use which contains the virus O1 Campos does not protect adequately animals primo-vaccinated against the virus O found. An agreed action plan is now being progressed to develop and assess a pilot vaccine containing the circulating O strain to determine improved efficacy and efficiency in the control of the disease.*

The vaccines used in the region are all single oil emulsions. O1 Campos and A24 Cruzeiro are used throughout the region, whilst C3 Indaial is included in Bolivia, Brazil and Paraguay. The justification has been the Amazonas outbreak in 2004. In Argentina, a tetravalent vaccine is used incorporating A ARG 2001 in addition to O Campos and A24 Cruzeiro and C3 Indaial. The role of the OIE reference Laboratories in advising on methodology and standards for vaccine control is considered extremely important. Cattle up to 2 years old are vaccinated every 6 months and thereafter annually, aiming for 100% coverage.

Considering the importance of vaccine potency, expectancy of protection (EPP) is also used to gauge antigenic match rather than relying on  $r_1$  values alone.

Apart from the O virus circulating in Ecuador, vaccine matching studies suggest that vaccines that are currently in use shown below should still protect against clinical disease when applied under systematic vaccination and revaccination schemes. However, this will now need very careful monitoring.

Vaccine strains recommended for use in the region\*:

Serotype	Internationally available	Locally produced
O	O <sub>1</sub> Campos,	O <sub>1</sub> Campos
A	A <sub>24</sub> Cruzeiro, Argentina 2001	A <sub>24</sub> Cruzeiro, Argentina 2001
C	C <sub>3</sub> Indaial	C <sub>3</sub> Indaial

\* PANAFTOSA recommendation is as High Priority: O<sub>1</sub> Campos, A<sub>24</sub> Cruzeiro, C<sub>3</sub> Indaial, and as medium priority: A Argentina 2001

NB: New vaccine for ‘O Ecuador 2010’ in development.

## 1.6 Clinical samples and FMDV isolates submitted to reference laboratories of the FMD network during 2010.

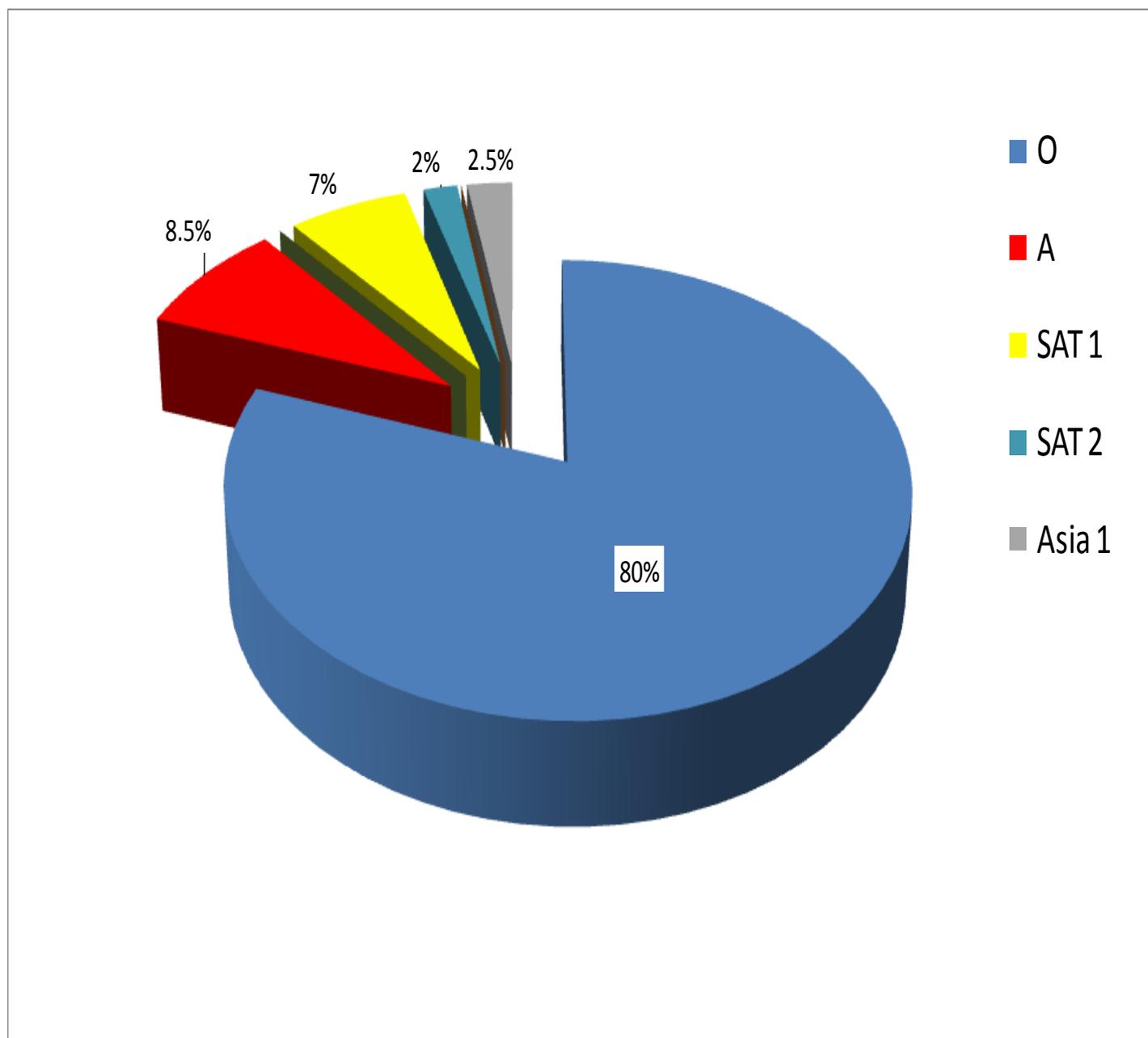
### 1.6.1 Overview of samples received and serotyping results

The network laboratories received and characterised more than 2,300 samples in 2010 from 38 countries, of which approximately 450 had been collected in previous years.

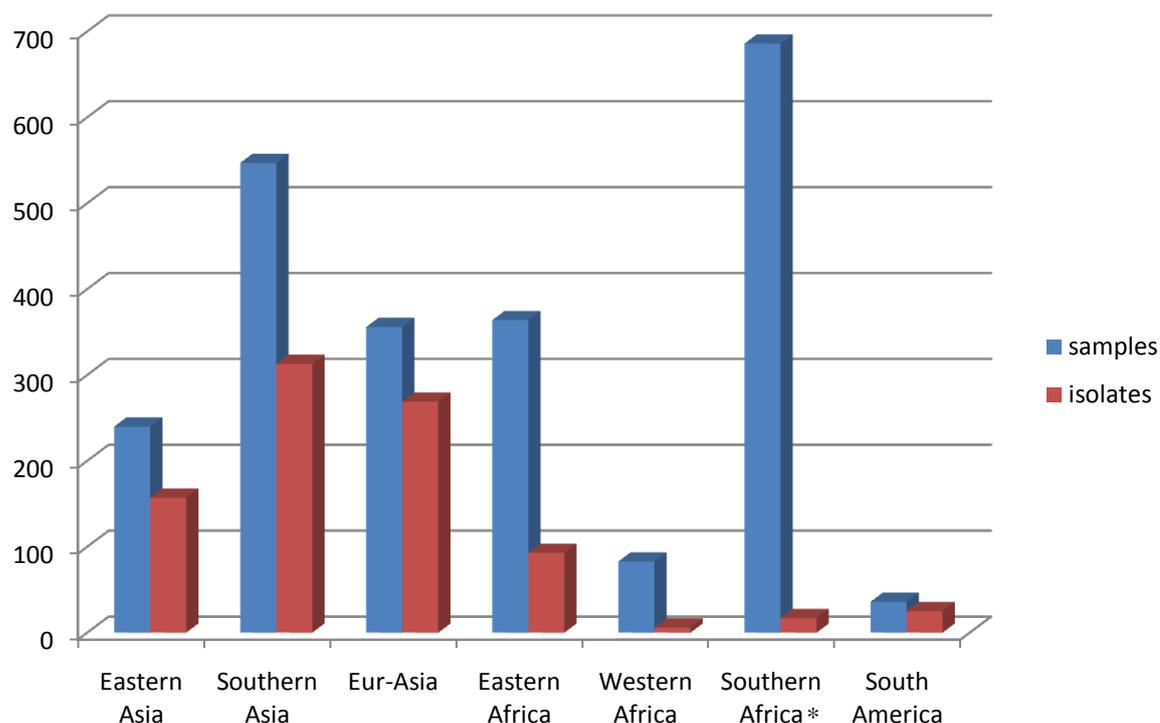
The proportion of the different serotypes detected in 2010 is shown below demonstrating that 80% of the samples characterised in 2010 were of the O serotype.

*Note that serotypes C and SAT 3 were not detected.*

**Figure 6.** Serotypes detected in 2010



The approximate number of samples and number of virus isolates made by region is shown below for samples collected in 2010:



**Figure 7.** Samples and virus isolates made by region. \* information on isolates not complete

The approximate numbers of samples received for FMDV detection and characterised in 2010 by the different network laboratories is tabulated below: In total 38 different countries submitted 2,338 samples in 2010 to the network laboratories with 53% being sent to WRLFMD and 27% sent to ARC-OVI.

**Table 4.** Samples received for FMDV detection

Laboratory	Collected in 2010		Collected earlier	
	Samples	Countries	Samples	Countries
<b>WRLFMD</b>	760	26	458**	14
<b>PANAFTOSA</b>	10	1		
<b>FGI-ARRIAH</b>	15	4		
<b>RRLSSA</b>	24	3		
<b>ARC-OVI</b>	642	10		
<b>PIADC-FADDL</b>	5	1		
<b>LVRI</b>	34	1		
<b>PDFMD</b>	244	1		
<b>RRLSEA</b>	98	4	10*	2
<b>LFADLCT</b>	22	1		
	1854	52 <sup>#</sup>	468	16 <sup>#</sup>

\*\* some samples collected in 2008 and 2009; \* samples collected in 2009. <sup>#</sup> Some countries submitted samples to more than one laboratory

A searchable on-line database of samples is available via the Reference Laboratories Information System (ReLaIS) for the OIE/FAO FMD Reference Laboratories Network <http://www.foot-and-mouth.org/>.

Characterisation results obtained on samples received by WRLFMD and PANAFTOSA can be found respectively at: <http://www.wrlfmd.org/> and at: <http://www.panaftosa.org.br>.

## 1.6.2. Details of serotyping and molecular detection results of samples collected and received in 2010

Country	No. of samples	Virus isolation in cell culture/ELISA									RT-PCR for FMD (or SVD) virus (where appropriate)					
		O	A	C	FMD virus serotypes				SVD virus	NVD	NT	Positive	Negative	NT	Laboratory	
					SAT 1	SAT 2	SAT 3	Asia 1								
<b>Pool 1: EASTERN ASIA</b>																
CAMBODIA	5	5	-	-	-	-	-	-	-	-	-	5	-	-	-	WRLFMD
CAMBODIA	20	12	-	-	-	-	-	-	-	8	-	-	-	-	-	RRLSEA
CHINA	34	17	3	-	-	-	-	-	-	-	-	-	-	-	-	LVRI
HONG KONG	26	19	-	-	-	-	-	-	-	7	-	25	1	-	-	WRLFMD
MYANMAR	5	3	-	-	-	-	-	-	-	1	1	4	-	1	-	WRLFMD
MYANMAR	3		2	-	-	-	-	-	-	1	-	-	-	-	-	RRLSEA
SOUTH KOREA	4	1	1	-	-	-	-	-	-	2	-	4	-	-	-	WRLFMD
THAILAND	6	6	-	-	-	-	-	-	-	-	-	6	-	-	-	WRLFMD
THAILAND	48	23	4	-	-	-	-	-	-	21	-	-	-	-	-	RRLSEA
VIETNAM	13	12	1	-	-	-	-	-	-	-	-	11	-	2	-	WRLFMD
VIETNAM	27	26	1	-	-	-	-	-	-	-	-	-	-	-	-	RRLSEA
<b>Pool 2: SOUTHERN ASIA</b>																
AFGHANISTAN*	109	-	-	-	-	-	-	-	-	4	105	100	9	-	-	WRLFMD
INDIA	244	200	24	-	-	-	-	20	-	-	-	-	-	-	-	PDFMD
NEPAL	20	10	-	-	-	-	-	-	-	10	-	15	5	-	-	WRLFMD
PAKISTAN**	76	54	5	-	-	-	-	-	-	18	-	72	4	-	-	WRLFMD
<b>Pool 3: EUR-ASIA</b>																
IRAN	225	155	21	-	-	-	-	-	-	49	-	200	25	-	-	WRLFMD
KAZAKHSTAN	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	FGI-ARRIAH
LEBANON	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	FGI-ARRIAH
MONGOLIA	18	8	-	-	-	-	-	-	-	10	-	9	3	6	-	WRLFMD
MONGOLIA	3	3	-	-	-	-	-	-	-	-	-	-	-	-	-	FGI-ARRIAH
QATAR	17	-	-	-	-	-	-	-	-	17	-	-	17	-	-	WRLFMD
RUSSIA	8	4	-	-	-	-	-	-	-	4	-	-	-	-	-	FGI-ARRIAH
TURKEY	39	23	6	-	-	-	-	-	-	10	-	36	3	-	-	WRLFMD
UNITED ARAB EMIRATES	7	4	-	-	-	-	-	-	-	3	-	4	3	-	-	WRLFMD

<b>Pool 4: EASTERN AFRICA</b>															
ETHIOPIA	22	3	-	-	-	1	-	-	-	18	-	16	6	-	WRLFMD
KENYA	133	2	-	-	53	-	-	-	-	69	9	73	51	9	WRLFMD
KENYA	211	63	2	-	91	2	-	-	-	-	-	-	-	-	Embakasi
TANZANIA	2	-	-	-	-	-	-	-	-	2	-	2	-	-	WRLFMD
<b>Pool 5: WESTERN AFRICA</b>															
SENEGAL	4	-	-	-	-	-	-	-	-	4	-	-	4	-	WRLFMD
<b>Pool 6: SOUTHERN AFRICA</b>															
BOTSWANA	5	-	-	-	-	5	-	-	-	-	-	5	-	-	WRLFMD
BOTSWANA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	RRLSSA
MALAWI	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ARC-OVI
MOZAMBIQUE	7	-	-	-	-	7	-	-	-	-	-	7	-	-	WRLFMD
MOZAMBIQUE	14	-	-	-	-	2	-	-	-	-	-	2	12	-	ARC-OVI
NAMIBIA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	RRLSSA
NAMIBIA	2	-	-	-	2	-	-	-	-	-	-	2	-	-	ARC-OVI
SOUTH AFRICA	45	-	-	-	12	1	-	-	-	-	-	22	23	-	ARC-OVI
ZAMBIA	4	3	-	-	-	-	-	-	-	1	-	4	-	-	WRLFMD
ZAMBIA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ARC-OVI
ZIMBABWE	4	-	-	-	-	2	-	-	-	2	-	4	-	-	WRLFMD
ZIMBABWE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	RRLSSA
ZIMBABWE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ARC-OVI
<b>Pool 7: SOUTH AMERICA</b>															
ECUADOR	9	9	-	-	-	-	-	-	-	-	-	9	-	-	WRLFMD
ECUADOR	5	5	-	-	-	-	-	-	-	-	-	5	-	-	PIADC-FADDL
ECUADOR	10	9	-	-	-	-	-	-	-	-	-	10	-	-	PANAFTOSA
ECUADOR	11	11	-	-	-	-	-	-	-	-	-	11	-	-	LFADLCT- SENASA
<b>Totals</b>	<b>1449</b>	<b>694</b>	<b>70</b>	<b>158</b>	<b>20</b>	<b>20</b>	<b>20</b>	<b>261</b>	<b>115</b>	<b>663</b>	<b>166</b>	<b>18</b>			

### 1.6.3. Details of serotyping and molecular detection results of samples collected prior to 2010 and received in 2010

Country	year	No. of samples	Virus isolation in cell culture/ELISA							NVD	NT	RT-PCR for FMD (or SVD) virus (where appropriate)			Laboratory
			FMD virus serotypes									Positive	Negative	NT	
			O	A	C	SAT 1	SAT 2	SAT 3	Asia 1						
<b>Pool 1: EASTERN ASIA</b>															
CAMBODIA	2009	2	-	-	-	-	-	-	-	2	-	-	-	-	RRLSEA
LAOS	2008	3	2	1	-	-	-	-	-	-	-	3	-	-	WRLFMD
LAOS	2009	1	1	-	-	-	-	-	-	-	-	1	-	-	WRLFMD
MYANMAR	2008	1	1	-	-	-	-	-	-	-	-	1	-	-	WRLFMD
MYANMAR	2009	5	5	-	-	-	-	-	-	-	-	5	-	-	WRLFMD
THAILAND	2009	23	15	7	-	-	-	-	-	1	-	23	-	-	WRLFMD
THAILAND	2009	8	5	-	-	-	-	-	-	3	-	-	-	-	RRLSEA
VIETNAM	2008	8	1	7	-	-	-	-	-	-	-	8	-	-	WRLFMD
VIETNAM	2009	8	-	8	-	-	-	-	-	-	-	8	-	-	WRLFMD
<b>Pool 2: SOUTHERN ASIA</b>															
AFGHANISTAN*	2009	67	-	-	-	-	-	-	-	-	67	58	9	-	WRLFMD
NEPAL	1997	2	-	-	-	-	-	-	-	2	-	2	-	-	WRLFMD
NEPAL	2001	2	-	-	-	-	-	-	-	2	-	-	2	-	WRLFMD
NEPAL	2002	1	-	-	-	-	-	-	-	1	-	-	1	-	WRLFMD
NEPAL	2004	7	-	-	-	-	-	-	-	7	-	7	-	-	WRLFMD
NEPAL	2006	6	-	-	-	-	-	-	-	6	-	1	5	-	WRLFMD
NEPAL	2007	11	-	-	-	-	-	-	-	11	-	1	10	-	WRLFMD
NEPAL	2008	2	-	-	-	-	-	-	-	2	-	1	1	-	WRLFMD
<b>Pool 3: EUR-ASIA</b>															
IRAN	2009	13	10	1	-	-	-	-	-	2	-	12	1	-	WRLFMD
TURKEY***	2007	5	-	-	-	-	-	-	-	-	5	-	-	5	WRLFMD
TURKEY***	2008	3	-	-	-	-	-	-	-	-	3	-	-	3	WRLFMD
TURKEY***	2009	14	3	9	-	-	-	-	-	2	-	14	-	-	WRLFMD
<b>Pool 4: EASTERN AFRICA</b>															
ERITREA	2005	1	-	-	-	-	-	-	-	1	-	1	-	-	WRLFMD
ERITREA	2006	6	-	2	-	-	-	-	-	4	-	6	-	-	WRLFMD

ERITREA	2007	8	-	1	-	-	-	-	-	7	-	6	2	-	WRLFMD
ERITREA	2008	5	-	2	-	-	-	-	-	3	-	4	1	-	WRLFMD
ERITREA	2009	69	-	2	-	-	-	-	-	67	-	19	50	-	WRLFMD
ETHIOPIA	2009	22	2	-	-	-	12	-	-	8	-	19	3	-	WRLFMD
KENYA	2009	6	1	-	-	2	1	-	-	2	-	6	-	-	WRLFMD
TANZANIA	2008	33	1	2	-	-	-	-	-	30	-	12	21	-	WRLFMD

**Pool 5: WESTERN AFRICA**

NIGERIA	2008	10	-	-	-	-	-	-	-	10	-	4	6	-	WRLFMD
NIGERIA	2009	40	2	3	-	-	-	-	-	35	-	13	27	-	WRLFMD
SENGAL	2009	29	-	-	-	-	1	-	-	28	-	5	24	-	WRLFMD

**Pool 6: SOUTHERN AFRICA**

None

**Pool 7: SOUTH AMERICA**

None

<b>Totals</b>		<b>421</b>	<b>49</b>	<b>45</b>		<b>14</b>				<b>236</b>		<b>240</b>	<b>163</b>		
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\* *all but 4 samples from Afghanistan were supplied in RNA later for PCR analysis*

\*\* *1 sample from Pakistan contained a mixture of type O and A FMDV's*

\*\*\* *8 type O samples from Turkey were submitted for full length genome sequencing*

## **1.7. Genetic and antigenic typing of FMD virus isolates submitted to the Reference Laboratories**

**1.7.1** FMDV isolates for which VP1 gene sequences (639 nucleotides) have been obtained by Network Laboratories during 2010.

In total 750 VP1 sequences were characterised for this report in 2010: **648 (86%) came from WRLFMD** while the remaining 102 (14%) came from other laboratories as listed below. Phylogenetic trees and observations on them can be found at <http://www.wrlfmd.org/> for all of the viruses that were analysed at WRLFMD. The VP1 gene sequences of a selection of virus isolates representative of all of the topotypes of FMDV can also be found at this website.

