

Summary report from the 10<sup>th</sup> OIE/FAO FMD Laboratory Network Meeting  
Brussels, Belgium: 24<sup>th</sup> – 26<sup>th</sup> November 2015



**Presentations by delegates:**

- An introductory welcome and overview of CODA-CERVA was provided by Dr Thierry van den Berg (Director of Viral Diseases) on the theme *“There is nothing permanent except change” Heraclitus*
- Global situation for FMD (Data from WRLFMD, presented by Dr Don King):  
This presentation reviewed the latest global information for FMD, particularly focussing on the spread of the O/ME-SA/Ind-2001 lineage in North Africa and the Middle East, and the detection by WRLFMD of a new serotype A lineage from the Indian sub-continent in Saudi Arabia. Recent serotype SAT 2 outbreaks in Mauritania and the Gulf States (Oman) were also described which further demonstrate the dynamic situation regarding the current global patterns of FMD distribution. The circulation of these new viral lineages raises obvious questions about suitable vaccines that might be deployed for control, and reinforces the importance of a global FMD Network to share laboratory data that monitor FMD outbreaks in different endemic settings.

**Summary of regional and country updates**

- Southeast Asia (from RRLSEA Pakchong presented by Dr Pranee Rodtian):  
During 2015, a total of 522 samples have been received by RRLSEA from Thailand and other countries in Southeast Asia. Results from these samples indicate that the prevailing FMDV lineages that are circulating in the region include O/SEA/Mya-98, O/ME-SA/PanAsia and A/ASIA/Sea-97. Initial ag-ELISA data for two samples collected in Cambodia suggests that serotype Asia-1 may also be circulating for the first time in the country since 2000 [this serotype has not been detected by Pakchong in SEA since 2008]; however, sequence data is required to confirm this observation. Vaccine matching data for locally produced vaccines was presented showing a good match for serotype O field strains with O/Udonthani 189/87, and most serotype A viruses with A/Lopburi/2012. An overview of the results from an annual PTS organized by RRLSEA-Pakchong was also presented with participants from 9 countries in the region. RRLSEA has also provided practical training to a Myanamese and two Australian scientists.
- China and East Asia (from LVRI, Lanzhou presented by Dr Jijun He)  
The results of samples collected recently in China were presented. Serotype A (A/ASIA/Sea-97) has been detected in clinical specimens collected from 3 outbreaks in the country (Anhui and Hubei (x2) Provinces), in contrast to previous years where both O and A have been found. These serotype A outbreaks have involved pigs which may represent a change in the FMD epidemiology. The source of FMD in China has been attributed to uncontrolled (illegal) animal movements from neighbouring countries. Wider FMD surveillance in the country has tested 3728 oropharyngeal fluid and tissue specimens by RT-PCR and detected serotype A in 11 samples. This work is supported by FMDV serology where >10k sera have been screened using LPB and 3ABC ELISAs to

detect SP and NSP antibodies, respectively. Dr He summarized vaccine-matching data for vaccine strains produced in China, in addition to the results of local PTS exercises and international exchanges that have been undertaken by LVRI staff during 2015.