<b>FMDV ID</b>	<b>Country of origin</b>	<b>Serotype</b>	<b>Topotype</b>	<b>Lineage/strain</b>	<b>Sub-lineage</b>	<b>Laboratory</b>	<b>Date received</b>
<b>Serotype A</b>							
<b>AFG/8/2009</b>	Afghanistan	A	ASIA	Iran-05	AFG-07	WRLFMD	09/04/2010
<b>AFG/9/2009</b>	Afghanistan	A	ASIA	Iran-05	AFG-07	WRLFMD	09/04/2010
<b>AFG/11/2009</b>	Afghanistan	A	ASIA	Iran-05	AFG-07	WRLFMD	09/04/2010
<b>AFG/13/2009</b>	Afghanistan	A	ASIA	Iran-05	AFG-07	WRLFMD	09/04/2010
<b>AFG/14/2009</b>	Afghanistan	A	ASIA	Iran-05	AFG-07	WRLFMD	09/04/2010
<b>AFG/15/2009</b>	Afghanistan	A	ASIA	Iran-05	AFG-07	WRLFMD	09/04/2010
<b>AFG/16/2009</b>	Afghanistan	A	ASIA	Iran-05	AFG-07	WRLFMD	09/04/2010
<b>AFG/17/2009</b>	Afghanistan	A	ASIA	Iran-05	AFG-07	WRLFMD	09/04/2010
<b>AFG/41/2009</b>	Afghanistan	A	ASIA	Iran-05	AFG-07	WRLFMD	09/04/2010
<b>AFG/3/2010</b>	Afghanistan	A	ASIA	Iran-05	AFG-07	WRLFMD	09/04/2010
<b>AFG/6/2010</b>	Afghanistan	A	ASIA	Iran-05	AFG-07	WRLFMD	09/04/2010
<b>AFG/9/2010</b>	Afghanistan	A	ASIA	Iran-05	AFG-07	WRLFMD	09/04/2010
<b>AFG/10/2010</b>	Afghanistan	A	ASIA	Iran-05	AFG-07	WRLFMD	09/04/2010
<b>AFG/15/2010</b>	Afghanistan	A	ASIA	Iran-05	AFG-07	WRLFMD	09/04/2010
<b>AFG/16/2010</b>	Afghanistan	A	ASIA	Iran-05	AFG-07	WRLFMD	09/04/2010
<b>AFG/17/2010</b>	Afghanistan	A	ASIA	Iran-05	AFG-07	WRLFMD	09/04/2010
<b>AFG/18/2010</b>	Afghanistan	A	ASIA	Iran-05	AFG-07	WRLFMD	09/04/2010
<b>AFG/47/2010</b>	Afghanistan	A	ASIA	Iran-05	AFG-07	WRLFMD	31/08/2010
<b>ERI/1/2006</b>	Eritrea	A	AFRICA	G-IV	unnamed	WRLFMD	20/01/2010
<b>ERI/5/2006</b>	Eritrea	A	AFRICA	G-IV	unnamed	WRLFMD	20/01/2010
<b>ERI/4/2007</b>	Eritrea	A	AFRICA	G-IV	unnamed	WRLFMD	20/01/2010
<b>ERI/1/2008</b>	Eritrea	A	AFRICA	G-IV	unnamed	WRLFMD	20/01/2010
<b>ERI/5/2008</b>	Eritrea	A	AFRICA	G-IV	unnamed	WRLFMD	20/01/2010
<b>ERI/16/2009</b>	Eritrea	A	AFRICA	G-IV	unnamed	WRLFMD	20/01/2010
<b>ERI/40/2009</b>	Eritrea	A	AFRICA	G-IV	unnamed	WRLFMD	20/01/2010
<b>IRN/78/2009</b>	Iran	A	ASIA	Iran-05	AFG-07	WRLFMD	18/03/2010
<b>IRN/7/2010</b>	Iran	A	ASIA	Iran-05	AFG-07	WRLFMD	18/03/2010
<b>IRN/9/2010</b>	Iran	A	ASIA	Iran-05	unnamed	WRLFMD	18/03/2010
<b>IRN/36/2010</b>	Iran	A	ASIA	Iran-05	BAR-08	WRLFMD	21/04/2010
<b>IRN/73/2010</b>	Iran	A	ASIA	Iran-05	AFG-07	WRLFMD	26/05/2010
<b>IRN/80/2010</b>	Iran	A	ASIA	Iran-05	AFG-07	WRLFMD	26/05/2010
<b>IRN/108/2010</b>	Iran	A	ASIA	Iran-05	AFG-07	WRLFMD	26/05/2010
<b>IRN/119/2010</b>	Iran	A	ASIA	Iran-05	AFG-07	WRLFMD	26/05/2010

<b>IRN/125/2010</b>	Iran	A	ASIA	Iran-05	AFG-07	WRLFMD	26/05/2010
<b>IRN/134/2010</b>	Iran	A	ASIA	Iran-05	AFG-07	WRLFMD	26/05/2010
<b>IRN/151/2010</b>	Iran	A	ASIA	Iran-05	AFG-07	WRLFMD	26/05/2010
<b>IRN/176/2010</b>	Iran	A	ASIA	Iran-05	AFG-07	WRLFMD	29/09/2010
<b>IRN/177/2010</b>	Iran	A	ASIA	Iran-05	AFG-07	WRLFMD	29/09/2010
<b>IRN/185/2010</b>	Iran	A	ASIA	Iran-05	AFG-07	WRLFMD	29/09/2010
<b>IRN/188/2010</b>	Iran	A	ASIA	Iran-05	AFG-07	WRLFMD	29/09/2010
<b>IRN/195/2010</b>	Iran	A	ASIA	Iran-05	AFG-07	WRLFMD	25/10/2010
<b>IRN/197/2010</b>	Iran	A	ASIA	Iran-05	AFG-07	WRLFMD	25/10/2010
<b>IRN/200/2010</b>	Iran	A	ASIA	Iran-05	AFG-07	WRLFMD	25/10/2010
<b>IRN/201/2010</b>	Iran	A	ASIA	Iran-05	AFG-07	WRLFMD	25/10/2010
<b>IRN/212/2010</b>	Iran	A	ASIA	Iran-05	AFG-07	WRLFMD	25/10/2010
<b>IRN/213/2010</b>	Iran	A	ASIA	Iran-05	AFG-07	WRLFMD	25/10/2010
<b>IRN/214/2010</b>	Iran	A	ASIA	Iran-05	AFG-07	WRLFMD	25/10/2010
<b>IRN/215/2010</b>	Iran	A	ASIA	Iran-05	AFG-07	WRLFMD	17/11/2010
<b>LAO/4/2008</b>	Laos	A	ASIA	Sea-97	unnamed	WRLFMD	05/02/2010
<b>MYA/4/2010</b>	Myanmar	A	ASIA	unnamed	unnamed	WRLFMD	25/11/2010
<b>NIG/3/2009</b>	Nigeria	A	AFRICA	G-IV	unnamed	WRLFMD	16/03/2010
<b>NIG/36/2009</b>	Nigeria	A	AFRICA	G-IV	unnamed	WRLFMD	16/03/2010
<b>NIG/38/2009</b>	Nigeria	A	AFRICA	G-IV	unnamed	WRLFMD	16/03/2010
<b>NIG/39/2009</b>	Nigeria	A	AFRICA	G-IV	unnamed	WRLFMD	16/03/2010
<b>PAK/12/2010</b>	Pakistan	A	ASIA	Iran-05	AFG-07	WRLFMD	09/04/2010
<b>PAK/13/2010</b>	Pakistan	A	ASIA	Iran-05	AFG-07	WRLFMD	09/04/2010
<b>PAK/23/2010</b>	Pakistan	A	ASIA	Iran-05	AFG-07	WRLFMD	09/04/2010
<b>PAK/24/2010</b>	Pakistan	A	ASIA	Iran-05	AFG-07	WRLFMD	09/04/2010
<b>PAK/37/2010</b>	Pakistan	A	ASIA	Iran-05	AFG-07	WRLFMD	31/08/2010
<b>SKR/2/2010</b>	South Korea	A	ASIA	Sea-97	unnamed	WRLFMD	17/05/2010
<b>Pochun/KOR/2010*</b>	South Korea	A	SEA	Mya-98	unnamed	NVRQS	08/01/2010
<b>TAI/5/2009</b>	Thailand	A	ASIA	Sea-97	unnamed	WRLFMD	05/02/2010
<b>TAI/6/2009</b>	Thailand	A	ASIA	Sea-97	unnamed	WRLFMD	05/02/2010
<b>TAI/7/2009</b>	Thailand	A	ASIA	Sea-97	unnamed	WRLFMD	05/02/2010
<b>TAI/8/2009</b>	Thailand	A	ASIA	Sea-97	unnamed	WRLFMD	05/02/2010
<b>TAI/9/2009</b>	Thailand	A	ASIA	Sea-97	unnamed	WRLFMD	05/02/2010
<b>TAI/10/2009</b>	Thailand	A	ASIA	Sea-97	unnamed	WRLFMD	05/02/2010
<b>TAI/14/2009</b>	Thailand	A	ASIA	Sea-97	unnamed	WRLFMD	05/02/2010
<b>TAI/12/2009</b>	Thailand	A	ASIA			RRLSEA	26/01/2009

<b>TAI/19/2009</b>	Thailand	A	ASIA			RRLSEA	5/02/2009
<b>TAI/20/2009</b>	Thailand	A	ASIA			RRLSEA	6/02/2009
<b>TAI/22/2009</b>	Thailand	A	ASIA			RRLSEA	17/02/2009
<b>TAI/25/2009</b>	Thailand	A	ASIA			RRLSEA	19/02/2009
<b>TAI/37/2009</b>	Thailand	A	ASIA			RRLSEA	7/09/2009
<b>TAN/11/2008</b>	Tanzania	A	AFRICA	G-I	unnamed	WRLFMD	10/03/2010
<b>TAN/12/2008</b>	Tanzania	A	AFRICA	G-I	unnamed	WRLFMD	10/03/2010
<b>TAN/4/2009</b>	Tanzania	A	AFRICA	G-I	unnamed	WRLFMD	10/03/2010
<b>TAN/9/2009</b>	Tanzania	A	AFRICA	G-I	unnamed	WRLFMD	10/03/2010
<b>TAN/11/2009</b>	Tanzania	A	AFRICA	G-I	unnamed	WRLFMD	10/03/2010
<b>TAN/42/2009</b>	Tanzania	A	AFRICA	G-I	unnamed	WRLFMD	10/03/2010
<b>TAN/45/2009</b>	Tanzania	A	AFRICA	G-I	unnamed	WRLFMD	10/03/2010
<b>TAN/47/2009</b>	Tanzania	A	AFRICA	G-I	unnamed	WRLFMD	10/03/2010
<b>TUR/8/2009</b>	Turkey	A	ASIA	Iran-05	EZM-07	WRLFMD	04/02/2010
<b>TUR/9/2009</b>	Turkey	A	ASIA	Iran-05	ARD-07	WRLFMD	04/02/2010
<b>TUR/10/2009</b>	Turkey	A	ASIA	Iran-05	ARD-07	WRLFMD	04/02/2010
<b>TUR/11/2009</b>	Turkey	A	ASIA	Iran-05	ARD-07	WRLFMD	04/02/2010
<b>TUR/12/2009</b>	Turkey	A	ASIA	Iran-05	unnamed	WRLFMD	04/02/2010
<b>TUR/13/2009</b>	Turkey	A	ASIA	Iran-05	ARD-07	WRLFMD	04/02/2010
<b>TUR/14/2009</b>	Turkey	A	ASIA	Iran-05	ARD-07	WRLFMD	04/02/2010
<b>TUR/18/2009</b>	Turkey	A	ASIA	Iran-05	BAR-08	WRLFMD	04/02/2010
<b>TUR/21/2009</b>	Turkey	A	ASIA	Iran-05	ARD-07	WRLFMD	04/02/2010
<b>TUR/2/2010</b>	Turkey	A	ASIA	Iran-05	ARD-07	WRLFMD	04/02/2010
<b>TUR/3/2010</b>	Turkey	A	ASIA	Iran-05	unnamed	WRLFMD	04/02/2010
<b>TUR/6/2010</b>	Turkey	A	ASIA	Iran-05	ARD-07	WRLFMD	04/02/2010
<b>TUR/28/2010</b>	Turkey	A	ASIA	Iran-05	AFG-07	WRLFMD	02/09/2010
<b>TUR/34/2010</b>	Turkey	A	ASIA	Iran-05	AFG-07	WRLFMD	02/09/2010
<b>TUR/20/2010(A)</b>	Turkey	A	ASIA	Iran-05	AFG-07	WRLFMD	02/09/2010
<b>TUR/99/2010*</b>	Turkey	A	ME-SA	Iran-05	AFG-07	FMDI	14/04/2010
<b>TUR/127/2010*</b>	Turkey	A	ME-SA	Iran-05	unnamed	FMDI	14/04/2010
<b>TUR/135/2010*</b>	Turkey	A	ME-SA	Iran-05	unnamed	FMDI	14/04/2010
<b>TUR/136/2010*</b>	Turkey	A	ME-SA	Iran-05	ARD-07	FMDI	14/04/2010
<b>TUR/138/2010*</b>	Turkey	A	ME-SA	Iran-05	ARD-07	FMDI	14/04/2010
<b>TUR/148/2010*</b>	Turkey	A	ME-SA	Iran-05	unnamed	FMDI	14/04/2010
<b>TUR/151/2010*</b>	Turkey	A	ME-SA	Iran-05	AFG-07	FMDI	14/04/2010
<b>TUR/152/2010*</b>	Turkey	A	ME-SA	Iran-05	ARD-07	FMDI	14/04/2010

<b>TUR/157/2010*</b>	Turkey	A	ME-SA	Iran-05	ARD-07	FMDI	14/04/2010
<b>TUR/158/2010*</b>	Turkey	A	ME-SA	Iran-05	ARD-07	FMDI	14/04/2010
<b>TUR/159/2010*</b>	Turkey	A	ME-SA	Iran-05	ARD-07	FMDI	14/04/2010
<b>TUR/160/2010*</b>	Turkey	A	ME-SA	Iran-05	ARD-07	FMDI	14/04/2010
<b>TUR/161/2010*</b>	Turkey	A	ME-SA	Iran-05	AFG-07	FMDI	14/04/2010
<b>TUR/20/2010(B)</b>	Turkey	A	ASIA	Iran-05	ARD-07	WRLFMD	02/09/2010
<b>VIT/2/2008</b>	Vietnam	A	ASIA	Sea-97	unnamed	WRLFMD	05/02/2010
<b>VIT/3/2008</b>	Vietnam	A	ASIA	Sea-97	unnamed	WRLFMD	05/02/2010
<b>VIT/4/2008</b>	Vietnam	A	ASIA	Sea-97	unnamed	WRLFMD	05/02/2010
<b>VIT/5/2008</b>	Vietnam	A	ASIA	Sea-97	unnamed	WRLFMD	05/02/2010
<b>VIT/6/2008</b>	Vietnam	A	ASIA	Sea-97	unnamed	WRLFMD	05/02/2010
<b>VIT/7/2008</b>	Vietnam	A	ASIA	Sea-97	unnamed	WRLFMD	05/02/2010
<b>VIT/8/2008</b>	Vietnam	A	ASIA	Sea-97	unnamed	WRLFMD	05/02/2010
<b>VIT/1/2009</b>	Vietnam	A	ASIA	Sea-97	unnamed	WRLFMD	05/02/2010
<b>VIT/2/2009</b>	Vietnam	A	ASIA	Sea-97	unnamed	WRLFMD	05/02/2010
<b>VIT/3/2009</b>	Vietnam	A	ASIA	Sea-97	unnamed	WRLFMD	05/02/2010
<b>VIT/4/2009</b>	Vietnam	A	ASIA	Sea-97	unnamed	WRLFMD	05/02/2010
<b>VIT/5/2009</b>	Vietnam	A	ASIA	Sea-97	unnamed	WRLFMD	05/02/2010
<b>VIT/6/2009</b>	Vietnam	A	ASIA	Sea-97	unnamed	WRLFMD	05/02/2010
<b>VIT/7/2009</b>	Vietnam	A	ASIA	Sea-97	unnamed	WRLFMD	05/02/2010
<b>VIT/8/2009</b>	Vietnam	A	ASIA	Sea-97	unnamed	WRLFMD	05/02/2010
<b>VIT/1/2010</b>	Vietnam	A	ASIA	Sea-97	unnamed	WRLFMD	05/11/2010
<b>VIT/2/2009</b>	Vietnam	A	ASIA			RRLSEA	8/06/2009
<b>VIT/3/2009</b>	Vietnam	A	ASIA			RRLSEA	8/06/2009
<b>VIT/7/2009</b>	Vietnam	A	ASIA			RRLSEA	8/06/2009
<b>VIT/8/2009</b>	Vietnam	A	ASIA			RRLSEA	8/06/2009
<b>VIT/9/2009</b>	Vietnam	A	ASIA			RRLSEA	8/06/2009
<b>VIT/11/2009</b>	Vietnam	A	ASIA			RRLSEA	8/06/2009
<b>VIT/12/2009</b>	Vietnam	A	ASIA			RRLSEA	8/06/2009
<b>VIT/12/2009</b>	Vietnam	A	ASIA			RRLSEA	8/06/2009
<b>VIT/15/2009</b>	Vietnam	A	ASIA			RRLSEA	8/06/2009
<b>VIT/16/2009</b>	Vietnam	A	ASIA			RRLSEA	8/06/2009
<b>VIT/17/2009</b>	Vietnam	A	ASIA			RRLSEA	8/06/2009
<b>VIT/20/2009</b>	Vietnam	A	ASIA			RRLSEA	8/06/2009
<b>VIT/21/2009</b>	Vietnam	A	ASIA			RRLSEA	8/06/2009
<b>VIT/22/2009</b>	Vietnam	A	ASIA			RRLSEA	8/06/2009

### Serotype O

<b>AFG/19/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/22/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/23/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/24/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/25/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/27/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/28/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/29/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/30/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/31/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/32/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/33/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/35/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/36/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/37/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/38/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/40/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/42/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/43/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/44/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/45/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/46/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/49/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/65/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/66/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/67/2009</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/1/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/2/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/4/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/5/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/11/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/12/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/13/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010

<b>AFG/19/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/20/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	PUN-10	WRLFMD	09/04/2010
<b>AFG/21/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/22/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/23/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/24/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/25/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/26/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/27/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	09/04/2010
<b>AFG/30/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	unnamed	WRLFMD	31/08/2010
<b>AFG/31/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	31/08/2010
<b>AFG/32/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/34/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/36/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/39/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/40/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/41/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/42/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/43/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/44/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/51/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/52/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	31/08/2010
<b>AFG/53/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	31/08/2010
<b>AFG/55/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/56/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/57/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/58/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/59/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/60/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/61/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/62/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/64/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/65/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/66/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/67/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/68/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010

<b>AFG/70/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/75/2010</b>	Afghanistan	O	ME-SA	unnamed	unnamed	WRLFMD	31/08/2010
<b>AFG/76/2010</b>	Afghanistan	O	ME-SA	unnamed	unnamed	WRLFMD	31/08/2010
<b>AFG/82/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/83/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/89/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/91/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/93/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	31/08/2010
<b>AFG/96/2010</b>	Afghanistan	O	ME-SA	unnamed	unnamed	WRLFMD	31/08/2010
<b>AFG/97/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	31/08/2010
<b>AFG/104/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>AFG/109/2010</b>	Afghanistan	O	ME-SA	PanAsia-2	BAL-09	WRLFMD	31/08/2010
<b>CAM/1/2010</b>	Cambodia	O	ME-SA	PanAsia	unnamed	WRLFMD	25/11/2010
<b>CAM/2/2010</b>	Cambodia	O	ME-SA	PanAsia	unnamed	WRLFMD	25/11/2010
<b>CAM/3/2010</b>	Cambodia	O	ME-SA	PanAsia	unnamed	WRLFMD	25/11/2010
<b>CAM/4/2010</b>	Cambodia	O	ME-SA	PanAsia	unnamed	WRLFMD	25/11/2010
<b>CAM/5/2010</b>	Cambodia	O	ME-SA	PanAsia	unnamed	WRLFMD	25/11/2010
<b>ECU/1/2010</b>	Ecuador	O	EURO-SA	unnamed	unnamed	WRLFMD	14/06/2010
<b>ECU/2/2010</b>	Ecuador	O	EURO-SA	unnamed	unnamed	WRLFMD	14/06/2010
<b>ECU/3/2010</b>	Ecuador	O	EURO-SA	unnamed	unnamed	WRLFMD	14/06/2010
<b>ECU/4/2010</b>	Ecuador	O	EURO-SA	unnamed	unnamed	WRLFMD	14/06/2010
<b>ECU/5/2010</b>	Ecuador	O	EURO-SA	unnamed	unnamed	WRLFMD	14/06/2010
<b>ECU/6/2010</b>	Ecuador	O	EURO-SA	unnamed	unnamed	WRLFMD	14/06/2010
<b>ECU/7/2010</b>	Ecuador	O	EURO-SA	unnamed	unnamed	WRLFMD	14/06/2010
<b>ECU/8/2010</b>	Ecuador	O	EURO-SA	unnamed	unnamed	WRLFMD	14/06/2010
<b>ECU/9/2010</b>	Ecuador	O	EURO-SA	unnamed	unnamed	WRLFMD	14/06/2010
<b>224-02</b>	Ecuador	O	EURO-SA			PANAFTOSA	
<b>224-08</b>	Ecuador	O	EURO-SA			PANAFTOSA	
<b>224-21</b>	Ecuador	O	EURO-SA			PANAFTOSA	
<b>224-36</b>	Ecuador	O	EURO-SA			PANAFTOSA	
<b>224-45</b>	Ecuador	O	EURO-SA			PANAFTOSA	
<b>224-47</b>	Ecuador	O	EURO-SA			PANAFTOSA	
<b>224-56</b>	Ecuador	O	EURO-SA			PANAFTOSA	
<b>224-61</b>	Ecuador	O	EURO-SA			PANAFTOSA	
<b>23-2010</b>	Ecuador	O	EURO-SA			SENASA	30/05/10
<b>10-2010</b>	Ecuador	O	EURO-SA			SENASA	27/03/10

<b>O/EC/2010-1</b>	Ecuador	O	EURO-SA			PIADC-FADDL	07/10
<b>O/EC/2010-2</b>	Ecuador	O	EURO-SA			PIADC-FADDL	07/10
<b>O/EC/2010-3</b>	Ecuador	O	EURO-SA			PIADC-FADDL	07/10
<b>O/EC/2010-4</b>	Ecuador	O	EURO-SA			PIADC-FADDL	07/10
<b>O/EC/2010-5</b>	Ecuador	O	EURO-SA			PIADC-FADDL	07/10
<b>ETH/59/2009</b>	Ethiopia	O	EA-3	unnamed	unnamed	WRLFMD	20/02/2010
<b>ETH/60/2009</b>	Ethiopia	O	EA-3	unnamed	unnamed	WRLFMD	20/02/2010
<b>ETH/7/2010</b>	Ethiopia	O	EA-3	unnamed	unnamed	WRLFMD	18/03/2010
<b>ETH/8/2010</b>	Ethiopia	O	EA-3	unnamed	unnamed	WRLFMD	18/03/2010
<b>ETH/9/2010</b>	Ethiopia	O	EA-3	unnamed	unnamed	WRLFMD	18/03/2010
<b>HKN/1/2010</b>	Hong Kong	O	SEA	Mya-98	unnamed	WRLFMD	05/03/2010
<b>HKN/4/2010</b>	Hong Kong	O	SEA	Mya-98	unnamed	WRLFMD	05/03/2010
<b>HKN/6/2010</b>	Hong Kong	O	SEA	Mya-98	unnamed	WRLFMD	05/03/2010
<b>HKN/7/2010</b>	Hong Kong	O	SEA	Mya-98	unnamed	WRLFMD	05/03/2010
<b>HKN/8/2010</b>	Hong Kong	O	SEA	Mya-98	unnamed	WRLFMD	05/03/2010
<b>HKN/9/2010</b>	Hong Kong	O	SEA	Mya-98	unnamed	WRLFMD	05/03/2010
<b>HKN/10/2010</b>	Hong Kong	O	SEA	Mya-98	unnamed	WRLFMD	05/03/2010
<b>HKN/11/2010</b>	Hong Kong	O	SEA	Mya-98	unnamed	WRLFMD	05/03/2010
<b>HKN/12/2010</b>	Hong Kong	O	SEA	Mya-98	unnamed	WRLFMD	05/03/2010
<b>HKN/13/2010</b>	Hong Kong	O	SEA	Mya-98	unnamed	WRLFMD	05/03/2010
<b>HKN/14/2010</b>	Hong Kong	O	SEA	Mya-98	unnamed	WRLFMD	05/03/2010
<b>HKN/15/2010</b>	Hong Kong	O	SEA	Mya-98	unnamed	WRLFMD	05/03/2010
<b>HKN/18/2010</b>	Hong Kong	O	SEA	Mya-98	unnamed	WRLFMD	05/03/2010
<b>HKN/19/2010</b>	Hong Kong	O	SEA	Mya-98	unnamed	WRLFMD	05/03/2010
<b>HKN/20/2010</b>	Hong Kong	O	SEA	Mya-98	unnamed	WRLFMD	16/03/2010
<b>HKN/24/2010</b>	Hong Kong	O	CATHAY	unnamed	unnamed	WRLFMD	08/12/2010
<b>HKN/25/2010</b>	Hong Kong	O	CATHAY	unnamed	unnamed	WRLFMD	08/12/2010
<b>HKN/26/2010</b>	Hong Kong	O	CATHAY	unnamed	unnamed	WRLFMD	08/12/2010
<b>IRN/79/2009</b>	Iran	O	ME-SA	PanAsia-2	FAR-09	WRLFMD	18/03/2010
<b>IRN/80/2009</b>	Iran	O	ME-SA	PanAsia-2	FAR-09	WRLFMD	18/03/2010
<b>IRN/81/2009</b>	Iran	O	ME-SA	PanAsia-2	FAR-09	WRLFMD	18/03/2010
<b>IRN/83/2009</b>	Iran	O	ME-SA	PanAsia-2	FAR-09	WRLFMD	18/03/2010
<b>IRN/84/2009</b>	Iran	O	ME-SA	PanAsia-2	FAR-09	WRLFMD	18/03/2010
<b>IRN/85/2009</b>	Iran	O	ME-SA	PanAsia-2	FAR-09	WRLFMD	18/03/2010
<b>IRN/86/2009</b>	Iran	O	ME-SA	PanAsia-2	unnamed	WRLFMD	18/03/2010
<b>IRN/87/2009</b>	Iran	O	ME-SA	PanAsia-2	FAR-09	WRLFMD	18/03/2010

<b>IRN/88/2009</b>	Iran	O	ME-SA	PanAsia-2	unnamed	WRLFMD	18/03/2010
<b>IRN/89/2009</b>	Iran	O	ME-SA	PanAsia-2	FAR-09	WRLFMD	18/03/2010
<b>IRN/1/2010</b>	Iran	O	ME-SA	PanAsia-2	FAR-09	WRLFMD	18/03/2010
<b>IRN/2/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	18/03/2010
<b>IRN/3/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	18/03/2010
<b>IRN/4/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	18/03/2010
<b>IRN/5/2010</b>	Iran	O	ME-SA	PanAsia-2	FAR-09	WRLFMD	18/03/2010
<b>IRN/8/2010</b>	Iran	O	ME-SA	PanAsia-2	FAR-09	WRLFMD	18/03/2010
<b>IRN/10/2010</b>	Iran	O	ME-SA	PanAsia-2	FAR-09	WRLFMD	18/03/2010
<b>IRN/11/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	18/03/2010
<b>IRN/12/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	18/03/2010
<b>IRN/13/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	18/03/2010
<b>IRN/14/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	18/03/2010
<b>IRN/15/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	18/03/2010
<b>IRN/17/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	18/03/2010
<b>IRN/18/2010</b>	Iran	O	ME-SA	PanAsia-2	FAR-09	WRLFMD	18/03/2010
<b>IRN/19/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	18/03/2010
<b>IRN/20/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	18/03/2010
<b>IRN/22/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	18/03/2010
<b>IRN/23/2010</b>	Iran	O	ME-SA	PanAsia-2	unnamed	WRLFMD	18/03/2010
<b>IRN/24/2010</b>	Iran	O	ME-SA	PanAsia-2	unnamed	WRLFMD	18/03/2010
<b>IRN/26/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	18/03/2010
<b>IRN/27/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	18/03/2010
<b>IRN/28/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	18/03/2010
<b>IRN/29/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	18/03/2010
<b>IRN/30/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	18/03/2010
<b>IRN/31/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	18/03/2010
<b>IRN/33/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	21/04/2010
<b>IRN/34/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	21/04/2010
<b>IRN/35/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	21/04/2010
<b>IRN/37/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	21/04/2010
<b>IRN/38/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	21/04/2010
<b>IRN/39/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	21/04/2010
<b>IRN/40/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	21/04/2010
<b>IRN/41/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	21/04/2010
<b>IRN/43/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	21/04/2010

<b>IRN/44/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	21/04/2010
<b>IRN/47/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	21/04/2010
<b>IRN/48/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	21/04/2010
<b>IRN/49/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	21/04/2010
<b>IRN/50/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	21/04/2010
<b>IRN/51/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	21/04/2010
<b>IRN/52/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	21/04/2010
<b>IRN/54/2010</b>	Iran	O	ME-SA	PanAsia-2	FAR-09	WRLFMD	21/04/2010
<b>IRN/55/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	21/04/2010
<b>IRN/59/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/60/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/61/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/62/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/64/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/65/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/66/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/67/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/68/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/69/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/71/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/75/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/76/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/78/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/79/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/81/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/82/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/83/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/85/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/86/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/88/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/89/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/90/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/91/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/92/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/93/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/94/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010

<b>IRN/95/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/96/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/97/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/98/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/99/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/100/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/101/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/102/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/104/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/105/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/106/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/109/2010</b>	Iran	O	ME-SA	PanAsia-2	FAR-09	WRLFMD	26/05/2010
<b>IRN/113/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/114/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/115/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/120/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/124/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/126/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/127/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/128/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/129/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/130/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/131/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/132/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/133/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/135/2010</b>	Iran	O	ME-SA	PanAsia-2	FAR-09	WRLFMD	26/05/2010
<b>IRN/136/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/137/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/138/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/139/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/140/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/141/2010</b>	Iran	O	ME-SA	PanAsia-2	unnamed	WRLFMD	26/05/2010
<b>IRN/142/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/143/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/144/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/146/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010

<b>IRN/147/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/148/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/149/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/150/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/154/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/155/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/156/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/157/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/158/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/160/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/161/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/162/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/170/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/172/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/173/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	26/05/2010
<b>IRN/174/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	29/09/2010
<b>IRN/175/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	29/09/2010
<b>IRN/178/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	29/09/2010
<b>IRN/179/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	29/09/2010
<b>IRN/180/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	29/09/2010
<b>IRN/181/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	29/09/2010
<b>IRN/182/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	29/09/2010
<b>IRN/183/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	29/09/2010
<b>IRN/184/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	29/09/2010
<b>IRN/186/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	29/09/2010
<b>IRN/187/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	29/09/2010
<b>IRN/191/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	25/10/2010
<b>IRN/192/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	25/10/2010
<b>IRN/194/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	25/10/2010
<b>IRN/196/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	25/10/2010
<b>IRN/198/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	25/10/2010
<b>IRN/199/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	25/10/2010
<b>IRN/202/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	25/10/2010
<b>IRN/203/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	25/10/2010
<b>IRN/204/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	25/10/2010
<b>IRN/205/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	25/10/2010

<b>IRN/206/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	25/10/2010
<b>IRN/207/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	25/10/2010
<b>IRN/208/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	25/10/2010
<b>IRN/209/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	25/10/2010
<b>IRN/210/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	25/10/2010
<b>IRN/211/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	25/10/2010
<b>IRN/217/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	17/11/2010
<b>IRN/219/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	17/11/2010
<b>IRN/221/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	17/11/2010
<b>IRN/222/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	17/11/2010
<b>IRN/223/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	17/11/2010
<b>IRN/224/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	17/11/2010
<b>IRN/225/2010</b>	Iran	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	17/11/2010
<b>JPN/2010*</b>	Japan	O	SEA	Mya-98	unnamed	NIAH	27/04/2010
<b>KAZ/2010*</b>	Kazakhstan	O	ME-SA	PanAsia-2	unnamed	ARRIAH	02/07/2010
<b>KEN/125/2009</b>	Kenya	O	EA-1	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/69/2010</b>	Kenya	O	EA-1	unnamed	unnamed	WRLFMD	06/04/2010
<b>KEN/100/2010</b>	Kenya	O	EA-4	unnamed	unnamed	WRLFMD	10/06/2010
<b>LAO/2/2008</b>	Laos	O	SEA	Mya-98	unnamed	WRLFMD	05/02/2010
<b>LAO/3/2008</b>	Laos	O	SEA	Mya-98	unnamed	WRLFMD	05/02/2010
<b>LAO/1/2009</b>	Laos	O	SEA	Mya-98	unnamed	WRLFMD	05/02/2010
<b>LAO/5/2009</b>	Lao PDR	O	SEA	Mya-98	unnamed	RRLSEA	29/01/2009
<b>O/Lebanon/2010</b>	Lebanon	O	ME-SA	PanAsia-2	unnamed	ARRIAH	2010
<b>MOG/1/2010</b>	Mongolia	O	SEA	Mya-98	unnamed	WRLFMD	12/10/2010
<b>MOG/2/2010</b>	Mongolia	O	SEA	Mya-98	unnamed	WRLFMD	12/10/2010
<b>MOG/3/2010</b>	Mongolia	O	SEA	Mya-98	unnamed	WRLFMD	12/10/2010
<b>MOG/4/2010</b>	Mongolia	O	SEA	Mya-98	unnamed	WRLFMD	12/10/2010
<b>MOG/5/2010</b>	Mongolia	O	SEA	Mya-98	unnamed	WRLFMD	12/10/2010
<b>MOG/6/2010</b>	Mongolia	O	SEA	Mya-98	unnamed	WRLFMD	12/10/2010
<b>MOG/7/2010</b>	Mongolia	O	SEA	Mya-98	unnamed	WRLFMD	12/10/2010
<b>MOG/9/2010</b>	Mongolia	O	SEA	Mya-98	unnamed	WRLFMD	12/10/2010
<b>MOG/CO3/2010*</b>	Mongolia	O	SEA	Mya-98	unnamed	ARRIAH	04/06/2010
<b>MOG/56/2010*</b>	Mongolia	O	SEA	Mya-98	unnamed	ARRIAH	16/11/2010
<b>MOG/66/2010*</b>	Mongolia	O	SEA	Mya-98	unnamed	ARRIAH	16/11/2010
<b>MOG/77/2010*</b>	Mongolia	O	SEA	Mya-98	unnamed	ARRIAH	16/11/2010
<b>MYA/3/2008</b>	Myanmar	O	SEA	Mya-98	unnamed	WRLFMD	05/02/2010

<b>MYA/9/2009</b>	Myanmar	O	SEA	Mya-98	unnamed	WRLFMD	05/02/2010
<b>MYA/10/2009</b>	Myanmar	O	SEA	Mya-98	unnamed	WRLFMD	05/02/2010
<b>MYA/11/2009</b>	Myanmar	O	SEA	Mya-98	unnamed	WRLFMD	05/02/2010
<b>MYA/12/2009</b>	Myanmar	O	SEA	Mya-98	unnamed	WRLFMD	25/06/2010
<b>MYA/13/2009</b>	Myanmar	O	SEA	Mya-98	unnamed	WRLFMD	25/06/2010
<b>MYA/1/2010</b>	Myanmar	O	SEA	unnamed	unnamed	WRLFMD	25/06/2010
<b>MYA/2/2010</b>	Myanmar	O	SEA	unnamed	unnamed	WRLFMD	25/06/2010
<b>MYA/3/2010</b>	Myanmar	O	SEA	Mya-98	unnamed	WRLFMD	25/06/2010
<b>MYA/1/2009</b>	Myanmar	O	SEA	Mya-98	unnamed	RRLSEA	24/01/2009
<b>MYA/2/2009</b>	Myanmar	O	SEA	Mya-98	unnamed	RRLSEA	28/01/2009
<b>NEP/3/2010</b>	Nepal	O	ME-SA	Ind-2001d	unnamed	WRLFMD	24/11/2010
<b>NEP/5/2010</b>	Nepal	O	ME-SA	Ind-2001d	unnamed	WRLFMD	24/11/2010
<b>NEP/6/2010</b>	Nepal	O	ME-SA	Ind-2001d	unnamed	WRLFMD	24/11/2010
<b>NEP/7/2010</b>	Nepal	O	ME-SA	Ind-2001d	unnamed	WRLFMD	24/11/2010
<b>NEP/9/2010</b>	Nepal	O	ME-SA	Ind-2001d	unnamed	WRLFMD	24/11/2010
<b>NEP/11/2010</b>	Nepal	O	ME-SA	Ind-2001d	unnamed	WRLFMD	24/11/2010
<b>NEP/12/2010</b>	Nepal	O	ME-SA	Ind-2001d	unnamed	WRLFMD	24/11/2010
<b>NEP/15/2010</b>	Nepal	O	ME-SA	Ind-2001d	unnamed	WRLFMD	24/11/2010
<b>NEP/16/2010</b>	Nepal	O	ME-SA	Ind-2001d	unnamed	WRLFMD	24/11/2010
<b>NEP/17/2010</b>	Nepal	O	ME-SA	Ind-2001d	unnamed	WRLFMD	24/11/2010
<b>NIG/3/2009</b>	Nigeria	O	EA-3	unnamed	unnamed	WRLFMD	16/03/2010
<b>NIG/15/2009</b>	Nigeria	O	EA-3	unnamed	unnamed	WRLFMD	16/03/2010
<b>PAK/1/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	09/04/2010
<b>PAK/2/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	09/04/2010
<b>PAK/3/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	09/04/2010
<b>PAK/4/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	09/04/2010
<b>PAK/5/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	09/04/2010
<b>PAK/6/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	09/04/2010
<b>PAK/7/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	09/04/2010
<b>PAK/8/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	09/04/2010
<b>PAK/9/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	09/04/2010
<b>PAK/14/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	09/04/2010
<b>PAK/15/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	09/04/2010
<b>PAK/16/2010</b>	Pakistan	O	ME-SA	PanAsia-2	FAR-09	WRLFMD	09/04/2010
<b>PAK/17/2010</b>	Pakistan	O	ME-SA	PanAsia-2	PUN-10	WRLFMD	09/04/2010
<b>PAK/18/2010</b>	Pakistan	O	ME-SA	PanAsia-2	PUN-10	WRLFMD	09/04/2010

<b>PAK/19/2010</b>	Pakistan	O	ME-SA	PanAsia-2	PUN-10	WRLFMD	09/04/2010
<b>PAK/20/2010</b>	Pakistan	O	ME-SA	PanAsia-2	PUN-10	WRLFMD	09/04/2010
<b>PAK/21/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	09/04/2010
<b>PAK/22/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	09/04/2010
<b>PAK/25/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>PAK/26/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>PAK/28/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>PAK/29/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>PAK/31/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>PAK/35/2010</b>	Pakistan	O	ME-SA	PanAsia-2	PUN-10	WRLFMD	31/08/2010
<b>PAK/36/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>PAK/37/2010</b>	Pakistan	O	ME-SA	PanAsia-2	PUN-10	WRLFMD	31/08/2010
<b>PAK/40/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>PAK/42/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	31/08/2010
<b>PAK/46/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>PAK/47/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>PAK/48/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>PAK/49/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>PAK/51/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>PAK/53/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>PAK/54/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>PAK/57/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>PAK/58/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>PAK/59/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>PAK/60/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>PAK/61/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>PAK/62/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>PAK/63/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>PAK/64/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>PAK/65/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>PAK/67/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>PAK/68/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>PAK/69/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>PAK/70/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>PAK/71/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>PAK/72/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010