- Asia (from FGBI-ARRIAH presented by Dr Don King on behalf of Dr Alexey Mischenko):  
Colleagues from the FMD Reference Laboratory in Vladimir, Russia were unable to attend the meeting, but provided results for 76 clinical samples that have been tested during 2015. One sample collected from Zabajkalskiy, in eastern Russia was positive for serotype A (A/ASIA/Sea-97), while 6/12 specimens received from three separate areas in Mongolia contained serotype O (O/ME-SA/PanAsia [two separate sub-lineages] and O/SEA/Mya-98). Sequence data for a further FMDV-positive sample received from central Asia (country not defined) was characterised as belonging to the HER-1 sub-lineage of A/ASIA/Iran-05, closely related to sequences previously obtained from Kyrgyzstan in 2014. Vaccine matching data was presented for FMDV vaccines (serotypes O and A) manufactured in Russia. FGBI-ARRIAH has coordinated a PTS exercise for 6 countries (Armenia, Belarus, Moldova, Kazakhstan, Kyrgyzstan and Tajikistan) and has provided practical training to Kazakhstan.
- Asia (from CSIRO, Australia presented by Dr Wilna Vosloo):  
Dr Vosloo provided an overview of recent studies that have evaluated the performance of vaccines included in most vaccine banks to protect against FMD viruses (serotypes O, A and Asia-1) that are circulating in Pool 1 (Southeast Asia). In order to better understand the changing antigenic profiles of field isolates of serotype A collected at RRLSEA, r-values have been determined and analysis of B-cell epitopes has been undertaken to highlight amino acid substitutions in VP1 that are proposed to impact upon viral phenotype driven by vaccine immunity. This presentation also reviewed the findings from a series of in-vivo challenge experiments in cattle and sheep using high-potency A-May-97 and A22-Iraq vaccines. Potency trials undertaken with monovalent O-Manisa vaccine and challenge with O/SEA/Mya-98 (SKR/2010) was also presented indicating that the vaccine will protect at 21 dpv but is not likely to be so effective at earlier times after vaccination. Results for a study in sheep using Asia-1 Shamir vaccine and challenge (via intranasal-pharyngeal instillation) with a Sindh-08 field virus demonstrated protection at 7 and 21 dpv.
- South Asia (from ICAR PD-FMD presented by Dr Don King on behalf of Dr Pattnaik):  
Colleagues from PD-FMD were unable to attend the meeting but submitted a presentation that summarised test results for India, where serotype O (O/ME-SA/Ind-2001) has dominated the sample submissions in 2014-15. Epidemiological and serosurveillance data was also included in the presentation highlighting where FMD is most frequently occurring in India.
- Turkey (from Şap Institute presented by Dr Fuat Özyörük):  
During 2015, the FMD Institute in Ankara, Turkey has tested 205 samples collected across the country representing serotypes O, A and Asia-1. Undoubtedly the most significant epidemiological event during 2015 has surrounded the detection of a new serotype A lineage that has emerged from the Indian sub-continent. A new phylogenetic tree with 4 new Turkish isolates (from Van [x3] and Bitlis) was presented showing their relationship to another recent FMDV isolate from Saudi Arabia. Data was also presented to indicate that in-house and IZSLER ag-ELISAs are able to detect viruses from this lineage. For serotype O, sequence data tentatively characterises isolates collected during 2015 within the FAR-09 sub-lineage of the O/ME-SA/PanAsia-2 strain, while Asia-1 isolates collected during 2015 represent two discrete genetic clusters within the Sindh-08 lineage. Unfortunately, the regional WELNET is currently proving not to be an effective forum to share data between countries.
- North Africa (from IZSLER presented by Dr Emi Brocchi):  
IZSLER has established collaboration and cooperation initiatives with countries in North Africa including Algeria, Tunisia, Libya and Egypt. During 2015, samples received from Egypt (n=10) were characterised as serotype O (O/EA-3) and A (A/ASIA/Iran-05<sup>BAR-08</sup>). Interestingly for three of these

samples, both serotype O and A could be isolated depending upon the cell line used (LFBK selected O, while IBRS-2 and BHK-21 selected A). In collaboration with ANSES, full genome data has been generated for 12 FMDVs (O/ME-SA/Ind-2001) collected from Tunisia (see below). Further serological work has been undertaken to support the use of vaccines to protect against O/ME-SA/Ind-2001 including vaccine potency tests, as well as field vaccination studies (in Tunisia) that have attempted to predict the efficacy of O-BFS vaccine. Results indicate that O-BFS is able to elicit a strong and rapid booster/recall response in cattle and sheep previously vaccinated with O-Maghreb/O-Manisa. In naïve animals, single vaccination with O-BFS induced seroconversion, but a proportion of sheep (30%) and cattle (15%) did not achieve antibody levels indicative of heterologous protection. IZSLER has coordinated a PT exercise for 3 Balkan countries (Bulgaria, Macedonia and Serbia) to evaluate serological ELISAs and virological assays.