<b>PAK/73/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>PAK/74/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>PAK/75/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>PAK/76/2010</b>	Pakistan	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	30/11/2010
<b>RUS/Jul-2010*</b>	Russia	O	SEA	Mya-98	unnamed	ARRIAH	19/07/2010
<b>RUS/Aug-2010*</b>	Russia	O	SEA	Mya-98	unnamed	ARRIAH	10/09/2010
<b>SKR/4/2010</b>	South Korea	O	SEA	Mya-98	unnamed	WRLFMD	17/05/2010
<b>Ganghwa/SKR/2010*</b>	South Korea	O	SEA	Mya-98	unnamed	NVRQS	12/04/2010
<b>Andong-1/SKR/2010*</b>	South Korea	O	SEA	Mya-98	unnamed	NVRQS	30/11/2010
<b>Yangju/SKR/2010*</b>	South Korea	O	SEA	Mya-98	unnamed	NVRQS	16/12/2010
<b>Yeoncheon/SKR/2010*</b>	South Korea	O	SEA	Mya-98	unnamed	NVRQS	16/12/2010
<b>Paju/SKR/2010*</b>	South Korea	O	SEA	Mya-98	unnamed	NVRQS	17/12/2010
<b>TAI/12/2009</b>	Thailand	O	SEA	Mya-98	unnamed	WRLFMD	05/02/2010
<b>TAI/13/2009</b>	Thailand	O	SEA	Mya-98	unnamed	WRLFMD	05/02/2010
<b>TAI/15/2009</b>	Thailand	O	SEA	Mya-98	unnamed	WRLFMD	05/02/2010
<b>TAI/16/2009</b>	Thailand	O	SEA	Mya-98	unnamed	WRLFMD	05/02/2010
<b>TAI/17/2009</b>	Thailand	O	SEA	Mya-98	unnamed	WRLFMD	05/02/2010
<b>TAI/18/2009</b>	Thailand	O	SEA	Mya-98	unnamed	WRLFMD	05/02/2010
<b>TAI/19/2009</b>	Thailand	O	SEA	Mya-98	unnamed	WRLFMD	05/02/2010
<b>TAI/20/2009</b>	Thailand	O	SEA	Mya-98	unnamed	WRLFMD	05/02/2010
<b>TAI/21/2009</b>	Thailand	O	SEA	Mya-98	unnamed	WRLFMD	05/02/2010
<b>TAI/22/2009</b>	Thailand	O	SEA	Mya-98	unnamed	WRLFMD	05/02/2010
<b>TAI/23/2009</b>	Thailand	O	SEA	Mya-98	unnamed	WRLFMD	05/02/2010
<b>TAI/24/2009</b>	Thailand	O	SEA	Mya-98	unnamed	WRLFMD	05/02/2010
<b>TAI/25/2009</b>	Thailand	O	SEA	Mya-98	unnamed	WRLFMD	05/02/2010
<b>TAI/26/2009</b>	Thailand	O	SEA	Mya-98	unnamed	WRLFMD	25/11/2010
<b>TAI/27/2009</b>	Thailand	O	SEA	Mya-98	unnamed	WRLFMD	25/11/2010
<b>TAI/1/2010</b>	Thailand	O	SEA	Mya-98	unnamed	WRLFMD	25/11/2010
<b>TAI/2/2010</b>	Thailand	O	SEA	Mya-98	unnamed	WRLFMD	25/11/2010
<b>TAI/3/2010</b>	Thailand	O	SEA	Mya-98	unnamed	WRLFMD	25/11/2010
<b>TAI/4/2010</b>	Thailand	O	SEA	Mya-98	unnamed	WRLFMD	25/11/2010
<b>TAI/5/2010</b>	Thailand	O	SEA	Mya-98	unnamed	WRLFMD	25/11/2010
<b>TAI/6/2010</b>	Thailand	O	SEA	Mya-98	unnamed	WRLFMD	25/11/2010
<b>TAI/4/2009</b>	Thailand	O	SEA	Mya-98	unnamed	RRLSEA	15/01/2009
<b>TAI/ 9/2009</b>	Thailand	O	SEA	Mya-98	unnamed	RRLSEA	12/01/2009
<b>TAI/11/2009</b>	Thailand	O	SEA	Mya-98	unnamed	RRLSEA	23/01/2009

<b>TAI/14/2009</b>	Thailand	O	SEA	Mya-98	unnamed	RRLSEA	20/01/2009
<b>TAI/15/2009</b>	Thailand	O	SEA	Mya-98	unnamed	RRLSEA	20/01/2009
<b>TAI/27/2009</b>	Thailand	O	SEA	Mya-98	unnamed	RRLSEA	24/02/2009
<b>TAI/35/2009</b>	Thailand	O	SEA	Mya-98	unnamed	RRLSEA	10/08/2009
<b>TAI/ 36/2009</b>	Thailand	O	SEA	Mya-98	unnamed	RRLSEA	25/08/2009
<b>TAI/ 38/2009</b>	Thailand	O	SEA	Mya-98	unnamed	RRLSEA	4/09/2009
<b>TAI/42/2009</b>	Thailand	O	SEA	Mya-98	unnamed	RRLSEA	6/10/2009
<b>TAI/43/2009</b>	Thailand	O	SEA	Mya-98	unnamed	RRLSEA	12/10/2009
<b>TAI/51/2009</b>	Thailand	O	SEA	Mya-98	unnamed	RRLSEA	5/11/2009
<b>TAI/53/2009</b>	Thailand	O	SEA	Mya-98	unnamed	RRLSEA	23/11/2009
<b>TAI/ 55/2009</b>	Thailand	O	SEA	Mya-98	unnamed	RRLSEA	23/11/2009
<b>TAI/64/2009</b>	Thailand	O	SEA	Mya-98	unnamed	RRLSEA	27/11/2009
<b>TAI/1-1/2010</b>	Thailand	O	SEA	Mya-98	unnamed	RRLSEA	12/02/2010
<b>TAI/1-2/2010</b>	Thailand	O	SEA	Mya-98	unnamed	RRLSEA	12/02/2010
<b>TAI/4/2010</b>	Thailand	O	SEA	Mya-98	unnamed	RRLSEA	12/02/2010
<b>TAI5/2010</b>	Thailand	O	SEA	Mya-98	unnamed	RRLSEA	12/02/2010
<b>TAI/9/2010</b>	Thailand	O	SEA	Mya-98	unnamed	RRLSEA	12/02/2010
<b>TAN/16/2008</b>	Tanzania	O	EA-2	unnamed	unnamed	WRLFMD	10/03/2010
<b>TAN/5/2009</b>	Tanzania	O	EA-2	unnamed	unnamed	WRLFMD	10/03/2010
<b>TAN/44/2009</b>	Tanzania	O	EA-2	unnamed	unnamed	WRLFMD	10/03/2010
<b>TUR/15/2009</b>	Turkey	O	ME-SA	PanAsia-2	TER-08	WRLFMD	04/02/2010
<b>TUR/17/2009</b>	Turkey	O	ME-SA	PanAsia-2	TER-08	WRLFMD	04/02/2010
<b>TUR/19/2009</b>	Turkey	O	ME-SA	PanAsia-2	TER-08	WRLFMD	04/02/2010
<b>TUR/1/2010</b>	Turkey	O	ME-SA	PanAsia-2	TER-08	WRLFMD	04/02/2010
<b>TUR/4/2010</b>	Turkey	O	ME-SA	PanAsia-2	SAN-09	WRLFMD	04/02/2010
<b>TUR/5/2010</b>	Turkey	O	ME-SA	PanAsia-2	SAN-09	WRLFMD	04/02/2010
<b>TUR/9/2010</b>	Turkey	O	ME-SA	PanAsia-2	SAN-09	WRLFMD	02/09/2010
<b>TUR/10/2010</b>	Turkey	O	ME-SA	PanAsia-2	FAR-09	WRLFMD	02/09/2010
<b>TUR/11/2010</b>	Turkey	O	ME-SA	PanAsia-2	FAR-09	WRLFMD	02/09/2010
<b>TUR/12/2010</b>	Turkey	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	02/09/2010
<b>TUR/18/2010</b>	Turkey	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	02/09/2010
<b>TUR/21/2010</b>	Turkey	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	02/09/2010
<b>TUR/22/2010</b>	Turkey	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	02/09/2010
<b>TUR/23/2010</b>	Turkey	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	02/09/2010
<b>TUR/26/2010</b>	Turkey	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	02/09/2010
<b>TUR/27/2010</b>	Turkey	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	02/09/2010

<b>TUR/29/2010</b>	Turkey	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	02/09/2010
<b>TUR/30/2010</b>	Turkey	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	02/09/2010
<b>TUR/31/2010</b>	Turkey	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	02/09/2010
<b>TUR/32/2010</b>	Turkey	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	02/09/2010
<b>TUR/33/2010</b>	Turkey	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	02/09/2010
<b>TUR/35/2010</b>	Turkey	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	02/09/2010
<b>TUR/36/2010</b>	Turkey	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	02/09/2010
<b>TUR/37/2010</b>	Turkey	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	02/09/2010
<b>TUR/38/2010</b>	Turkey	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	02/09/2010
<b>TUR/39/2010</b>	Turkey	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	02/09/2010
<b>TUR/4/2010*</b>	Turkey	O	ME-SA	PanAsia-2	TER-08	FMDI	19/02/2010
<b>TUR/32/2010*</b>	Turkey	O	ME-SA	PanAsia-2	TER-08	FMDI	19/02/2010
<b>TUR/34/2010*</b>	Turkey	O	ME-SA	PanAsia-2	SAN-09	FMDI	19/02/2010
<b>TUR/38/2010*</b>	Turkey	O	ME-SA	PanAsia-2	SAN-09	FMDI	19/02/2010
<b>TUR/46/2010*</b>	Turkey	O	ME-SA	PanAsia-2	SAN-09	FMDI	19/02/2010
<b>TUR/49/2010*</b>	Turkey	O	ME-SA	PanAsia-2	TER-08	FMDI	19/02/2010
<b>TUR/51/2010*</b>	Turkey	O	ME-SA	PanAsia-2	SAN-09	FMDI	19/02/2010
<b>TUR/54/2010*</b>	Turkey	O	ME-SA	PanAsia-2	SAN-09	FMDI	19/02/2010
<b>TUR/58/2010*</b>	Turkey	O	ME-SA	PanAsia-2	SAN-09	FMDI	19/02/2010
<b>TUR/59/2010*</b>	Turkey	O	ME-SA	PanAsia-2	TER-08	FMDI	19/02/2010
<b>TUR/60/2010*</b>	Turkey	O	ME-SA	PanAsia-2	SAN-09	FMDI	19/02/2010
<b>TUR/71/2010*</b>	Turkey	O	ME-SA	PanAsia-2	FAR-09	FMDI	26/02/2010
<b>TUR/72/2010*</b>	Turkey	O	ME-SA	PanAsia-2	FAR-09	FMDI	26/02/2010
<b>TUR/73/2010*</b>	Turkey	O	ME-SA	PanAsia-2	FAR-09	FMDI	26/02/2010
<b>TUR/131/2010*</b>	Turkey	O	ME-SA	PanAsia-2	SAN-09	FMDI	14/04/2010
<b>TUR/132/2010*</b>	Turkey	O	ME-SA	PanAsia-2	SAN-09	FMDI	14/04/2010
<b>TUR/149/2010*</b>	Turkey	O	ME-SA	PanAsia-2	SAN-09	FMDI	14/04/2010
<b>TUR/153/2010*</b>	Turkey	O	ME-SA	PanAsia-2	ANT-10	FMDI	14/04/2010
<b>TUR/154/2010*</b>	Turkey	O	ME-SA	PanAsia-2	ANT-10	FMDI	14/04/2010
<b>TUR/155/2010*</b>	Turkey	O	ME-SA	PanAsia-2	SAN-09	FMDI	14/04/2010
<b>TUR/156/2010*</b>	Turkey	O	ME-SA	PanAsia-2	SAN-09	FMDI	14/04/2010
<b>TUR/163/2010*</b>	Turkey	O	ME-SA	PanAsia-2	TER-08	FMDI	14/04/2010
<b>UAE/1/2010</b>	United Arab Emirates	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	28/04/2010
<b>UAE/2/2010</b>	United Arab Emirates	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	28/04/2010
<b>UAE/3/2010</b>	United Arab Emirates	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	28/04/2010
<b>UAE/4/2010</b>	United Arab Emirates	O	ME-SA	PanAsia-2	ANT-10	WRLFMD	28/04/2010

VIT/1/2008	Vietnam	O	CATHAY	unnamed	unnamed	WRLFMD	05/02/2010
VIT/2/2008*	Vietnam	O	CATHAY	unnamed	unnamed	TRRL	07/03/2010
VIT/3/2008*	Vietnam	O	CATHAY	unnamed	unnamed	TRRL	07/03/2010
VIT/2/2010	Vietnam	O	SEA	Mya-98	unnamed	WRLFMD	05/11/2010
VIT/3/2010	Vietnam	O	ME-SA	PanAsia	unnamed	WRLFMD	05/11/2010
VIT/4/2010	Vietnam	O	ME-SA	PanAsia	unnamed	WRLFMD	05/11/2010
VIT/5/2010	Vietnam	O	SEA	Mya-98	unnamed	WRLFMD	05/11/2010
VIT/6/2010	Vietnam	O	ME-SA	PanAsia	unnamed	WRLFMD	05/11/2010
VIT/7/2010	Vietnam	O	ME-SA	PanAsia	unnamed	WRLFMD	05/11/2010
VIT/8/2010	Vietnam	O	ME-SA	PanAsia	unnamed	WRLFMD	05/11/2010
VIT/9/2010	Vietnam	O	ME-SA	PanAsia	unnamed	WRLFMD	05/11/2010
VIT/10/2010	Vietnam	O	ME-SA	PanAsia	unnamed	WRLFMD	05/11/2010
VIT/11/2010	Vietnam	O	ME-SA	PanAsia	unnamed	WRLFMD	05/11/2010
VIT/12/2010	Vietnam	O	ME-SA	PanAsia	unnamed	WRLFMD	05/11/2010
VIT/13/2010	Vietnam	O	ME-SA	PanAsia	unnamed	WRLFMD	05/11/2010
ZAM/1/2010	Zambia	O	EA-2	unnamed	unnamed	WRLFMD	09/11/2010
ZAM/3/2010	Zambia	O	EA-2	unnamed	unnamed	WRLFMD	09/11/2010
ZAM/4/2010	Zambia	O	EA-2	unnamed	unnamed	WRLFMD	09/11/2010

### Serotype SAT-1

KEN/121/2009	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	06/04/2010
KEN/123/2009	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	06/04/2010
KEN/1/2010	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	06/04/2010
KEN/3/2010	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	06/04/2010
KEN/4/2010	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	06/04/2010
KEN/5/2010	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	06/04/2010
KEN/6/2010	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	06/04/2010
KEN/8/2010	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	06/04/2010
KEN/10/2010	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	06/04/2010
KEN/12/2010	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	06/04/2010
KEN/13/2010	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	06/04/2010
KEN/14/2010	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	06/04/2010
KEN/71/2010	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	06/04/2010
KEN/72/2010	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	06/04/2010
KEN/75/2010	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	06/04/2010
KEN/76/2010	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	06/04/2010

<b>KEN/77/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	06/04/2010
<b>KEN/78/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	06/04/2010
<b>KEN/80/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	06/04/2010
<b>KEN/88/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/90/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/93/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/94/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/96/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/97/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/98/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/101/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/102/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/103/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/104/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/105/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/106/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/107/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/109/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/110/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/111/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/112/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/113/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/114/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/115/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/116/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/118/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/119/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/120/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/121/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/122/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/123/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/124/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/125/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/126/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/128/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/130/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010

<b>KEN/131/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/132/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>KEN/133/2010</b>	Kenya	SAT-1	I (NWZ)	unnamed	unnamed	WRLFMD	10/06/2010
<b>NAM/1/10*</b>	Namibia	SAT 1	III (WZ)	unnamed	FAR-09	BVI	15/06/2010
<b>NAM/2/10*</b>	Namibia	SAT 1	III (WZ)	unnamed	unnamed	BVI	09/07/2010
<b>NAM/1/10/1</b>	Namibia	SAT 1	III (WZ)			ARC-OVI	04/10
<b>SAR/2/10-</b>	South Africa PZ	SAT 1				ARC-OVI	08/10
<b>SAR/21/10/1</b>	South Africa PZ	SAT 1				ARC-OVI	08/10

### Serotype SAT-2

<b>BOT/1/2010</b>	Botswana	SAT-2	III	unnamed	unnamed	WRLFMD	09/11/2010
<b>BOT/2/2010</b>	Botswana	SAT-2	III	unnamed	unnamed	WRLFMD	09/11/2010
<b>BOT/3/2010</b>	Botswana	SAT-2	III	unnamed	unnamed	WRLFMD	09/11/2010
<b>BOT/4/2010</b>	Botswana	SAT-2	III	unnamed	unnamed	WRLFMD	09/11/2010
<b>BOT/5/2010</b>	Botswana	SAT-2	III	unnamed	unnamed	WRLFMD	09/11/2010
<b>ETH/64/2009</b>	Ethiopia	SAT-2	XIII	unnamed	unnamed	WRLFMD	20/02/2010
<b>ETH/65/2009</b>	Ethiopia	SAT-2	XIII	unnamed	unnamed	WRLFMD	20/02/2010
<b>ETH/67/2009</b>	Ethiopia	SAT-2	XIII	unnamed	unnamed	WRLFMD	20/02/2010
<b>ETH/68/2009</b>	Ethiopia	SAT-2	XIII	unnamed	unnamed	WRLFMD	20/02/2010
<b>ETH/69/2009</b>	Ethiopia	SAT-2	XIII	unnamed	unnamed	WRLFMD	20/02/2010
<b>ETH/70/2009</b>	Ethiopia	SAT-2	XIII	unnamed	unnamed	WRLFMD	20/02/2010
<b>ETH/72/2009</b>	Ethiopia	SAT-2	XIII	unnamed	unnamed	WRLFMD	20/02/2010
<b>ETH/73/2009</b>	Ethiopia	SAT-2	XIII	unnamed	unnamed	WRLFMD	20/02/2010
<b>ETH/74/2009</b>	Ethiopia	SAT-2	XIII	unnamed	unnamed	WRLFMD	20/02/2010
<b>ETH/75/2009</b>	Ethiopia	SAT-2	XIII	unnamed	unnamed	WRLFMD	20/02/2010
<b>ETH/76/2009</b>	Ethiopia	SAT-2	XIII	unnamed	unnamed	WRLFMD	20/02/2010
<b>ETH/77/2009</b>	Ethiopia	SAT-2	XIII	unnamed	unnamed	WRLFMD	20/02/2010
<b>ETH/2/2010</b>	Ethiopia	SAT-2	XIII	unnamed	unnamed	WRLFMD	20/02/2010
<b>KEN/122/2009</b>	Kenya	SAT-2	IV	unnamed	unnamed	WRLFMD	06/04/2010
<b>MOZ/1/2010</b>	Mozambique	SAT-2	I	unnamed	unnamed	WRLFMD	09/11/2010
<b>MOZ/2/2010</b>	Mozambique	SAT-2	I	unnamed	unnamed	WRLFMD	09/11/2010
<b>MOZ/3/2010</b>	Mozambique	SAT-2	I	unnamed	unnamed	WRLFMD	09/11/2010
<b>MOZ/4/2010</b>	Mozambique	SAT-2	I	unnamed	unnamed	WRLFMD	09/11/2010
<b>MOZ/5/2010</b>	Mozambique	SAT-2	I	unnamed	unnamed	WRLFMD	09/11/2010
<b>MOZ/6/2010</b>	Mozambique	SAT-2	I	unnamed	unnamed	WRLFMD	09/11/2010
<b>MOZ/7/2010</b>	Mozambique	SAT-2	I	unnamed	unnamed	WRLFMD	09/11/2010

<b>SAR/1/10/2</b>	South Africa PZ	SAT-2	I			ARC-OVI	20/05/10
<b>SEN/27/2009</b>	Senegal	SAT-2	VII	unnamed	unnamed	WRLFMD	18/01/2010
<b>TAN/43/2009</b>	Tanzania	SAT-2	IV	unnamed	unnamed	WRLFMD	10/03/2010
<b>ZIM/2/2010</b>	Zimbabwe	SAT-2	II	unnamed	unnamed	WRLFMD	09/11/2010
<b>ZIM/4/2010</b>	Zimbabwe	SAT-2	II	unnamed	unnamed	WRLFMD	09/11/2010

\* virus name is not a WRLFMD ref. no.

### 1.7.2. Summary of antigenic typing

Vaccine efficacy is influenced by both vaccine potency and vaccine match and it is possible that a poor match may to some extent be compensated by high potency vaccines and by administering more than one dose at suitable intervals. The use of oil adjuvant is also expected to improve efficacy. Thus, a vaccine with a weak antigenic match to a field isolate, as determined by serology, may nevertheless afford some protection if it is of sufficiently high potency. Therefore, in the absence of a good match, or where the match is unknown, vaccines of high potency should preferably be used. The  $r_1$  values shown below, represent the one way serological match between vaccine strain and field isolate, calculated from the comparative reactivity of an antiserum, raised against the vaccine in question, to the vaccine virus and the field isolate.

### 1.7.3. Antigenic characterisation of field isolates by matching with vaccine strains at FGI-ARRIAH

Antigenic characterisation of FMD field isolates by matching with vaccine strains  $r_1$  values were obtained by VNT.

#### FMDV Serotype O

Table 5. Antigenic characterisation of field isolates by matching with vaccine strains

FMDV ID	$r_1$ value in VNT	
	Vaccine strain O <sub>1</sub> Manisa	Vaccine strain O/Russia/2000
O/Mongolia/2010	0,4	0,63
O/Kazakhstan/2010	Pending	Pending
O/Russia/JUL2010	0,31	0,36
O/Russia/AUG2010	0,33	0,62
O/Lebanon/2010	0,25	0,43

#### Interpretation of $r_1$ values

##### In the case of VNT:

$r_1 = \geq 0.3$  suggests that there is a close relationship between field isolate and vaccine strain. A potent vaccine containing the vaccine strain is likely to confer protection.

$r_1 = < 0.3$  suggests that the field isolate is so different from the vaccine strain that the vaccine is unlikely to protect.

#### 1.7.4. Antigenic characterisation of field isolates from Ecuador by matching with vaccine strain O1 Campos- $r_1$ values obtained by VNT at SENASA Laboratory.

Test	Results
Virus Neutralization	<p>“<math>r_1</math>” Value: Sample 46-2010 (Napo): <b>0.08</b></p> <p>Sample 23-2010 (Tsáchila): <b>0.09</b></p>

The EPP was estimated using serum from revaccinated animals kept at the SENASA serum bank which were tested against a commercial trivalent vaccine (A,O,C) and the field strain N° 46 of Ecuador. The results are presented in the following table (Table 6.) .

**Table 6.** EPP estimation

	O1 Campos			O1Ecuador N°46-2010					
	30 DPRV Serum	VN titer1 100DI50%	% EEP VN1	VN Titer1 100 DI50%	% EPP VN1	VN titer2 100 DI 50%	% EPP VN2	Average VN1+VN2	% EPP VN1+VN2.
Trivalent (O,A,C) Commercial Vaccine for Export.	912	2,00	90,59	1,51	66,39	1,37	55,44	1,44	60,27
	913	2,42	97,47	1,46	61,84	1,26	45,58	1,36	53,81
	915	2,33	96,73	1,70	78,15	1,58	70,65	1,64	74,58
	917	2,40	97,3	1,39	57,07	1,45	61,84	1,42	58,68
	918	2,30	96,28	1,94	88,76	1,79	83,26	1,87	86,63
	919	2,55	98,39	1,70	78,15	1,50	64,90	1,60	72,00
	921	2,33	96,73	1,26	45,58	1,41	58,68	1,34	52,17
	922	2,64	98,76	2,09	93,05	1,43	60,27	1,76	81,34
	923	2,33	96,73	1,26	45,58	1,26	45,58	1,26	45,58
	925	2,45	97,77	1,51	66,39	1,26	45,58	1,39	57,07
	926	2,31	96,51	1,36	53,81	1,29	48,87	1,32	50,52
	931	2,00	90,59	1,26	45,58	1,26	45,58	1,26	45,58
	932	2,55	98,39	1,36	53,81	1,29	48,87	1,32	50,52
	935	1,81	84,16	1,26	45,58	1,26	45,58	1,26	45,58
	936	2,34	96,73	1,26	45,58	1,31	50,52	1,29	48,87
940	1,74	80,32	1,26	45,58	1,46	61,84	1,36	53,81	

	942	2,14	93,86	1,65	75,81	1,41	58,68	1,53	67,84
Mean		2,27	94,55	1,49	61,57	1,39	55,98	1,44	59,11
DS %		0,25	5,25	0,25	16,21	0,14	10,64	0,18	12,86

### Cross Protection Test

It was performed with a strain isolated from the outbreaks of 2010 collected in June at the province of Napo.

Two groups of 16 cattle, one vaccinated and the other revaccinated at 30 days with an experimental oil vaccine formulated with O1 Campos virus antigen with a mass of 20 ug per dose were used.

Two animals were used as controls.

The dose per animal was 2 ml.

The experimental vaccine had been assessed by the Protection against Podal Generalisation Test showing a 75% protection to challenge with the homologous strain.

The animals were challenged using 10,000 suckling mouse infective dose (DIRL) per ml.

Dates:

- Date of vaccination for single vaccination group: October 5
- Date of vaccination for revaccinated group: September 8 and October 5
- Dates of virus challenge for three groups: November 3
- Date of observation of lesions: November 10

Results:

- Group single vaccination:
  - One animal protected / fifteen unprotected animals (6% protection).
- Group Revaccinated:
  - Three animals protected /thirteen unprotected animals (18% protection).

### 1.7.5. Antigenic characterization of field isolates from Ecuador by matching with vaccine strain O1 Campos- r<sub>1</sub> values obtained at PANAFTOSA Laboratory.

**Table 7.** Antigenic characterization of field isolates from Ecuador

FMDV ID	r <sub>1</sub> value by FC50%	EPP/ELISA-CFL	EPP/VN	r <sub>1</sub> value by LPBE	r <sub>1</sub> value by VN	Comments
	Vaccine strain O1 Campos	Vaccine strain O1 Campos	Vaccine strain O1 Campos	Vaccine strain O1 Campos	Vaccine strain O1 Campos	
224-02	0.49					Epidemiological data from the country correlates with vaccine matching results obtained using r <sub>1</sub> FC50% and EPP. Field observations do not support the r <sub>1</sub> values obtained by LPBE and VN, (which suggest the field virus is different from the
224-08	0.49					
224-21	0.71					
224-36	0.45	91.4	99.68	Average: 0.106	Average: 0.26	
224-45	0.45			Note: r <sub>1</sub> values ranged from 0.053 to 0.26 depending on sera used to perform	Note: r <sub>1</sub> values ranged from 1.0 to 0.09 depending on sera used to perform	
224-47	0.37					
224-56	0.37					
224-61	0.61					

				the test	the test	vaccine strain). In our experience vaccine matching tests using FC50% and EPP are a more reliable methodology. A revision and harmonization of vaccine matching methodologies recommended by OIE is needed, considering laboratory data and epidemiological observations.
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***Expected Percentage of Protection of Vaccine strain against a field O1 strain Ecuador 2010***

	EPP ELISA –CFL *		EPP VN***
Field Strain	30 Dpv	30dprv **	30 dprv
O1 Ecuador 2010	47,85	91,4	99,98

\* calculated using 30 bovine serum

\*\* Values  $\geq 70\%$  in revaccinated animals indicate that vaccine strain is protective

\*\*\* calculated using 10 bovine serum

PANAFTOSA also reported that it has observed differences between the findings of different results for “r1” when performing VNT or ELISA depending on the sera used in the study while the assessment by EPP plus “r1” by FC50% gives more reliable information in areas under systematic vaccination campaigns. The preference for using EPP combined with “r1” by complement fixation is a recommendation of PANAFTOSA based on the correlation between their epidemiological observations over the years and laboratory data in South American countries.

**1.7.6. Antigenic characterisation of field isolates by matching with vaccine strains by WRLFMD:  $r_1$  values were obtained by VNT or ELISA at WRLFMD**

**Vaccine matching data for 2010 from the WRLFMD Pirbright Laboratory, UK.**

**January-March 2010**

Twelve FMDV type O isolates (See table 8 Type O) from Bangladesh, Bhutan, Turkey and Thailand collected in 2008, 2009 and 2010 were analysed antigenically by two dimensional virus neutralisation test (2dmVNT) and/or LPBE. All isolates except O Tai 7/2008 were matched with O IND R2/75. Two isolates from Bangladesh and Bhutan, respectively, one virus from Thailand but none from Turkey were matched with O Manisa by VNT. Two isolates from both Bangladesh and Bhutan, three out of four from Thailand and one out of four isolates from Turkey showed antigenic match with O BFS by VNT. Two, 2 and 3 isolates from Turkey, Thailand and Thailand were matched with O Tunisia 89, O HKN 6/83 and O TAW 189/87 by LPBE, respectively. All isolates from Thailand were antigenically close with O 4171 by LPBE; and all isolates from Bangladesh and Bhutan were closely related to O TAI 189/87 vaccine strains by LPBE..

Two FMDV type A viruses (see table 8) from Iran collected in 2009 were analysed for antigenic relationships with various vaccine strains by 2dmVNT and/or LPBE. Both isolates showed antigenic matches with A TUR 06 but only one with A<sub>22</sub> Irq and A SAU 41/91 vaccine strains. No virus matched with A IND 17/82, A Eri 98 or A Iran 87. However, both viruses were antigenically close with A May 97 by LPBE.

One Asia1 isolate from Pakistan collected in 2009 was received and analysed for vaccine matching by both VNT and LPBE. Surprisingly, it did not match with Asia1 Shamir but was antigenically close with Asia1 WBN 117/85 by VNT.

Two FMDV SAT 1 viruses from Kenya collected in 2009, were analysed for antigenic relationship with two vaccine strains by 2dmVNT and/or LPBE. They were both antigenically matched with SAT 1 Rho vaccine by both VNT and LPBE.

Two FMDV SAT 2 viruses from Ethiopia collected in 2009 were analysed for antigenic relationship with various vaccine strains by 2dmVNT and/or LPBE. Both isolates showed a good match with SAT 2 Eritrea (by both VNT and LPBE) and Sat 251 vaccines (by VNT). No virus was antigenically close with SAT 2 BOT 3/77 by LPBE.

**TABLE 8:** Antigenic characterisation of FMD field isolates by matching with vaccine strains by VNT and/or LPBE – r1 value data from 1<sup>st</sup> January to 31<sup>st</sup> March 2010

**Type O:**

Field isolates	r1 values by 2dmVNT and LPBE									
	O Manisa		O Ind R2/75	O Bfs		O Tai 189/87	O 4174	O Tunisia 89	O Hkn 6/83	O Taw 189/87
	VNT	LPBE	VNT	VNT	LPBE	LPBE	LPBE	LPBE	LPBE	LPBE
Ban 04/2009	0.32	≥1.50	>0.95	0.68	0.67	≥1				
Ban 30/2009	0.39	>1	>1.0	0.46	0.59	>1	0.19			
Bhu 06/2009	0.44	>1	>0.98	0.44	0.50	>1	0.11			
Bhu 40/2009	0.40	>1	>0.90	0.40	0.42	>1	0.29			
Tur 15/2009	0.19	0.5	0.50	0.20	DNT		DNT	DNT		
Tur 17/2009	0.21	0.17	0.54	0.25	DNT		DNT	DNT		
Tur 01/2010	0.26	0.59	0.72	0.49	0.38		DNT	>1		
Tur 05/2010	0.23	0.50	0.76	0.10	0.38		DNT	>1		
O Tai 7/2008	0.06	>1	0.13	0.09			0.25			
O Tai 1/2009	0.24	>1	0.67	0.34	0.25		0.59			0.42
O Tai 2/2009	0.28	>1	0.79	0.43	0.22		0.53		1.00	0.84
O Tai 4/2009	0.45	>1	>0.83	0.65	0.17		0.59		≥0.75	0.84

**Type A:**

Field isolates	r1 values by 2dmVNT and LPBE										
	A22 Irq		A Tur06	A Ind 17/82	SAU 41/91	Eri98	Im 87	May-97		IRN99	
	VNT	LPBE	VNT	VNT	VNT	LPBE	VNT	LPBE	VNT	LPBE	LPBE
Im 50/2009	0.50	0.63	1.00	0.12	0.44	0.11	0.08	0.25	0.09	0.22	
Im 73/2009	0.11	0.08	0.41	0.10	0.03	0.5	0.06	0.38	0.26	0.5	0.25

**Type Asia1:**

Field Isolates	VNT			LPBE		
	Asia1 Sham	Asia1 Ind	WBN 117/85	Asia1 Shamir	Asia1 117/85	Wbn
Asia1 29/2009 Pak	0.14	0.13	0.43	0.22	0.00	

**Type SAT 1:**

Field Isolate	r1 value against vaccine Sat1 Rho	
	VNT	LPBE
Sat1 Ken 55/2009	0.51	0.75
Sat1 Ken 119/2009	0.95	0.25

**Type SAT 2:**

Field isolates	r1 values by 2dmVNT and LPBE				
	Sat2 Eri 3218		SAT251		Sat2 Bot 3/77
	VNT	LPBE	VNT	LPBE	LPBE
Eth 51/2009	0.84	0.5	0.39	DNT	0.16
Eth 56/2009	0.59	0.5	0.33	0.19	0.08

**April-June 2010  
Vaccine matching**

Five FMDV type O isolates (See Table 9 Type O) from Hong Kong SAR of China, Iran and South Korea (SKR) collected in 2009 and 2010 were analysed antigenically by two dimensional virus neutralisation test (2dmVNT) and/or LPBE. All isolates were matched with O Manisa, O IND R2/75 and O BFS by 2dmVNT. The isolate from SKR was also matched with O TAW 98 and O TAI 189/87 by VNT and LPBE, respectively. One isolate from Iran was antigenically close to O 4174 by LPBE .

Three FMDV type A viruses (see table 9 Type A) from Iran and SKR collected in 2009 and 2010 were analysed for antigenic relationships with various vaccine strains by 2dmVNT and/or LPBE. Both isolates from Iran showed antigenic matches with A TUR 06, A IND 17/82, A Eri98 and A IRN 87 but not with A<sub>22</sub> Irq, A SAU 41/91, A IRN 96 and A IRN 99. The isolate from SKR showed matches with A Eri98, A May 97 and A IRN 99 with no antigenic matches to A<sub>22</sub> IRQ, A TUR06, A IND 17/82, A IRN87 and A IRN 96 .

**TABLE 9:** Antigenic characterisation of FMD field isolates by matching with vaccine strains by VNT and/or LPBE – r1 value data from 1<sup>st</sup> April to 30<sup>th</sup> June 2010

**Type O:**

r1 values by 2dmVNT and LPBE for serotype O virus									
Vaccine strains	O Manisa		O Ind R2/75	O BFS		O Taw98	O Tai 189/87	O 4174	O Hkn 6/83
Field isolates	LPBE	VNT	VNT	LPBE	VNT	VNT	LPBE	LPBE	LPBE
HKN 01/2010		0.50	>1.0		0.51	0.72			
IRN 80/2009	0.50	0.39	>1.0		0.47				
IRN 01/2010	0.75	0.31	>1.0	0.25	0.36			0.13	
IRN 30/2010	0.59	0.41	>0.95	0.36	0.78			0.62	
SKR 4/2010	0.42	0.57	0.71	0.06	0.36	0.48	1.00		0.15

**Type A:**

r1 values by 2dmVNT and LPBE for serotype A virus												
Vaccine strains	A22 Irq		A Tur06	A Ind 17/82	SAU 41/91	Eri98	Im 87	IRN96	May-97		IRN99	
Field isolates	LPBE	VNT	VNT	VNT	VNT	LPBE	LPBE	VNT	VNT	LPBE	VNT	LPBE
A IRN 78/2009	0.05	0.23	0.43	0.45	0.11	0.38	0.26	0.32	0.28			0.21
A IRN 09/2010		0.13	0.34	0.44	0.13	0.36	0.29	0.21	0.16			0.05
A SKR 2/2010	0.1	0.13	0.28	0.17		0.29		0.16	0.10	0.42	0.44	0.25

## July-September 2010

### Vaccine matching

Fourteen FMDV type O isolates (See Table 10, Type O) from Ecuador, Iran, Pakistan and United Arab Emirates (UAE) collected in 2009 and 2010 were analysed antigenically by the two dimensional virus neutralisation test (2dmVNT) and/or LPBE. All isolates from Iran, Pakistan and UAE were matched with O Manisa, O IND R2/75, O TAW 98 and O BFS by 2dmVNT or LPBE except one isolate, respectively. Four isolates from Pakistan and UAE also showed close match with O Campos, O IND 53/79 and O TAI 189/87 by VNT or LPBE. Two isolates from Ecuador showed no match with any of the testing vaccine strains. Five isolates from IRAN were antigenically close to O 4174 by LPBE.

Nine FMDV type A viruses from Iran, Nigeria, Pakistan and Tanzania collected in 2009 and 2010 were analysed for antigenic relationships with various vaccine strains by 2dmVNT and/or LPBE. All four isolates from Iran showed a close match with A TUR 06 but not with all other tested vaccine strains except one which matched with A IND 17/82. The isolate from Nigeria was antigenically close to A Eri 98, A TUR 06 and A IND 17/82 but not to all other vaccine strains. Two isolates from Pakistan were close match with A22 Irq and A SAU 41/91 strains. Both isolates from Tanzania showed a close match with Eri 98 but only one matched with A22 Irq, A SAU 41/91 and A TUR 06 (Table 10).

Two FMDV type SAT2 virus (see table V3, Type SAT2 for details) from Kenya and Tanzania collected in 2009 were antigenically matched with SAT2 3218 strain, but only one isolate was a close match with SAT2 ZIM vaccine strain (Table 10).

**TABLE 10:** Antigenic characterisation of FMD field isolates by matching with vaccine strains by VNT and/or LPBE – r1 value data from 1<sup>st</sup> July to 30<sup>th</sup> September 2010

#### Type O:

r1 values by 2dmVNT and LPBE for type O virus –WRL, Pirbright													
Vaccine strains	O 4174	O 4625	O BFS	O Campos	O Hkn 6/83	O Ind 53/79	O Ind R2/75	O Isr 2/88	O Manisa	O Tai 189/87	O Taw98		
WRL ref for virus	LPBE	VNT	LPBE	VNT	VNT	LPBE	LPBE	VNT	LPBE	LPBE	VNT	LPBE	VNT
Ecu 01/2010		0.16		0.10	0.23			0.12		0.13	0.05		0.02
Ecu 09/2010				0.06	0.17			0.12		0.08	0.06		0.04
Iran 05/2010			0.21	0.52				0.61		0.67	0.27		0.48
Iran 08/2010	0.54		0.38	0.87				>1.0		0.44	0.72		>0.85
Iran 17/2010	≥0.75	0.55	0.29	0.40				0.85		0.40	0.19		0.31
Iran 27/2010	0.28		0.25	0.36				>0.78		0.38	0.26		0.38
Iran 33/2010	1		0.32	0.48				>0.94		0.38	0.25		0.46
Iran 44/2010	0.44		0.63	0.39				0.91		0.75	0.29		0.31
Iran 49/2010			0.32	0.29				>1.0		0.15	0.25		0.67
Iran 89/2009			0.20	0.45				>0.76		≥0.75	0.27		0.73
Pak 01/2010			0.30	0.34	0.74		0.50	>0.95		0.38	0.46	0.59	0.60
Pak 20/2010		0.55	0.10	0.11	0.31	0.21	0.42	0.61	0.06	0.38	0.19	1.00	0.21
UAE 02/2010			0.36	0.31	0.72	0.50	1.00	0.90		0.50	0.39	≥1.00	0.38
UAE 04/2010			0.22	0.23	0.72	0.50	0.84	>1.0		0.50	0.32	1.00	0.38

## Type A:

r1 values by 2dmVNT and LPBE for type A virus --WRL, Pirbright										
Vaccine strains	A Eri98		A Ind 17/82	A Irn 87		A Irn96	A22 Irq		A SAU 41/91	A Tur06
WRL ref for virus	LPBE	VNT	VNT	LPBE	VNT	VNT	LPBE	VNT	VNT	VNT
Irn 36/2010		0.17	0.55					0.12	0.13	0.37
Nig 38/2009		0.68	0.38					0.16	0.09	0.19
Pak 12/2010		0.19	0.09					0.53	0.31	0.85
Pak 24/2010		0.23	0.08					0.34	0.47	0.69
Tan 11/2009		0.37	0.20					0.14	0.19	0.10
Tan 42/2009		0.52	0.06					0.33	0.38	0.32
Irn 73/2010		0.20	0.21	0.08	0.11	0.17*	0.19	0.24	0.12	>0.68
Irn 80/2010	0.22	0.22	0.22	0.15	0.16	0.15*	0.16	0.23	0.09	0.44
Irn 125/2010	0.06	0.08	0.09	0.05	0.05	0.05*	0.25	0.24	0.27	>0.68

## Type SAT2:

r1 values by 2dmVNT for type SAT2 virus --WRL, Pirbright		
Vaccine strains	SAT2 3218	SAT2 ZIM
WRL ref for virus	VNT	VNT
Ken 122/2009	0.41	0.42
Tan 43/2009	0.38	0.19

## October-December 2010

### Vaccine matching

Twenty five FMDV type O isolates (See Table 11 Type O) from Ethiopia, Iran, Kenya, Mongolia, Myanmar, Nigeria, Pakistan, Thailand, Turkey, Tanzania and Zambia collected in 2009 and 2010 were analysed antigenically by the two dimensional virus neutralisation test (2dmVNT) and/or LPBE. Two isolates from Ethiopia showed antigenic matching with O K77/73, O BFS, and O Manisa by VNT and /or LPBE. Out of 7 isolates from Iran, all were antigenically matched with O IND R2/75 and O TAW 98 vaccines; 5 were antigenically close to O 4625, 2/7 and 3/7 were matched with O BFS and O Manisa respectively. Two viruses collected from Kenya were antigenically similar to O IND R2/75 but not to O Manisa, O BFS and O TAW 98. 3 viruses isolated from Mongolia showed an antigenic match with O 4625 vaccine but not with O Manisa or O BFS. Two field isolates from Myanmar showed a match with both O Manisa and O IND R2/75. Two isolates from Pakistan were closely matched with all of O 4625, O BFS, O Manisa, O IND R2/75, O TAW 98 and O TNN 24/84 vaccine strains. Two isolates from Turkey were a close match with all of O 4625, O BFS, O IND R2/75, O PanAsia 2 and O TNN 24/84 vaccine strains with only one antigenically matched with O Manisa. The single isolates from Nigeria, Tanzania and Zambia all showed antigenic similarity to O Manisa strain (Table 11).

Four FMDV type SAT 1 viruses from Kenya collected in 2010 were analysed for antigenic relationships with two vaccine strains by 2dmVNT and/or LPBE. Only one virus showed an antigenic match with SAT 1 RHO and SAT 1 BOT 1/68 by VNT and LPBE, respectively (Table 11).