- North Africa (from ANSES presented by Dr Don King on behalf of Dr Labib Bakkali Kassimi): Unfortunately, Dr Kassimi was unable to attend the meeting and sent his apologies on behalf of ANSES which has been recently recognised as an OIE Reference Laboratory for FMD. During 2015, samples (n=54) have been received from Tunisia and tested by ANSES. Twenty-one FMD virus isolates have been generated. Training on FMD diagnostic methods has been provided to Morocco and a delegate from ANSES has participated in a workshop in Tunisia.
- East Africa (from Embakasi presented by Dr Abraham Sangula): Serotypes O, A, SAT 1 and SAT 2 have been detected in recent samples (n=101) collected from Kenya, while recent Ugandan samples (n=34) comprised serotype O and SAT 1 [sequence data is pending for all these samples]. Locally produced vaccines (from KEVEVAPI) include K77/78 (for O/EA1), K5/80 (for A/AFRICA/GI), T155/71 (for SAT 1/NWZ) and K52/84 (for SAT 2/IV). A brief overview of collaborative projects was provided.
- Ethiopia (from NAHDIC presented by Dr Daniel Gizaw): During 2015, 83 samples have been collected from outbreaks in Ethiopia and characterised at NAHDIC by Ag-ELISA (supported by additional testing and sequencing at OIE/FAO Reference Laboratories). FMDV serotypes O (O/EA-3) and SAT 2 (SAT 2/VII/Alx-12) have been detected (serotype A has not been detected during 2015). Wider surveillance indicates that seroprevalence in small ruminants is 9.2%, while 11.5% of cattle samples (n=6469) were FMDV antibody positive using the 3ABC NSP ELISA. NAHDIC have just started a new 3-year OIE Twinning Project with the Pirbright Institute that aims to improve diagnostic capability.
- Nigeria (from NVRI presented by Dr Wungak Yiltawe): A brief overview of work to build a new BSL-3 laboratory at NVRI was presented. This new facility will house the FMD diagnostic and research laboratory as well as work with other highly infectious diseases. In addition to the OIE twinning project with CODA-CERVA (described below), NVRI is undertaking a project to develop improved FMDV vaccines that are tailored for use in Nigeria funded by the West African Agricultural Productivity Project (WAAPP). During 2015, 22 samples from domesticated livestock have been tested and typed using Ag-ELISA [sequence analysis is pending]. Serological analysis of wildlife (eland, wildebeest and waterbuck) was been performed: preliminary analysis reveals the presence of antibodies to serotypes O, A, SAT 1 and SAT2.
- Nigeria (OIE Twinning Project): A study to investigate the epidemiology of FMD in Nigeria has been carried out by investigating the ~30% of NSP-positive cases in non-vaccinated cattle and sheep (in the Kachia grazing reserve). These positive samples were tested by SPCE, which showed that the dominant serotype was O followed by A. Many sera generated antibody positive results for multiple serotypes and rRT-PCR analysis of probang samples was able to detect FMDV, although no viruses were isolated.
- Senegal (from LISRA-LNERV presented by Dr Mariame Diop): There is currently no virus isolation facility or capacity to carry out sequencing at the FMD Reference Laboratory in Senegal and only a small number (n=2, serotype A) of sample

submissions have been received during 2015. Retrospective analysis indicates that serotypes O and A are responsible for 37% and 51% of FMD outbreaks, respectively [~12% are currently untyped]. A collaborative project with ANSES is on-going which aims to deploy and evaluate molecular detection systems in Senegal.

- Sub-Saharan Africa (from BVI-SSARRL presented by Dr George Matlho):  
This presentation summarised the results for 159 samples sent to BVI during 2015 from 7 countries (Botswana, Mozambique, Namibia, Niger, Uganda, Zambia and Zimbabwe). Recent FMD outbreaks in north-west Botswana have been due to SAT 1, which is a change from previous years where SAT 2 has dominated. Elsewhere, both SAT 1 and SAT 2 have been implicated in recent FMD outbreaks in Namibia. In October 2015, new outbreaks in western Zambia were due to serotype SAT3 which poses new risks to the region. Vaccine matching results were presented for BVI vaccines, which included data for a vaccine strain (SAT2035), which is used in combination with SAT251.
- Sub-Saharan Africa (from ARC-OVI presented by Dr Francois Maree):  
During 2015, samples (n=13) have been received from Mozambique (SAT 2), Namibia (SAT 2) and Swaziland (NVD). OVI also undertakes serological testing for export, suspected outbreaks, trials and wider surveillance in the region, and during 2015 has tested 2126 sera from Mozambique (n=47), Namibia (n=1857) and Swaziland (n=222), in addition to 13,545 sera tested from South Africa. An overview of work to improve the antigenic characterisation and vaccine matching of FMD viruses (serotypes SAT 1, SAT 2 and SAT 3) was presented and has highlighted gaps in the coverage of existing vaccines.
- South America (from PANAFTOSA presented by Dr Rossana Allende):  
Since no FMD has been reported in the continent, no samples have been received during 2015 for outbreak investigation. Ecuador has been recently recognised by the OIE as a country FMD-free with vaccination and the FMD control program in Venezuela has now been recognized by the OIE. A PT exercise has been coordinated during 2015 for FMDV and VSV typing by RT-PCR involving 13 participants.
- South America (from SENASA presented by Dr Andrea Pedemonte):  
Further to the presentation from PANAFTOSA, Dr Pedemonte described the situation in Argentina where five zones have been established: two that are FMD-free with vaccination, and three that are FMD-free without vaccination (including Valles de Calingasta and Patagonia Norte A which were granted the new FMD-free status in 2015). An overview of on-going research projects was also provided which include the development of a new generation of non-infectious capsid-based vaccines.
- USA (From NVSL-VS-STAS-APHIS FADDL presented by Dr Consuelo Carillo):  
Dr Carillo provided an update on the situation in the US regarding the recent emergence of Seneca Virus A/Seneca Valley Virus as a virus that can cause vesicular-like clinical signs in pigs. A similar pattern in pigs has also been seen in South America and Canada (reported by Dr Allende and Dr Nfon). Cases of vesicular disease due to VSV have also been reported in the US during 2015. A range of training courses for FMD have been offered including two International Transboundary Animal Disease (ITAD) courses to 56 participants.

### **Acknowledgements:**

The OIE and FAO were thanked for providing financial support for delegates to travel to the meeting, and the European Commission were acknowledged for providing support (via EuFMD) to WRLFMD. This meeting was kindly hosted by CODA-CERVA, Brussels, Belgium and the hospitality of Drs Kris De Clercq, David Lefebvre and colleagues in the face of the prevailing “difficulties in the city” was very much appreciated by the delegates. The OIE/FAO FMD Laboratory Network warmly thanked Dr Thomas Struckmeyer and Thermo Fisher Scientific for kindly hosting the evening meal. Thanks also go to Sarah Belgrave who provided assistance to organize this meeting at WRLFMD.

## Reports from the Network Working Groups

In response to discussions at last year's OIE/FAO FMD Laboratory Network meeting (Brescia), two working groups have been established to focus the expertise of the Network members specific issues relating to the control of FMD and laboratory analysis of field strains of FMDV. A summary of the progress made by these working groups was provided by the respective coordinating secretaries:

- Virus Nomenclature (presented by Dr Kasia Bankowska from WRLFMD)

The aim of this working group is to address isolate, lineage and topotype nomenclature and to provide recommendations about coherent naming of FMD viruses. Members of this working group are: Francois Maree (OVI), Fuat Özyörük (Şap), Wilna Vosloo (CSIRO), Nick Knowles (WRLFMD), Jitendra Biswal (PD-FMD), Jijun He (LVRI) and Alexei Scherbakov (ARRIAH – when he is able to attend). Four teleconferences have been arranged since July 2015, although it is recognised that there have been some technical and logistical difficulties to arrange the meetings so that all members are able to attend. The terms of reference and priorities of the group are to [1] To propose common nomenclature to be used to describe samples and sequences, [2] To define topotype nomenclature for all serotypes (including nucleotide sequence cut-offs for different serotypes), and [3] To explore formal approaches (such as establishing a standing Network sub-group committee) to oversee the naming of new FMD viral lineages. Progress on work to redefine a common topotype nomenclature for SAT viruses was presented, which has considered the degree of nucleotide identity between representative viruses. Two peer-reviewed publications are planned to outline this new proposed SAT nomenclature, as well as to publicise the role of this working group to oversee the naming of new FMD viral lineages.
- Vaccine recommendations for endemic countries (presented by Dr Anna Ludi from WRLFMD)