Five FMDV type SAT2 viruses from Ethiopia, Kenya and Tanzania collected in 2009 and 2010 were antigenically matched with different vaccine strains. All viruses showed antigenic matching with SAT 2 Eritrea vaccines. All three virus from Ethiopia were also closely matched with SAT 2 K65/82 by LPBE. One virus each from Kenya and Tanzania were antigenically close to K 65/82 vaccine strain by LPBE. The virus from Kenya was also antigenically matched with SAT 2 Zim vaccine strain by VNT (Table 11).

**TABLE 11:** Antigenic characterisation of FMD field isolates by matching with vaccine strains by VNT and/or LPBE from 1<sup>st</sup> October to 31<sup>st</sup> December 2010

**Type O:**

Vaccine matching for type O FMDV by VNT and LPBE - WRL FMD																	
Isolates	O 3039		O K77/78		O 4625		O BFS		O IND R2/75		O Manisa		O PanAsia 2	O TAI 189/87	O TAW 98	O TNN 24/84	O 4174
	LPBE	VNT	LPBE	LPBE	VNT	LPBE	VNT	VNT	LPBE	VNT	VNT	LPBE	VNT	VNT	LPBE		
Eth 07/2010			M			M	N	M	M	M				M			
Eth 09/2010			M			M	N	N	M	N				N			
lrrn 88/2010				M		M	N	M	N	N				M			
lrrn 92/2010							N	M		N				M			
lrrn 99/2010				M			N	M	M	N				M			
lrrn 143/2010							N	M	M	M				M			
lrrn 149/2010					M		N	M		N				M			
lrrn 174/2010		M		M	M	N	M	M	M	M				M			M
lrrn 187/2010		M		M	M		M	M	N	M				M			
Ken 100/2010							N	M		N				N			
Ken 125/2009							N	M		N				N			
Mog 3/2010					M	N	N	M	N	N			M				
Mog 4/2010					M	N	N	N	N	N			M	N			
Mog 9/2010					M	N	N	M	N	N			N	N			
Mya 13/2009	M						N	M	M	N							
Mya 3/2010							N	M		M							
Nig 15/2009			M			M	N	M	M	M				N			
Pak 25/2010					M	M	M	M	N	M				M		M	
Pak 42/2010					M	M	M	M	N	M				M		M	
Tai 2/2009						M											
Tai 4/2009						N											
Tan 05/2009			M			M	N	M	M	M				M			
Tur 18/2010					M	M	M	M	N	N	M					M	
Tur 39/2010					M	M	M	M	N	M	M					M	
Zam 04/2010		M			M		M			M				M			

**Type SAT 1:**

Vaccine matching for type SAT 1 FMDV by VNT and LPBE - WRL FMD			
Isolates	Sat 1 Rho		Sat 1 Bot 1/68
	VNT	LPBE	LPBE
Ken 01/2010	N	N	M
Ken 77/2010	N	N	N
Sat1 Ken 101/2010	N	N	
Sat1 Ken 133/2010	M		

**Type SAT2:**

Vaccine matching for type SAT 2 FMDV by VNT and LPBE - WRL FMD									
Isolates	Eri 3218		SAT 2 Zim		K65/82	Sat2 Bot 3/77	Sat2 Zim 11/89	Sat2 K65/82	Sat2 Zam 3/81
	VNT	LPBE	VNT	LPBE				LPBE	
Eth 74/2009	M	M	N	N			N		M
Eth 75/2009	M	N	N	N			M	N	N
Eth 02/2010	M	M	N				N		M
Ken 122/2009	M		M		M				
Tan 43/2009	M		N		M				

M: the isolate was antigenically matched with the vaccine strain  
 N: the isolate showed no antigenic match with the vaccine strain

## Interpretation of $r_1$ values

### In the case of VNT:

$r_1 = \geq 0.3$ . Suggests that there is a close relationship between field isolate and vaccine strain. A potent vaccine containing the vaccine strain is likely to confer protection.

$r_1 = < 0.3$ . Suggests that the field isolate is so different from the vaccine strain that the vaccine is unlikely to protect

### In the case of LPB ELISA:

$r_1 = 0.4-1.0$ . Suggests that there is a close relationship between field isolate and vaccine strain. A potent vaccine containing the vaccine strain is likely to confer protection.

$r_1 = 0.2-0.39$ , Suggests that the field isolate is antigenically related to the vaccine strain. The vaccine strain might be suitable for use if no closer match can be found provided that a potent vaccine is used and animals are preferably immunised more than once.

$r_1 = < 0.2$ . Suggests that the field isolate is so different from the vaccine strain that the vaccine is unlikely to protect

## 1.7.6.1 Summary of all $r_1$ vaccine matching tests carried out by WRLFMD in 2010

Date	Isolates tested	$r'$ values	Cumulative isolate ' $r'$ values
21.01.10	Asia1 Irn 30/04	1	
22.01.10	Asia1 Irn 30/04	1	
25.01.10	Sat2 Eth 51,56/09	4	4
28.01.10	Sat2 Eth 51,56/09	4	8
28.01.10	A Irn 51, 73/09	12	20
29.01.10	A Irn 51, 73/09	12	32
04.02.10	O Ban 4, 30/09, Bhu 6, 40/09, Irn 51, 76/09	18	50
05.02.10	O Ban 4, 30/09, Bhu 6, 40/09, Irn 51, 76/09	18	68
05.02.10	Asia1 Irn 30/04	1	69
01.02.10	A Irn 51, 73/09	3	72
09.02.10	A Eri 1/06, 4/07, 1/08, 16, 40/09	15	87
10.02.10	Sat2 Sen 27/09	4	91
10.02.10	A Eri 1/06, 4/07, 1/08, 16, 40/09	15	106
18.02.10	A Eri 1/06, 4/07, 1/08, 16, 40/09	15	121
18.02.10	Sat2 Sen 27/09	4	125
19.02.10	Asia1 Irn 30/04	1	126
19.02.10	A Eri 1/06, 4/07, 1/08, 16, 40/09	15	141
22.02.10	O Irn 72/09	3	144
22.02.10	O Irn 72/09	3	147
25.02.10	A Eri 1/06, 4/07, 1/08, 16, 40/09	5	152
25.02.10	A Eri 1/06, 4/07, 1/08, 16, 40/09	5	157
25.02.10	A Eri 1/06, 4/07, 1/08, 16, 40/09	5	162
25.02.10	A Eri 1/06, 4/07, 1/08, 16, 40/09	5	167
26.02.10	A Eri 1/06, 4/07, 1/08, 16, 40/09	5	172
26.02.10	A Eri 1/06, 4/07, 1/08, 16, 40/09	5	177
01.03.10	O Tur 5/10	3	180
01.03.10	O Tur 5/10	3	183
25.03.10	O Tur 15,17/09, 1/10	9	192
25.03.10	O Tur 15,17/09, 1/10	9	201
26.03.10	Asia1 Irn 30/04	1	202
29.03.10	O Tur1,5/10	3	205
29.03.10	O Tur1,5/10	3	208

01.04.10	A Tur 12,18/09, 2,6/10	12	220
01.04.10	A Tur 12,18/09, 2,6/10	12	232
15.04.10	O Irn 34/07 x2, Irn72/09, Tur 17/09, 5/10	5	237
15.04.10	O Irn 34/07 x2, Irn72/09, Tur 17/09, 5/10	5	242
16.04.10	O Irn 34/07 x2, Irn72/09, Tur 17/09, 5/10	5	247
15.04.10	A Tur 12,18/09, 2,6/10	4	251
16.04.10	A Tur 12,18/09, 2,6/10	8	259
16.04.10	A Tur 12,18/09, 2,6/10	4	263
15.04.10	O Taw 1/2009	2	267
15.04.10	O Taw 1/2009	2	269
16.04.10	O Taw 1/2009	2	269
16.04.10	O Taw 1/2009	2	271
19.04.10	A Tur 12,18/09, 2,6/10 Vit 4/08, 1/09, 8/09	7	278
19.04.10	A Tur 12,18/09, 2,6/10 Vit 4/08, 1/09, 8/09	7	285
19.04.10	Asia1 Irn 30/04	1	286
22.04.10	O Taw 1/2009	2	288
22.04.10	O Hkn 1/2010, Irn 34/06 x2	5	293
22.04.10	O Hkn 1/2010, Irn 34/06 x2	5	298
23.04.10	O Hkn 1/2010, Irn 34/06 x2	5	303
29.04.10	O Irn 34/07 x2	6	309
29.04.10	O Irn 34/07 x2	6	311
29.04.10	A Vit 4/08, 1/09, 8/09	3	318
30.04.10	A Vit 4/08, 1/09, 8/09	3	321
13.05.10	A Vit 4/08, 1/09, 8/09	3	324
13.05.10	A Vit 4/08, 1/09, 8/09	3	327
13.05.10	Asia1 Irn 30/04	1	328
13.05.10	Asia1 Irn 30/04	1	329
20.05.10	A Irn 78/09, 9/10	12	341
20.05.10	O Irn 80/09. 1/10, 30/10	9	350
20.05.10	O Irn 80/09. 1/10, 30/10	9	359
22.05.10	A Irn 78/09, 9/10	12	371
23.05.10	O Irn 1/10, 30/10, Taw 1/09, Hkn 1/10	6	377
27.05.10	A Irn 78/09, Skr 2/10	11	388
27.05.10	A Skr 2/10	6	394
28.05.10	O Skr 4/10	4	398
28.05.10	O Skr 4/10	4	402
18.06.10	Asia1 Irn 30/04	1	403
18.06.10	O 3039, O TNN 24/84	2	405
21.06.10	O Hkn 1/10, Skr 4/10	2	441
21.06.10	O Hkn 1/10, Skr 4/10	2	409
25.06.10	O Irn 89/09, 5,8,17,27,33,44,49/10	16	425
28.06.10	O Irn 89/09, 5,8,17,27,33,44,49/10	16	441
01.07.10	O Irn 89/09, 5,8,17,27,33,44,49/10	16	457
02.07.10	O Irn 89/09, 5,8,17,27,33,44,49/10	16	473
01.07.10	O Irn 89/09, 5,27,44,49/10	5	478
05.07.10	O UAE 2,4/10, Pak 1,20/10, Ecu 1,9/10	12	490
05.07.10	O UAE 2,4/10, Pak 1,20/10, Ecu 1,9/10	6	496
08.07.10	O UAE 2,4/10, Pak 1,20/10, Ecu 1,9/10	18	514
09.07.10	O UAE 2,4/10, Pak 1,20/10, Ecu 1,9/10	12	526
12.07.10	O UAE 2,4/10, Pak 1,20/10, Ecu 1,9/10	12	538
13.07.10	Sat1 Ken 1, 77/10	2	540
13.07.10	Sat1 Ken 1, 77/10	2	542
16.07.10	A Irn 36/09, Pak 12,24/10, Nig 38/09, Tan 11,42/09	18	560
16.07.10	O 4625 (Geshur Isr2/85)	2	562

18.07.10	A Irn 36/09, Pak 12,24/10, Nig 38/09, Tan 11,42/09	18	580
22.07.10	A Irn 36/09, Pak 12,24/10, Nig 38/09, Tan 11,42/09	12	592
22.07.10	Asia1 Irn 30/04	1	593
23.07.10	A Irn 36/09, Pak 12,24/10, Nig 38/09, Tan 11,42/09	12	605
23.07.10	Sat2 Eth 74,75/09, 2/10	6	611
23.07.10	Sat2 Eth 74,75/09, 2/10	6	617
29.07.10	O Eth 7,9/10, Nig 15/09, Tan 5/09	8	625
30.07.10	O Eth 7,9/10, Nig 15/09, Tan 5/09 A Irn 36/09, Pak 12,24/10, Nig 38/09, Tan 11,42/09	8	633
26.07.10	A Irn 36/09, Pak 12,24/10, Nig 38/09, Tan 11,42/09	12	645
09.08.10	A Pak 13/10	2	647
09.08.10	A Pak 13/10	2	649
12.08.10	Sat1 Ken 101, 133/10	2	651
12.08.10	Sat1 Ken 101, 133/10	2	653
12.08.10	O Ken 125/09, 100/10, Mya 13/09, 3/10	12	665
13.08.10	O Ken 125/09, 100/10, Mya 13/09, 3/10	12	677
19.08.10	A Irn 73,80,125/10	15	692
16.08.10	A Sau 41/91 bvs SI94-96	2	694
16.08.10	O Mya 13/09, 3/10	3	697
16.08.10	O Mya 13/09, 3/10	2	699
20.08.10	A Irn 73,80,125/10	15	714
21.08.10	A Irn 73,80,125/10	6	720
21.08.10	A Irn 73,80,125/10	6	726
22.08.10	O Irn 88,92,99,143,149/10	10	736
22.08.10	O Irn 88,92,99,143,149/10	10	746
23.08.10	O Irn 88,92,99,143,149/10	10	756
23.08.10	O Irn 88,92,99,143,149/10	10	766
26.08.10	Sat2 Ken 122/10, Tan 43/09	2	768
26.08.10	Sat2 Ken 122/10, Tan 43/09	2	770
26.08.10	A Tur06 Phase XX sera pool	2	772
16.09.10	A Irn96 B50 used, fresh, B193/10 O Ecu 1/10, Irn 17,149/10, Pak 20/10, Tur 5/10	6	778
17.09.10	O Ecu 1/10, Irn 17,149/10, Pak 20/10, Tur 5/10	5	783
20.09.10	O Ecu 1/10, Irn 17,149/10, Pak 20/10, Tur 5/10	5	788
27.09.10	O Pak 25,42/10	12	800
29.09.10	A Irn 2005 VV	4	804
30.09.10	O Pak 25,42/10	12	816
30.09.10	A Irn 2005 VV	4	812
08.09.10	A Irn 2005 VV	2	822
08.09.10	Asia1 Shamir (Isr 3/89) VV	4	826
08.09.10	O Pak 42/10	1	827
11.10.10	A Tur 20,34/10	12	839
11.10.10	A Tur 20,34/10	4	843
15.10.10	A Tur 20,34/10	12	855
18.10.10	A Tur 20,34/10	6	861
21.10.10	O Tur 18, 39/10	12	873
21.10.10	A Tur 20/10	1	874
22.10.10	O Tur 18, 39/10	12	886
25.10.10	A Irn 176,185/10	8	894
29.10.10	O Irn 174,187/10	8	902
29.10.10	A Irn 176,185/10	8	910
25.10.10	O Tur 18, 39/10	4	914
04.11.10	O Irn 174,187/10	8	922
05.11.10	O Irn 174,187/10	7	929
05.11.10	A Irn 185/10	1	930

05.11.10	Asia1 Irn 30/04	1	931
08.11.10	O Campos VV	3	934
08.11.10	Asia1 Shamir (Isr 3/89) VV	3	937
08.11.10	O Irn 174,187/10	6	943
08.11.10	A Egy 6/06, 16/09	2	945
08.11.10	A Irn 185/10	1	946
11.11.10	A Egy 6/06, 16/09	2	948
11.11.10	O Mog 3,4, 9/10 Hkn 20/10	16	964
12.11.10	O Mog 3,4, 9/10 Hkn 20/10	16	980
15.11.10	O Mog 3,Hkn 20/10	4	984
15.11.10	O Mog 3,Hkn 20/10	4	988
22.11.10	O Mog 4, 9/10 Hkn 20/10	12	1000
26.11.10	O Mog 4, 9/10 Hkn 20/10	12	1012
22.11.10	A Iran 2005 VV	6	1018
26.11.10	A Iran 2005 VV	6	1024
29.11.10	O Irn 191,194,196/10	3	1027
29.11.10	A Irn 195,214/10	2	1029
02.12.10	O Irn 191,194/10, Mog 4,9/10 Vit 2,12/10	26	1055
02.12.10	O Irn 191,194/10, Vit 2,12/10	16	1071
06.12.10	O Irn 191,194/10, Vit 2,12/10	8	1079
06.12.10	A Irn 195,214/10, Vit 1/10	9	1088
06.12.10	O Irn 191,194/10, Vit 2,12/10	11	1099
09.12.10	O Irn 191,194/10, Vit 2,12/10, Zam 4/10	20	1119
09.12.10	A Irn 195,214/10, Vit 1/10	6	1125
10.12.10	O Tur 5/09	3	1128
10.12.10	Sat2 Bot1,5/10, Moz 1/10, Zim 2/10	8	1136
10.12.10	A Irn 195,214/10, Vit 1/10	6	1142
13.12.10	Sat2 Bot1,5/10, Moz 1/10, Zim 2/10	8	1150
15.12.10	A Irn 195,214/10, Vit 1/10	9	1159
13.12.10	O Tur 18,39/10	4	1163
15.12.10	O Tur 18,39/10	4	1167
16.12.10	A Irn 195,214,215/10, Vit 1/10	12	1179
16.12.10	O Zam 4/10, Irn 225/10	12	1191
16.12.10	Sat2 Bot1,5/10,Zim 2/10	6	1197
23.12.10	A Irn 215/10, Vit 1/10	6	1203
23.12.10	O Irn 225/10	6	1209

**Average number of  $r_1$  values generated per week = 23.22**

**Total number of  $r_1$  values generated in 2010 = 1209**

### 1.7.7. WRLFMD Vaccine Recommendations

The recommendations made by the WRLFMD are drawn principally from a list of vaccine strains for which master seed vaccine viruses are believed to be available within the portfolios of vaccine suppliers able to fulfill the quality requirements for use in Europe. The ranking of the utility of the viruses is based on the results obtained by the WRLFMD from *in vitro* serological tests to match these vaccine viruses to recent field isolates. As such, the WRLFMD can only recommend vaccine virus strains for which it has received supplies of both the vaccine virus and the homologous antiserum. Since these vaccine strains are chosen to protect against threats from outside of Europe, it can be anticipated that the vaccines should also be useful to counter such threats at source. However, other vaccine viruses may have been produced, for example by vaccine

manufacturers located in the regions from which the threats arise and using local isolates, that would also provide an equivalent or even better antigenic match to the field isolates that pose the threat (see Regional Recommendations at section 1.5).

### **HIGH PRIORITY**

O Manisa  
O BFS or O Campos  
*PanAsia 2\**  
A24 Cruzeiro  
Asia 1 Shamir  
A Iran 05  
A22 Iraq  
SAT 2 Saudi Arabia or equivalent

### **MEDIUM PRIORITY**

A Eritrea  
A Iran 96  
SAT 2 Zimbabwe  
A Iran 87 or A Saudi 23/86  
SAT 1 South Africa  
A Malaysia 97  
A Argentina 2001  
O Taiwan 97 (or equivalent pig-adapted strain)  
A Iran 99

### **LOW PRIORITY**

A15 Bangkok related strain  
A87 Argentina related strain  
C Noville  
SAT 2 Kenya  
SAT 1 Kenya  
SAT 3 Zimbabwe  
A Kenya

*NB Strains are not listed in order of importance within each priority grouping.*

*\*Very recent introduction: PanAsia 2 vaccine use will be monitored carefully during 2011*

### **Acknowledgements**

For the work carried out at Pirbright, the majority of the vaccine strains and vaccine antisera used for matching tests have been supplied to the WRLFMD by Merial. Some strains and/or antisera were supplied to WRLFMD by Intervet, ARRIAH and the Thai Regional Reference Laboratory at Pakchong

## PART 2

# *Improving the quality of laboratory tests from international and national reference laboratories*

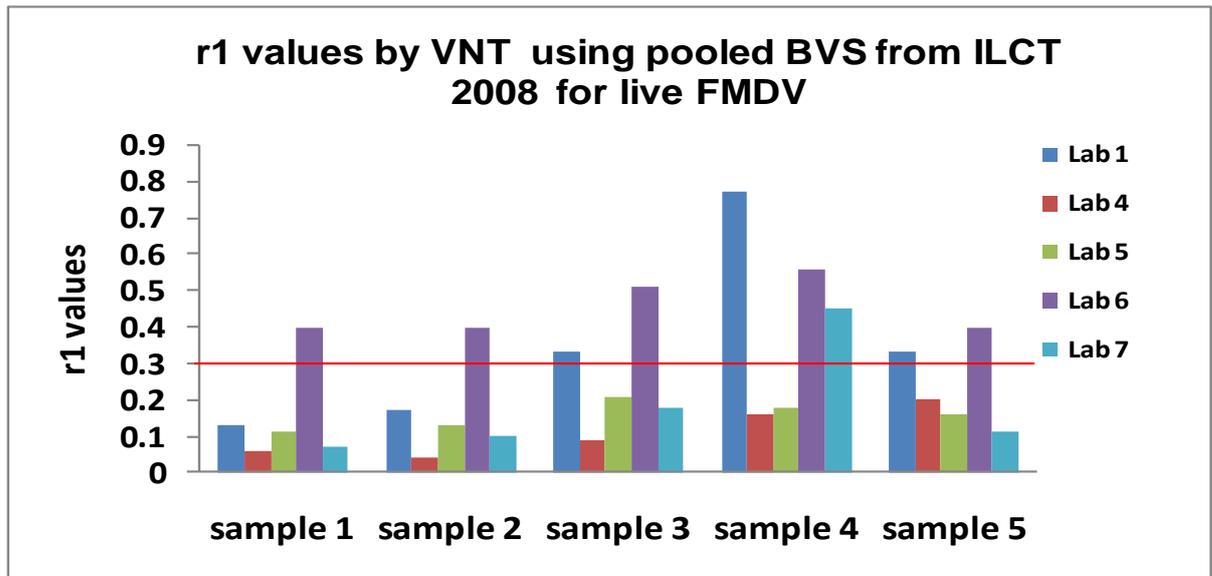
## 2. Inter-laboratory comparative testing exercises

### 2.1 Vaccine Matching by serology

A vaccine matching inter-laboratory comparative test (ILCT) trial was organised for the first time in 2008. It involved distribution of an A<sub>22</sub> vaccine strain along with five bovine anti-A<sub>22</sub> sera and five FMDV type A field isolates with r<sub>1</sub> values ranging from high, moderate and low. Guinea-pig and rabbit anti-A<sub>22</sub> sera were also distributed for ELISA testing. Eight laboratories were invited to participate. Some laboratories achieved reproducible results but others did not. Using the five antisera in a pool gave very similar results to the mean of using them individually and was considered the best approach. Full methodologies and raw test data were not received from all participants. For 2009, it was decided to extend the study by supplying additional viruses to be matched and by using the pooled BVS only. Four more (twelve in total) laboratories were invited to participate and eleven agreed to do so. All were encouraged to carry out the tests using their own methodology as well as that supplied by WRLFMD. Great difficulties were experienced with the processing of documents and the sending of samples for the exercise. More documents including various certificates and contracts than previous years were required from WRLFMD for some countries to obtain the import permits from their authorities. Also, some airlines which were available in the past have refused to take on board biological infectious materials or shipments containing dry ice since the beginning of 2009. This greatly delayed the progress of the work and consequently the exercise was not completed by the end of 2009 and was extended into 2010. One laboratory had still not received the panels and one lab has just received the materials by the time of the Network Meeting in October 2010. Results from nine participants were received by the end of 2010 and they were decoded and collated. The analysis of the results received suggested that the correlation between different labs has improved compared to the results observed in 2008 studies, where no lab showed comparable results for all 5 samples either by VNT for the live virus panel (Figure 1a) or by LPBE for the inactivated panel (Figure 1b). For the results from the 2009/2010 exercise, comparable results for all nine samples were observed in four out of seven labs testing live virus panels using LPBE and/or VNT (Figure 2 a, b), and three out of four labs testing inactivated samples using LPBE (Figure 2c), respectively. The overall interpretations were consistent from seven out of nine laboratories. This reduction of discrepancy between different labs could be due to the distribution of detailed protocols for both VNT and LPBE from WRLFMD to all the participants at the time of sending the samples. Two labs (6 and 7) showed a great improvement in VNT (compare Figure 1 and Figure 2). The methodology from different labs has been harmonised to some extent by this exercise. Participating laboratories have been requested to send details of their methodologies as well as the raw data of their test results including titre values as well as calculated r values from both the 2008 and 2009/2010 testing exercises.