This working group was also established in July 2015 and has 9 members: Alexey Mischenko (ARRIAH), David Paton (WRLFMD), Emiliana Brocchi (IZSLER), Gaurav Sharma (PD-FMD), George Matlho (BVI), Jijun He/Yanmin Li (LVRI), Kees Van Mannen (EuFMD), Kris De Clercq (CODA-CERVA) and Rossana Allende (PANAFTOSA). The group has held three meetings and will also explore alternative communication methods to improve participation from all the delegates. The goal of the working group is to prepare harmonized guidance for approaches that can be used to select FMDV vaccines (in endemic and FMD-free with vaccination settings). Broadly, this work can be broken-down into 4 activities: [1] Developing approaches and generation of new reagents to explore whether or not alternative serological approaches are more appropriate for vaccine matching recommendations in endemic settings where multivalent vaccines provided by local or international suppliers are employed. If so, the group should consider developing standardized laboratory methods and reagents for this purpose that can be rolled-out to members within the Network, [2] Inter-laboratory robustness of serological data: review data from previous PT exercise with a view to publishing this data, [3] Calibration of different test approaches: plan a further practical study that can be used to harmonise in-vitro vaccine matching methods (VNT and LPBE) used in different laboratories within the Network, [4] Validation of methods: ensure this advice is supported by appropriate data from field and epidemiological studies. During 2015, new BVS (against Asia1 Shamir, A<sub>22</sub> IRQ, A MAY/97, O Manisa, O 3039, SAT 2 Eritrea) has been prepared by WRLFMD for use by FMD Reference Laboratories. Further discussion will ensure coordination of reagent product with other Network partners (FADDL and IZSLER).

### **Special topic for discussion: Serotype C**

A special session of the Network meeting considered the current status of FMDV serotype C. As reported previously by Network partners, this serotype has not been detected anywhere in the world since the last clinical samples representing this serotype were collected in 2004 (in Kenya and Brazil). Three invited presentations provided an overview the historical distribution of FMDV serotype C and recent serological data:

- Dr Aldo Dekker from CVI: *FMD serology for freedom of infection: absence of proof is not proof of absence*
- Dr Abraham Sangula from Embakasi (with Dr Graham Belsham - DTU): *FMDV serotype C in Kenya (1967-2004)*
- Dr Rossana Allende from PANAFTOSA: *FMDV serotype C history in South America.*

Discussion among the delegates considered the difficulties of interpreting serotype-specific serological data, and other epidemiological approaches that might be adopted to substantiate the “extinction” of this serotype.

### **Draft recommendations arising from discussion between the Network partners:**

#### **Research priorities to provide evidence that serotype C is no longer circulating**

- Follow up investigation of serotype C serologically positive samples:
  - Investigate whether heterologous cross-reactivity (for other serotypes) can account for the signal detected in these positive samples
  - Consider whether or not the positive/negative cut-off adopted in serological tests (often based on “negative” sera from FMD-free settings) is appropriate for use to screen sera (collected in Africa)
  - Evaluate whether or not there is significant spatial or temporal clustering of serotype C positive samples which would indicate active circulation of FMDV
  - Where possible, undertake resampling and testing of animals (and other individuals within the epidemiological units) where serotype C-specific responses have been detected
- Develop serotype C-specific molecular tests for use to pro-actively screen samples collected from the field (particularly those where virus recovery might be challenging)

#### **On the use of serotype C in vaccines**

- In-vitro “live” virus work with serotype C should only be performed in facilities that conform to (EU or equivalent) minimum standards (BSL3+)
- In-vivo challenge (and potency tests) studies using serotype C should no longer continue
- Consideration be given to halting the production of serotype C vaccines
- Risk-based approaches should consider the continued use of serotype C in vaccines (in South America) and inclusion in vaccine antigen banks (FMD-free countries)

## **Formal meeting of OIE and FAO Reference Laboratories to discuss organisation and management of the Network:**

Apologies from PD-FMD, ARRIAH and ANSES

### **10 year review of Network History and Membership:** (presentation from Don King, WRLFMD)

The purpose of the OIE/FAO FMD Reference Laboratory Network is to make available accurate and timely data to support global surveillance and control of FMD.