Figure 8.  $r_1$  values using pooled BVS generated by participants from FMD vaccine matching ILCT 2008 by VNT (a) on the live virus and LPBE (b) on the inactivated virus

a.



b.

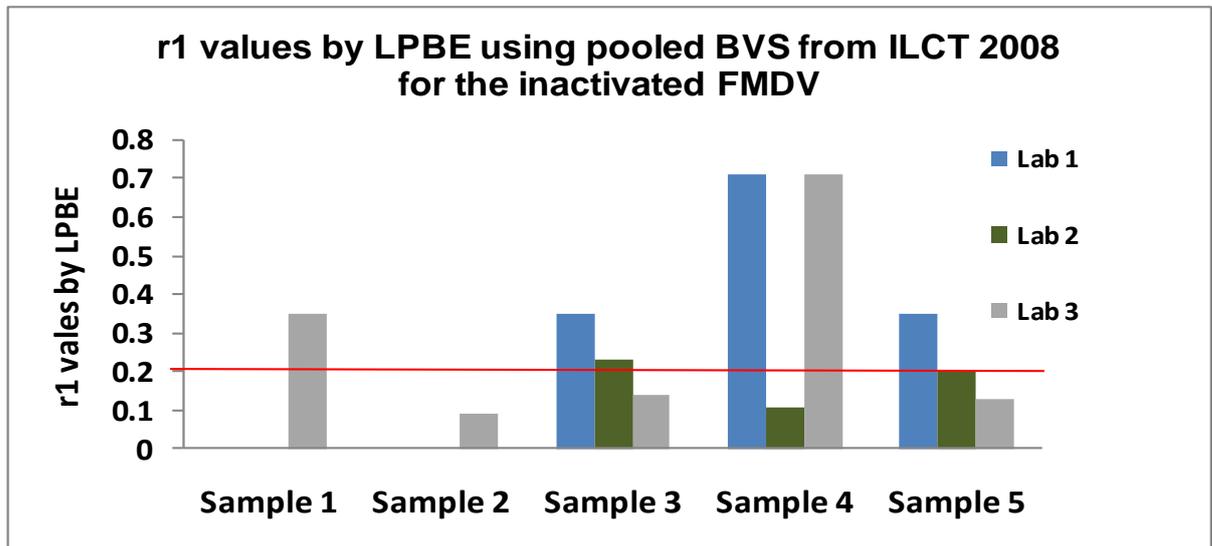
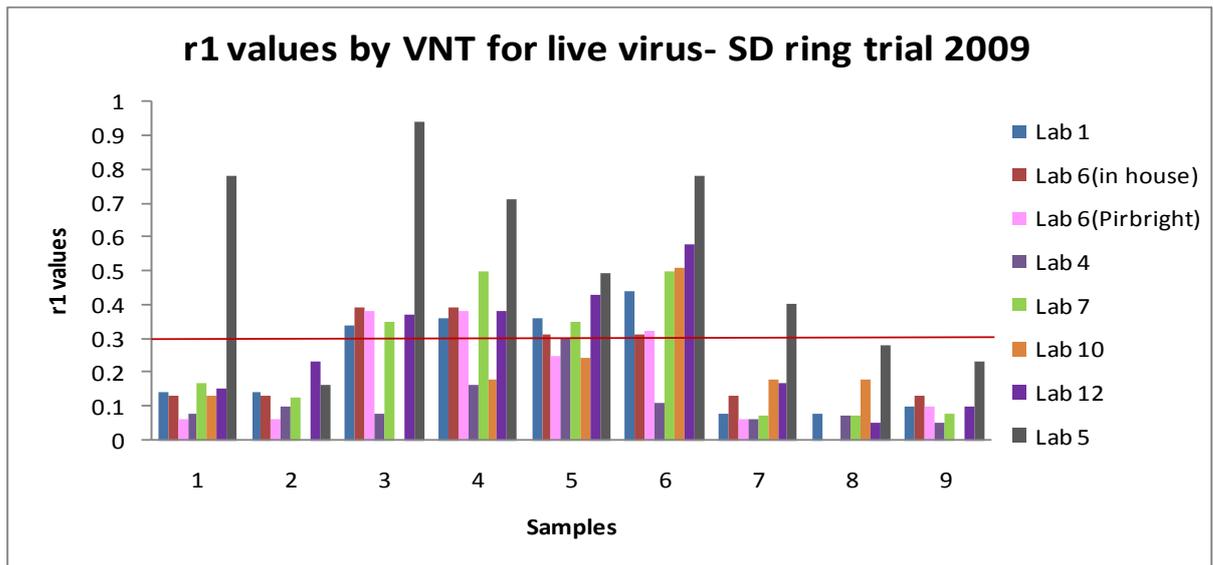
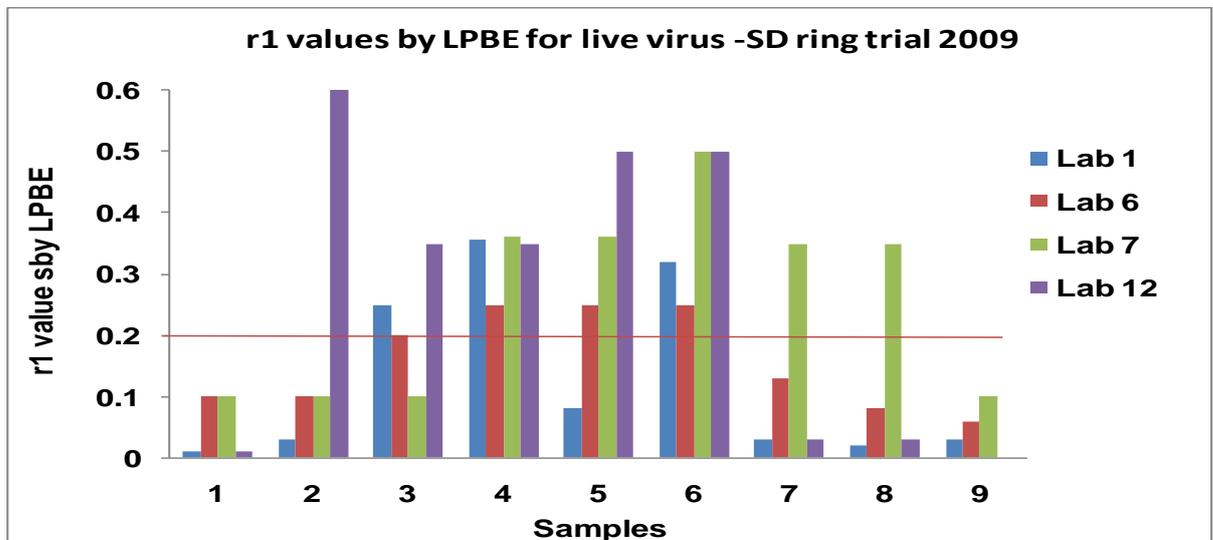


Figure 9.  $r_1$  values using pooled BVS generated by participants from FMD vaccine matching ILCT 2009/2010 for live virus panel by VNT (a) and LPBE (b) and for BEI inactivated virus panel by LPBE (c)

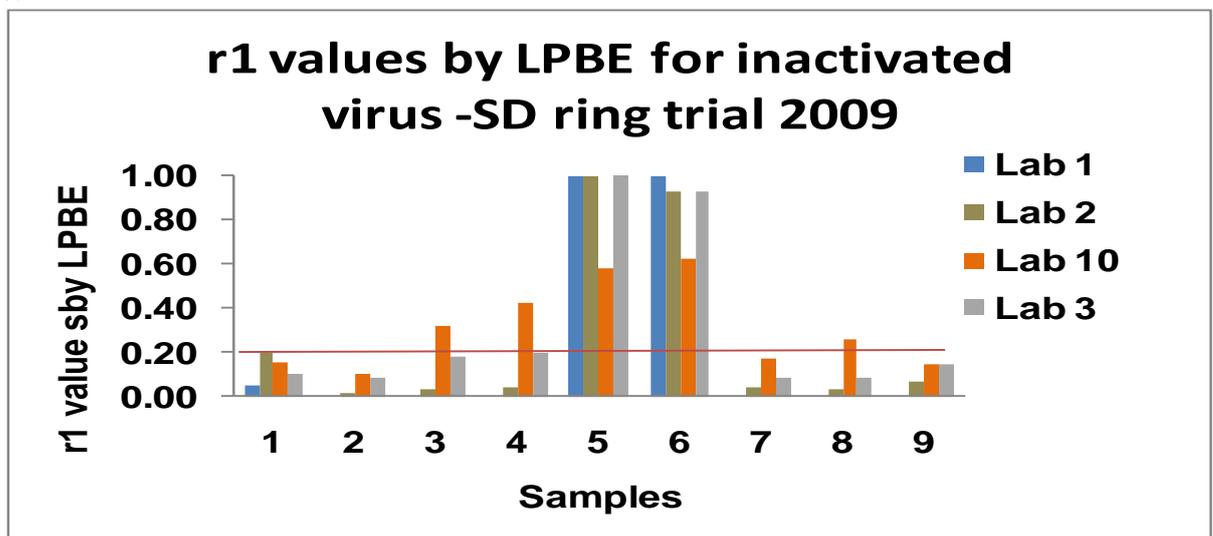
a.



b.



c.



## 2.2 Virus isolation and serology

### 2.2.1. Proficiency testing study (PTS) organised by WRLFMD/CRLFMD-SVD

During 2010, the European Community Reference Laboratories for FMD and SVD, in association with WRLFMD, organised a round of inter-laboratory proficiency testing to help quality assure FMD and SVD diagnosis. The first priority was to supply proficiency panels to member states of the EU and of the EUFMD, but the panels were also made available more widely, including targeting of the OIE/FAO FMD Network Laboratories.

The test purposes evaluated in this PTS were outbreak detection by virus and virus genome detection and screening the importation samples by serology. The serology panel therefore involved samples for testing after both vaccination and non-vaccination. All samples were analysed sufficiently prior to selection to ensure that they would give consistent results in tests by index methods. One panel included live virus so that virus isolation testing could be evaluated. Virus in other panels was inactivated so that they can be evaluated in laboratories that do not work at the highest containment levels.

Seventy seven labs from seventy five countries were invited to take part in this study supported by the EC and the EUFMD. Of the 60 labs that agreed to participate, 27 labs were from EU member countries; 33 labs from Non-EU countries. Participants were sent a package containing uniquely coded and labelled samples as described below. The aim of the exercise was to complete a proficiency testing study for virology and serology diagnosis for FMD and SVD during 2010. Particular tests were not specified, but labs were invited to select tests and interpret results based upon:

- a. Virus detection – as if samples were from suspect outbreaks.
- b. Serology - as if serum samples were from FMD /SVD endemic countries with vaccination (for FMD samples) or non-vaccination (for SVD samples) history for importation screening.

Participants were asked to give results for individual tests on each sample and where multiple tests were used, an overall result for each sample. Overall interpretation for each suspect case under investigation for panels 1 was also requested. Participants were also requested to provide information on national surveillance for FMD and/or SVD. This was to enable a clear picture of the scale of activities, the state of QA accreditation and the tests actually being used by participants during 2009/2010 to be compiled.

Results of this study will be presented at the joint meeting of FMD/SVD national reference laboratory's in Brussels, Belgium in May 2011 and a feedback letter including the overall results from all participants for 4 panels, comments and recommendations on each test for each panel will be prepared and sent to each laboratory.

Details of panels:

**Panel 1: infectious material from 2 cases of suspected vesicular disease for virus detection**

Case 1a: 2 epithelial and 1 faecal suspension samples from pigs in a herd affected with a vesicular condition.

Case 1b: 2 epithelial and 1 faecal suspension samples from pigs in a herd affected with a vesicular condition.

**Panel 2: non-infectious material<sup>1</sup> from cattle or pigs for virus genome/antigen detection by RT-PCR and/or Ag-ELISA**

7 samples from cattle or pigs, with each originating from a different case of a herd with a vesicular condition.

**Panel 3: non-infectious material<sup>2</sup> for FMD serology**

6 bovine sera from FMD endemic country Pakistan for importation screening. The cattle were vaccinated against both FMDV vaccines A<sub>22</sub> Iraq and Asia1 Shamir.

**Panel 4: non-infectious material<sup>2</sup> for SVD serology**

6 sera from pigs for importation screening.

Summary of the exercise

1. Seventy seven labs from seventy five countries were invited to take part in this study and sixty labs that agreed to participate.
2. 20, 49, 58 and 37 labs required and received panel 1, panel 2, panel 3 and panel 4, respectively.
3. Information was collected on tests in use, strains of virus used in tests, extent of ongoing testing, and quality accreditation status of tests. The decoding, collating and analysing of the results received is in progress while expecting some labs to return their results before the end of February 2011.

**2.2.2. South American initiatives on laboratory testing harmonization**

During 2010, PANAFTOSA organized its annual rounds of inter-laboratory test for FMD diagnosis and sero-surveillance. This year the rounds involved panels of materials for testing by NSP/NCP-serology for 14 laboratories. Testing will include I-ELISA 3ABC/EITB System. All samples will have been analyzed sufficiently prior to selection to ensure that they would give consistent results in tests in use in the laboratories of the region. Laboratories will be given individual feedback on their results including observations and non-conformities according to predefined criteria. Laboratories with non-conformities will receive technical cooperation to identify and correct potential problems.

**2.2.3. North American initiatives on diagnostic harmonization**

**North America Animal Health Network:**

In February, 2007, under the auspices of the Security and Prosperity Partnership of North America (SPP), representatives from the animal health laboratory networks of Canada (CN), the United States of America (USA) and Mexico (MX) met in Winnipeg, Manitoba, Canada to initiate discussions on the terms of cooperation and guidance towards the harmonization of tests used for the diagnosis of animal diseases. Initial harmonization included activities such as training of laboratory staff, sharing of diagnostic test protocols and Inter-laboratory tests of harmonization panels. These efforts were concentrated in 3 areas: Vesicular Diseases (FMD, VSV, SVD), Avian Influenza and Bovine Tuberculosis. The committee representatives agreed to the following definition of harmonization: "Ensuring an equivalency of diagnostic test results between the laboratories, regardless of protocols practiced by each country". This statement guided each working group when analyzing the diagnostic tests used for each disease in their respective country. For each disease category, a working group was assembled to include subject matter experts, a coordinator and a statistician. The basic approach is to develop harmonization panels to address performance of each method utilized for

specific analyte. In 2010, the following FMD diagnostic techniques were harmonized among the three countries: AgELISA, virus isolation, realtime RT-PCR and virus neutralization test were harmonized among the three laboratories. Furthermore, research collaboration and exchange of assays were established as a results of this effort.

Specifically in 2010, FADDL provided FMD proficiency panel for rRT-PCR for 200 participants. FADDL supports the NAHLN by setting quality standards which are followed in the laboratories along with adequate facility biosafety and biosecurity levels. These premises are then reinforced with scenario testing. In addition, FADDL and the NAHLN work together to provide input on methods validation and approval for animal testing, review of available methods and associated gaps and identifying potential new technologies. FADDL participates with the NAHLN in determining equivalency of modified methods or platforms, monitoring performance of implemented assays, and development of performance characteristic summary documents.

FADDL develops and may share training materials with other national laboratory networks in a move to standardize training practices across agencies.

#### **2.2.4. Harmonisation of tests in the SADC region**

##### **Harmonisation of LPBE SOP for SADC**

The FMD reference centres at ARC-OVI and Botswana Vaccine Institute (BVI) collaborated to harmonise the LPBE SOP for SADC. Dr G Thobokwe from BVI together with RM Dwarka, B. Botha, J. Esterhuysen and N. Cassim from ARC-OVI met on 13-14 May 2010 to harmonise the LPBE SOP for SADC. The harmonised SOP was used in a training workshop conducted at BVI during July 2010 and funded by FAO. Each participating country needed to set up the test in their laboratories with BVI providing all reagents. BVI conducted a proficiency test in November 2010 to determine the competency of the laboratories and feedback will be given to each country.

### **2.3. Training**

**2.3.1 WRLFMD, IAH Pirbright laboratory** hosted its annual 2-week FMDV / SVDV diagnostics training course for overseas scientists between 12th and 23<sup>rd</sup> April 2010.

#### **Diagnostic Course Attendees:**

- |                            |                                  |           |
|----------------------------|----------------------------------|-----------|
| • Natalia Lugovskaya       | FGI                              | Russia    |
| • Chanasa Mpelumbe-Ngeleja | Central Veterinary Lab           | Tanzania  |
| • Claudia Beascochea       | OIE/FMD Ref Lab SENASA           | Argentina |
| • Kamila Gorna             | AFSSA                            | France    |
| • Andrzej Fitzner          | Vet Research Institute in Pulawy | Poland    |
| • Peter Hostnik            | NRL for FMDV                     | Slovenia  |

A number of IAH Pirbright staff also gave key lectures in the Defra Notifiable Diseases Course held at IAH Pirbright on the 20<sup>th</sup> and 21<sup>st</sup> May. Notifiable Diseases Attendees: Approx 30 animal health officers attended.

During the year a number of overseas laboratory staff attended for various periods of training.

### Period training

- Dr Unal Parlak from the SAP FMD institute, Ankara, Turkey for 2 months to undertake training and analysis of full-genome sequences of FMD viruses from the Middle East
- Dr Christopher Kasanga for 3 months. From: Sokoine University of Agriculture, Tanzania. Collaborator on Wellcome Trust Project (SACIDS) Southern Africa Centre for Infectious Disease Surveillance. Dr. Kasanga was trained for NSP ELISA, VNT , LPBE and PrioCHECK type O ELISA.
- Dr Youjin Shang, 3 months. From: Lanzhou Veterinary Research Institute, China. Collaborator on one year EU project – “FMD in Asia”
- Mr Shane Riddell 2 weeks. Collaboration with Australian Animal Health Laboratory (AAHL). Development and validation of new FMD assays.
- Luc Gilbert Aplogan from: Benin. Two training mission funded by IAEA for LAMP detection assays
- JiJin He from Lanzhou Veterinary Research for 2 months. Full-genome sequencing studies of Asian FMD viruses as part of EPIZONE (internal call project: FMDV in Asia)
- Belinda Blignaut from Onderstepoort , South Africa has been trained for VNT for antibody detection and Vaccine matching tests.

### 2.3.2 SENASA:

Date	Venue of training	Subject of training	Supplier of the training	Recipient of the training
August	SENASA Argentina	FMDV characterization	SENASA	G. Salgado-National Institute of Hygiene Ecuador
April	IAH Pirbright	FMDV diagnosis	IAH Pirbright	C. Perez-SENASA Argentina
May	IICAB-Ames-USA	Veterinary Biologics Training	IICAB	V. Barros – SENASA Argentina
Nov	Senacsa Paraguay	Twinning project	SENASA	Senacsa Paraguay.

### 2.3.3 PANAFTOSA:

Date	Venue of training	Subject of training	Supplier of the training	Recipient of the training
July 19 <sup>th</sup> to 30 <sup>th</sup>	PANAFTOSA	Cell culture production and maintenance of cell lines	PANAFTOSA	Professionals from South American FMD laboratories
July 5 <sup>ve.</sup> To 16 <sup>th</sup> .	PANAFTOSA	Vesicular disease diagnosis and differential diagnosis by PCR	PANAFTOSA	Professionals from South American FMD laboratories
July 26 <sup>th</sup> to August 6 <sup>th</sup> .	PANAFTOSA	Virus Neutralization test for VSV antibodies	PANAFTOSA	Professionals from South American FMD laboratories
August	PANAFTOSA	LPBE for FMD	PANAFTOSA	Professionals from

9 <sup>th</sup> to 20 <sup>th</sup> .		antibody detection		South American FMD laboratories
August 23 <sup>rd</sup> to 27 <sup>th</sup> .	PANAFTOSA	The use of control charts to monitor process in the laboratory	PANAFTOSA	Professionals from South American FMD laboratories

### 2.3.4 PIADC-FADDL:

#### Training

##### NAHLN Activities for 2010

- PVSS provided training for 3 laboratories, and trainers within the NAHLN trained 2 laboratories to bring the total number of laboratories trained for FMDv detection by real-time PCR to 40 state laboratories and 2 federal laboratories.
- A recording system was developed and introduced to capture the results of the proficiency tests and an accompanying database mining system was developed to inspect the results.
- A negative cohort study for the real-time PCR for FMDv detection is being conducted in cooperation with 11 NAHLN laboratories and FADDL and refresher training was given to the laboratories on extraction and PCR. Standard operating procedures were edited to accommodate the reporting of false negatives during this study.
- Positive extraction controls and positive amplification controls for the negative cohort study were produced from a defective Q-beta phage construct and sent to laboratories participating in the negative cohort study.
- Sixteen tabletop exercises were conducted by the NAHLN in 2 months looking at responses of the NAHLN labs should FMD occur in the US.
  - Future plans include adapting FMD serological methods to a robotic system. The NAHLN laboratories will be trained and tested in this system for response readiness, but will not actively be using the method for testing.

Date	Venue of training	Subject of training	Supplier of the training	Recipient of the training
July, 2010	PIADC-FADDL	rRT-PCR platform	PIADC-FADDL	NAHLN
Jan, March, June, Aug Nov	PIADC-FADDL	Field diagnostician training	PIADC-FADDL	Veterinarian; US military, State, Federal and International

### 2.3.5 RRLSEA:

Date	Venue of training	Subject of training	Supplier of the training	Recipient of the training
13-16 September 2010	Regional Reference Laboratory	FMD diagnosis and vaccine production	Animal Production and Health ,	Dr. Ranjith Wijewardana, senior veterinary

	for FMD, Pakchong And Bureau of Veterinary Biologics, Pakchong, Thailand		Sri Lanka	officer from Animal Production and Health , Sri Lanka
25-29 October 2010	Singapore	Biosafety management training	FAO and USDA collaboration project.	Rompruke Udon, senior Veterinary Officer and Mr. Charouy Yothakaew Machanic engineer RRLSEA, Thailand
11-22 October 2010,	Regional Reference Laboratory for FMD, Pakchong, Thailand	FMD diagnosis, serology test, antigen typing test, virus isolation	OIE Sub-Regional Representative (SRR)	Dr. January Magcalas, Veterinarian of National Foot and Mouth Disease Task Force (NFMDF), Bureau of Animal Industry of the Philippines
22 November – 3 December 2010	Regional Reference Laboratory for FMD, Pakchong, Thailand	FMD diagnosis , serology test, antigen typing test, virus isolation	FAO on FMD control in Bhutan project	Dr. N.K. Thapa, Principle Animal Health Officer and Mr. Dawa Tshering, Senior lab technician from NCAH, Serithang, Bhutan

### 2.3.6 ARC-OVI:

Date	Venue of training	Subject of training	Supplier of the training	Recipient of the training
Feb-March 2010	ARC-OVI-TADP	Laboratory diagnostics methods for FMD	ARC	David Lazurus ( Nigeria) and Iolanda Anahory (Mozambique)

### 2.3.7 ARRIAH:

Date	Venue of training	Subject of training	Supplier of the training	Recipient of the training
23 February – 5 March	Russia (FGI ARRIAH)	FMD diagnostics	ARRIAH	Scientific-Production Enterprise "Biological products" (Tadzhikistan)
12-23 April 2010	UK	FMD diagnostics	Institute for Animal Health (Pirbright)	Natalia Lugovskaya
23-27 August 2010	Belarus	FMD diagnostics	ARRIAH	Belarusian State Veterinary Centre (Minsk)

### 2.3.8 Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy

Activities conducted in 2009-2010

#### Training

Two training courses were organised and carried out, one for six trainees (three from Afghanistan and three from Pakistan) in the framework of the FAO project GTFS/INT/907/ITA, FAO scientific leader Dr Giancarlo Ferrari, and a second one in November 2010 for nine trainees from Countries involved in long term projects funded by EUFMD (Armenia, Azerbaijan, Georgia, Iran). Objective of the training was learning of principles and practice of ELISAs for FMD diagnosis, including:

- antigen detection and typing in clinical samples,
- detection of anti-NSP antibodies,
- detection of serotype-specific antibodies (types O, A, Asia1)

#### Reagents supplied

As part of the FAO project GTFS/INT/907/ITA, aimed at acquiring initial knowledge on the presence, distribution and level of circulation of FMD viruses in five countries of central Asia (Afghanistan, Pakistan, Turkmenistan, Tajikistan, Uzbekistan), by serosurveys and virus detection in clinical samples with transfer of laboratory capacity into the beneficiary countries, the following products were delivered:

- In-house stabilised kits for analysis of 6000 sera in each country (30,000 in total) for the detection of anti-NSP antibodies by 3ABC-trapping ELISA;
- In-house stabilised ELISA kits (prototype) for analysis of 120 clinical samples in each country 600 in total) for antigen detection and typing (types O,A,Asia1).

#### Other Diagnostic Support Provided

Antibody profile of serum samples listed below was determined through the analysis of each sample in four in-house ELISAs for titration of antibodies to FMDV serotypes O, A, Asia 1 and to NSP.

- 5100 sera from Transcaucasus regions (1100 from Armenia, 3300 from Georgia, 700 from Azerbaijan), for a total of 20,000 single quantitative analyses (EUFMD project). Objectives: evaluation of level and distribution of vaccine induced immunity and virus circulation.
- Approximately 400 experimental sera, for a total of 1600 single quantitative analyses, for the evaluation of potency and purity of two trivalent vaccines (local production and commercial product).
- A subset of sera from serosurveillance carried out in countries beneficiary of the FAO project GTFS/INT/907/ITA, namely: 480 selected samples from Tajikistan, 720 selected sera from Pakistan and 629 selected sera from Afghanistan, for a total of 1829 samples and 7316 single quantitative analyses.
- 800 sera from CHAD; in addition to assays described above these samples were also analysed for antibodies to FMDV serotypes SAT1 and SAT2, then six different immunoassays for a total of 4800 single analyses.

## **2.4 Reagent and test kit supply**

### **2.4.1 WRLFMD:**

For the period January to March, viruses, reagents or kits have been sent to Croatia, Italy, Greece, Turkey, Bulgaria, Kuwait, Iran, Lebanon, South Africa, Russia, Ethiopia, Philippines, New Zealand and Korea.

For the period April to June, viruses, reagents or kits have been sent to Uzbekistan, Georgia, Morocco, Switzerland, Iraq, Sudan, Saudi Arabia, Indonesia, Mongolia, Japan, Qatar, Vietnam and Belarus.

For the period July to September, viruses, reagents or kits have been sent to Spain, Israel, Thailand, Taiwan, Switzerland, Romania, USA, Tanzania, Korea, Vietnam, Pakistan and Belgium.

For the period October to December, viruses, reagents or kits have been sent to Russia, Mali, New Zealand, Taiwan, Rwanda, Vietnam, Germany, Slovenia, Egypt and Austria.

- OIE Reference sera are available for serotypes O, A, Asia 1 and C. Rabbit and guinea pig antisera against O1 Manisa, SAT1, SAT 2 and SAT 3 are available for strain differentiation studies and other serology tests. Rabbit and guinea pig antisera against A22 Iraq, A Iran and SAT2 Eritrea are also available for strain differentiation by Liquid Phase Blocking Elisa. Bovine sera against O1 Manisa is also available.
- The FMDV reference laboratory provided reference plasmids and encapsidated controls for real-time RT-PCR to Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy

### WRLFMD: Reagents Supplied

Reagents produced	Supplied nationally	Supplied to Other Countries
Rabbit and Guinea Pig Antisera	300ml	350ml
FMD Reference sera	None	320ml
Inactivated virus	100ml	1250ml
Live virus	1000ml	200ml
Antigen ELISA kits	None	31
Antibody ELISA kits	None	72

### 2.4.2 SENASA: Reagents Supplied/Received

Type of reagent	Quantity	Supplier of the reagent	Recipient of the reagent
Hyper-immune guinea pig serum	37 vials x 1ml e	SENASA Argentina	Argentina, Brazil, Paraguay
Vaccine strains	2	SENASA Argentina	Argentina
LP-ELISA kit	60 kits	CEVAN Argentina	Argentina, Brazil
Typing ELISA kit	11	CEVAN Argentina	Argentina, Brazil

### 2.4.3 PANAFTOSA: Reagents Supplied/Received

National Laboratories of the South American countries by producing, controlling and distributing reference reagents for their diagnosis, sero-surveillance and vaccine control activities.

Tests	Total (TEST)
I-ELISA 3ABC	319.440
EITB	31.600
ELISA-IS Typing	7.350
FC 50% (hyperimmune sera)	39.500
Lp-ELISA FMD Seroepidemiology	28.000
Lp-ELISA VSV Seroepidemiology	32.000
Lp-ELISA FMD Vaccine quality control	382.000
Lp-ELISA VSV Vaccine quality control	4.000
3D-AGID	37.000
Sp-ELISA BT	7.000
AGID BT	2.000
Sp-ELISA IBR	3.000

### 2.4.4 PIADC-FADDL: Reagents Supplied/Received

Type of reagent	Quantity	Supplier of the reagent	Recipient of the reagent
AgELISA reagents	NA	PIADC-FADDL	LADIVES Panama
PCR positive control	USA	PIADC-FADDL	National Animal Health Lab Network

### 2.4.5 RRLSEA: Pakchong Reagents Supplied/Received

Type of reagent	Quantity	Supplier of the reagent	Recipient of the reagent
ELISA reagent 1. Rabbit trapping antibody type O, A, Asia1 2. Guinea pig detecting antibody type O, A, Asia1 3. Inactivated antigen type O, A, Asia1 4. Control serum for strong, weak and negative	51 sets  (1 set can test ~ 1000 samples)	Regional reference Laboratory for FMD, Pakchong, Thailand	FMD laboratory within Thailand and South East Asia member countries under the SEAFMD control campaign

#### 2.4.6 NVRI: Nigeria Reagents Supplied/Received

Type of reagent	Quantity	Supplier of the reagent	Recipient of the reagent
FMD Elisa Kit (LPB) Immuno-Assay For Antibody Detection	1	Institute For Animal Health, Pirbright, UK.	National Veterinary Research Institute, Vom- Nigeria.
FMD Elisa Kit For Antigen Detection	1	Institute For Animal Health, Pirbright, UK	National Veterinary Research Institute, Vom-Nigeria

#### 2.4.7 ARRIAH: Russia Reagents Supplied/Received

Type of reagent	Quantity	Supplier of the reagent	Recipient of the reagent
FMDV antisera	3 ml	ARRIAH	Republic of Kazakhstan
FMDV antigen	3 ml	ARRIAH	Republic of Kazakhstan
FMDV antibody kits	201	ARRIAH	Republic of Kazakhstan, Syria, Belarus, Taiwan
FMD vaccinated cattle sera	6 ml	ARRIAH	Belarus
FMD recovered cattle sera	6 ml	ARRIAH	Belarus

## 2.5. Collaborative Research

**2.5.1. WRLFMD** is a founder member of the Global FMD Research Alliance (GFRA) that seeks to bring together FMD researchers from around the world with the aim of developing recommendations on research priorities and collaborative research projects. A major initiative in 2010 was to further broaden membership of GFRA and to arrange a meeting to discuss the Immune response to FMD virus and vaccines. IAH organised and hosted this GFRA meeting which was held on 21<sup>st</sup>-22<sup>nd</sup> January, 2010 at the Pirbright Laboratory, UK.

The IAH FMD research groups and WRLFMD maintain close research links with a wide range of partner laboratories worldwide.

WRLFMD chaired the annual meeting of the European FMD/SVD National Reference Laboratories in Brussels, Belgium in January 2010.

WRLFMD organised, hosted and chaired the OIE/FAO FMD Reference Laboratories Network Meeting held at the IAH ,Pirbright, UK. 4th-6th October 2010.

There has been continuation of participation in/coordination of the following collaborative research projects on FMD sponsored by the European Commission: (i) EU Network of Excellence EPIZONE (<http://www.epizone-eu.net/>) (ii) Disconvac (<http://fmddisconvac.net/>).

The EPIZONE project has funded work to develop and share full genome sequencing approaches with laboratories in Turkey, PR China, Denmark, Italy and Belgium. This work continues previous studies undertaken by a 1 year pilot project funded by FAO.

Staff members are participating in new projects funded by BBSRC/DfID/Scottish Executive Combating Infectious Diseases in Livestock for International Development (CIDLID) and the Wellcome Trust (Southern African Centre for Infectious Disease Surveillance) which aim to improve FMD control in East and Southern Africa.

FMD type O and type Asia 1 ELISA kits developed in Brescia, Italy and an FMDV NSP ELISA developed by The Australian Animal Health Laboratory (AAHL), have been validated in SAU, WRL/EU RL FMD in Pirbright. The specificity and sensitivity for each test has been evaluated and the full data and comments on tests were provided.

### **2.5.2 SENASA**

RIIDFA (SENASA, INTA, CEVAN, Biogenesis Bago): Coordinated research and development actions in FMD to grant the status of country without FMD:

Outcomes;

- Development and optimization of alternative FMD vaccine quality and efficacy control methods
- FMD virus transmission quantification in vaccinated and non vaccinated bovines
- Cross protection evaluation in bovines between vaccine and heterologous strains
- Real time PCR development for the rapid FMD diagnostics
- Development and scale up production of diagnostic reagents for the FMD virus and its antibodies
- Study of FMDV molecular evolution in Argentine outbreaks using A Argentina 2001 strain as model
- Biosafety training program

7<sup>th</sup> Framework Programme UE FMD DISCONVAC: Development, enhancement and complementation of animal sparing, FMD vaccine based control strategies for free and endemic regions-

Outcomes:

- WP2 Reduction and refinement of in vivo vaccine quality test by in vitro methods
- WP3 Assessment and improvement of heterologous protection by FMD vaccines
- WP4 Development of vaccines and alternatives (antivirals) with rapid onset of immunity and based on safer production methods

### **2.5.3 ARRIAH**

VAR Institute, Belgium (ALTANDI): FMD vaccines testing  
7-th Framework program EU-Russia (PLAPROVA)

### **2.5.4 PIADC-FADDL**

Mongolia, Pakistan and Ireland: Evaluation of LFD Svanova Universal  
IAH Pirbright: FMD Universal primers for P1 sequence : Completed and publication is in preparation

Nigeria, Mongolia and Egypt Vet labs: Development and validation of 3D ELISA –  
Nigeria, Mongolia and Egypt

Mongolia: Twinning with Mongolia ‘state central veterinary laboratory’

### **2.5.6 ARC-OVI**

**Sampling of buffalo in SADC**

The FMD reference centres at ARC-OVI and BVI, in collaboration with the SADC TADs project undertook to sample buffalo herds in Zambia, Malawi, Mozambique and Tanzania. The SADC TADs project intends sampling buffalo in different national parks within these countries over a period of 3 years to determine the current status of FMD virus strains circulating in the buffalo herds. During the period August- September 2010 buffalo as well as cattle at the park interface was sampled as follows:

Country	National Park	Number of buffalo probangs and sera	Number of Cattle probangs	Number of cattle sera
Zambia	Kafue National Park	25	25	50
	Lochnivar National Park	25	25	50
Malawi	Lengwe National Park	25	25	50
Mozambique	Marromeu National Park	25	20	45
Tanzania	Katavi National Park	30	30	60

The samples from Zambia were driven to BVI and thereafter distributed to ARC-OVI and IAH, Pirbright for analysis. The samples from Malawi, Mozambique and Tanzania were shipped to ARC-OVI. Samples were processed and aliquots shipped to BVI and IAH, Pirbright for testing.

#### **2.5.7 PANAFTOSA**

MAPA/Brazil: Development of an ELISA kit for FMD vaccine control

#### **2.5.8 NVRI Nigeria**

Agricultural Research Council of Nigeria: Counterpart Funding

European Commission for The Control of FMD (EuFMD): Funding, Training and Joint Research

Institute for Animal Health, Pirbright, UK: Classical and Molecular Typing of FMD Virus

### 3. Summary

The overall number of samples submitted to FMD reference laboratories in 2010 remained at similar levels to 2009. There was a decrease in submissions from a number of countries in the Middle East compared with 2009 but with Turkey and Iran reporting increased FMD activity. In total 38 different countries submitted 2,338 samples in 2010 to the network laboratories with 53% being sent to WRLFMD and 27% sent to ARC-OVI. Seven hundred and fifty VP1 sequences were reported with 86% of those coming from WRLFMD.

Notably, both Japan and Republic of Korea reported FMD outbreaks in 2010 thus losing their status as countries listed by OIE as FMD-free without vaccination. These critical events served to highlight the continual risk faced by free countries of the introduction of this economically devastating disease. Millions of animals were slaughtered and the necessary control and eradication processes cost millions, if not billions, of Euros.

The majority of FMD viruses were isolated from samples submitted from Africa and Asia which remain the major reservoirs for the disease. In South America, FMDV circulation has mainly been detected in Ecuador and Venezuela where the circulating serotype O virus has become sufficiently different to vaccine strains that it has been proposed a new vaccine should be developed.

The predominant serotype detected and reported throughout 2010 year was type O (>80%) with a decrease in detection of serotype A and reports of Serotype Asia 1 activity coming only from within India. There were no reports of serotypes C or SAT 3 during the year and it is of great interest to note that serotype C has not been reported since 2004.

With regards to vaccine matching, towards the end of the year, laboratory derived evidence began to accumulate that the O Manisa vaccine was not providing the broad protection coverage previously exhibited when used against a number of field isolates of the toptotype ME-SA, lineage PanAsia-2 from pool 3. Such observations had been reported from the field previously but until recently little laboratory evidence had been available to lend credence to these reports. Since the O Manisa based vaccines are such an important component of the global FMD vaccination toolkit, it is vital that this situation is closely monitored and that samples from this region and others are regularly submitted to the reference laboratories for matching.

The International harmonisation of laboratory tests has progressed a great deal during the time of the network. In 2010 results show that correlation of specified diagnostic assay results between a number of laboratories has improved as a direct result of the proficiency testing activities initiated by network members. Work is in progress to further harmonise methodologies and materials used by network laboratories in order to produce comparable results to be utilized by any member of the network regardless of the laboratory of origin. This will be essential for provision of the necessary information and for management of the inevitable increased workload for virus characterization, and most importantly, vaccine matching in the coming years as the joint OIE/FAO initiative to progressively control FMD increases momentum.

#### **4. Final comments**

It is clear that the activities of the OIE/FAO FMD reference laboratory network provide essential information which serves to inform and influence national, regional and global FMD control policy. Importantly, there is now, and will be into the future, increasing pressure on those network laboratories to maintain and increase their outputs and required quality of service. It is vital that the fundamental activities of these laboratories are recognized and supported by funding bodies to enable continual improvement of this service and provide the necessary recognized expertise in training and building of additional regional laboratory capability and capacity that will be required if the joint FMD control initiative is to succeed in the coming years.

*Jef Hammond March 2011.*