### **Network goals**

- Understanding global FMD virus distribution and patterns in order to identify threats and make vaccine recommendations
- Improving the quality of laboratory tests from international and national reference laboratories
- Building up local capability in support of regional control programmes

This Network arose from a meeting of the OIE Ad Hoc group of Antigen and Vaccine Banks (in Paris 2004) where it was decided to generate two forums to coordinate international activities: a vaccine bank network (now the IVSRN), and this FMD Reference Laboratory Network. Currently there are 14 core members, with two new members joining the network in 2015: ANSES (OIE) and Winnipeg (FAO). Attendance at the meeting by delegates from affiliate FMD laboratories is an essential component of the Network and provides an approach to ensure that the most relevant data is collected regarding FMD outbreaks and surveillance. A history of meetings held by the Network is outlined below:

- 2004: Paris
- 2005: Pirbright
- 2006: 1<sup>st</sup> Meeting in Florianopolis, Brazil
- 2007: 2<sup>nd</sup> Meeting in Gaborone, Botswana
- 2008: 3<sup>rd</sup> Meeting in Lanzhou, China
- 2009: 4<sup>th</sup> Meeting in Delhi, India
- 2010: 5<sup>th</sup> Meeting in Pirbright, UK
- 2011: 6<sup>th</sup> Meeting in Pirbright, UK
- 2012: 7<sup>th</sup> Meeting in Jerez, Spain
- 2013: 8<sup>th</sup> Meeting Bangkok, Thailand
- 2014: 9<sup>th</sup> Meeting Brescia, Italy
- 2015: 10<sup>th</sup> Meeting in Brussels, Belgium

### **Memorandum of Understanding (MoU)**

It was agreed that this document is central to the philosophy of the Network: however, in the past only a few laboratories have formally signed this agreement. In some respects, the current lack of a formal agreement limits the open exchange of unpublished data between partners. The previously drafted document was reviewed by all of the partners that attended the meeting and there was broad agreement that the text appeared to be still relevant and fit-for-purpose.

**ACTION (by April 2016): All partners agreed to send a draft version of this document to their Institutional Administrators, with the view to get an opinion of whether are any obstacles that might prevent signing of this document. If any changes are required these will be provide back to the partners. The goal is to prepare a final draft by July 2016, for circulation to all partners by October 2016. This document has been circulated by email – please contact Don King ([donald.king@pirbright.ac.uk](mailto:donald.king@pirbright.ac.uk)) if you need a copy.**

## **Draft Work Plan for 2016**

### 1: Continued activities of the OIE/FAO FMD Laboratory Network:

- With assistance from OIE and FAO, the network will obtain and analyse samples from under-sampled endemic pools (particularly West and Central Africa and central Asia)
- Network partners will provide a central resource of expertise and advice regarding FMD control, vaccines and diagnostics
- The network will continue to explore (and support) tools for real-time sharing of Laboratory data generated within the Network
  - Continue to engage with OIE and FAO regarding the design and implementation of tools to exchange sequence data
  - Local tools being developed at WRLFMD (VibaSys) will be circulated to partners when completed
- Core OIE and FAO Network partners to consider the organization of the network and opportunities to make it a more inclusive network to maximize data collected from the field

### 2: Continue the work of the Network Working Groups

- Virus nomenclature
- FMD vaccines and recommendations for vaccine matching

Anyone interested in joining these groups should contact Dr Anna Ludi or Dr Kasia Bankowska.

### 3: Communication:

- WRLFMD to coordinate the preparation of an Annual Report
  - Agreed timelines for preparation of 2015 report: - Network partners to provide feedback on pools they work closely with. Network members to provide an update to WRLFMD for report (include final data for November and December 2015)
    - Final summaries: January 2016
    - Draft Report: February 2016
    - Report Published: March 2016
- WRLFMD to organise an Annual meeting (location to be agreed after discussion with OIE and FAO) – will be at the end of the year. Agreed that (where possible) this should be hosted by a member lab of the network
- WRLFMD to host new website outlining Network activities:
  - Feedback and suggestions from partners are welcome
  - Website will contain “Public” and “Private” areas
  - Links to institutional websites and GFRA
- Proposal to enhance real-time exchange of data between partners, possibly in each of the pools – communicate new virus strains in real-time or other information; or quarterly conference call; link with EUFMD update monthly report (calendar to have specific times to write/edit for each lab). However, this will not require another report. [Dr Rossana Allende agreed to look at ways that this might be accomplished]

### 4: Formal agreement

- OIE/FAO Reference laboratories agreed to review the formal MoU that covers work of the Network and data exchange between partners
  - March 2016 – comments on draft and suggested revisions
  - July 2016 – prepare final draft
  - October 2016 – circulate document or signatures

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