The 13th Annual Meeting of the OIE/FAO FMD Reference Laboratories Network
7th – 8th November 2018
Gorse Hill Hotel, Woking, UK – hosted by The Pirbright Institute, UK

Global Headlines (WRLFMD)

Over the last ten years, long-distance trans-pool movement of FMD viral lineages has been a common theme of reports generated by laboratories within the Network. An example is represented by the O/ME-SA/Ind-2001 lineage, where viral genetic data has been shared between the Network to show that this lineage has "escaped" from the South Asian countries (Pool 2) on many occasions to now become an important endemic virus lineage in the Gulf States of the Middle East (Pool 3) and Southeast/East Asia (Pool 1). Based on data presented by PD-FMD and WRLFMD at last year's meeting, this lineage has been divided into two sublineages (O/ME-SA/Ind-2001d and O/ME-SA/Ind-2001e). O/ME-SA/Ind-2001e appears to now dominate the situation in Pool 1 where the O/ME-SA/IND-2001d has not been reported since 2015. Other recent trans-pool movements out of Pool 2 include the spread of A/ASIA/G-VII to countries to the West (most recently causing outbreaks in northern Israel in 2018) and serotype Asia 1 causing outbreaks in Myanmar in 2017.

During 2017/18, attention has been focussed on the emerging situation in North Africa where outbreaks due to serotypes O and A have been reported. During 2018, FMDV causing outbreaks in Algeria has been characterised as belonging to the O/EA-3 topotype, most closely related to viruses circulating in West African countries (>99% with sequences collected by ANSES in Guinea), where there appears to have been upsurge in cases due to FMD. These O/EA-3 sequences collected from West Africa are phylogenetically distinct from the O/EA-3 strains seen in Egypt, Palestine and Israel. A lineage specific real-time RT-PCR developed by WRLFMD via a twinning project with NAHDIC (Ethiopia) is now available. Vaccine matching tests have been reported; but, there is a lack of in vivo evidence to support the use of vaccines for O/EA-3 viruses, since no potency tests have been performed, and filling this gap should now become a priority for the Network. Taken together with the outbreaks that have recently occurred in Algeria and Tunisia due to A/AFRICA/G-IV (reported in 2017), the emergence of these new FMD lineage in Maghreb is a significant change of epidemiological status which may substantiate new trans-Saharan connections between North and West Africa which raise the onward risks to FMD-free countries in Europe.

Elsewhere, in Southern Africa there has been a southwest spread of serotype O/EA-2. In Zambia, this lineage is normally only found close to the border with Tanzania – but has been recent isolated in

central Zambia (Chisamba). In Pakistan, a new serotype O antigenic variant has been observed. This lineage appears to have very poor antigenic match with commercial vaccines from MSD and Boehringer-Ingelheim (new antigenic variant); however, only two isolates have been detected to date in the Punjab. The spread of this lineage needs to be closely monitored.

Pool 1: South East Asia (RRLSEA, Thailand)

During 2018, samples have been received from Thailand (n=111) and Lao PDR (n=24). In Southeast Asia, the predominant serotype is serotype O, with the most common viral lineages being O/ME-SA/PanAsia, O/ME-SA/Ind2001e and O/SEA/Mya-98. Recent sero-surveillance undertaken in Thailand indicates that ~21% of the cattle population are positive for FMDV non-structural protein antibodies. VNT and LPBE vaccine matching against the locally produced O/189/98 vaccine shows that there is a good match for serotype O viruses. However, for serotype A, locally produced A118/87 and A/Sakolnakorn/97 appear not to be matched against all of the FMD virus isolates that have been tested (but the locally produced A/Lopburi/ 2012 vaccine is well matched against these viruses). Serotype Asia 1 has not been found in the region – except for the cases that occurred in Myanmar during 2017. A regional inter-laboratory PT scheme continues to be offered by the RRLSEA; where the latest exercise has involved 16 laboratories (9 outside of Thailand).

Pool 1: East Asia and China (LVRI, China)

Twenty four FMD outbreaks have been reported in China in 2018 affecting 12 different provinces. Samples collected show that the majority of these outbreaks are due to serotype O (with only one serotype A [A/ASIA/ASIA/SEA-97] detected in the year). The epidemiological picture for serotype O is complicated by the co-circulation of four different FMDV lineages in the country (O/CATHAY – 7 outbreaks, O/ME-SA/Ind-2001e – 8 outbreaks, O/SEA/Mya-98 – 3 outbreaks and O/ME-SA/PanAsia – 5 outbreaks). O/CATHAY is dominant in pigs and is poorly matched to vaccines used, while recent outbreaks of O/PanAsia appear to be due to a new introduction of the lineage into China during 2018. New risks are evident via an increase in illegal movements of animals from Southeast Asia. In addition, it is anticipated that recent outbreaks of ASF will have a dramatic impact on pig production that may influence the circulation of FMD in the country.

China has withdrawn the use of serotype Asia 1 from vaccines. During 2018, a cross challenge study in pigs has been performed using type O vaccine and O/Myr98 isolate O/17016 as the challenging virus, where monovalent and bivalent formulations both protected animals.

Pool 1: Korea and East Asia (APQA, Republic of Korea)

Outbreaks of serotype A/ASIA/SEA-97 were reported in two pig farms close to the North Korean border (12km distance between farms). This outbreak affected 1 province and lasted 7 days. The source of the outbreak is unknown. There have been changes to the vaccination protocol used in the country; fattening pigs are now vaccinated twice. Post vaccination is being carried out by SP(O) ELISA and the antibody levels recorded in fattening pigs have increased due to this change. Additional changes now mean that pigs receive multivalent vaccine (O-3039, O_1 Manisa and A_{22}), or O Primorksy and A Zabaikalsky vaccine strains. Across the country, sero-surveilance appears to highlight a decrease in the occurrence of NSP positive farms.

Pools 1 and 3: (FGBI-ARRIAH, Russia)

During 2018, samples have been tested from Russia (O/ME-SA/PanAsia), Mongolia (O/ME-SA/PanAsia, O/ME-SA/Ind-2001) and Pakistan (O/ME-SA/PanAsia-2, A/ASIA/Iran-05, Asia 1/Sindh-08). The FMDV outbreaks in Russia (Zabajkalkiy) occurred near the Mongolian border and were most genetically

related to viruses collected in Mongolia during 2017/18. The results from vaccine-matching studies were presented describing results using representative viruses collected during the year (from Russia, Mongolia and Pakistan).

Pool 2: India (ICAR-DFMD, India) - Presented by Don King on behalf of the laboratory (NB: note change in the formal name of the laboratory).

Results for samples collected in India from suspect FMD cases (n=169) were presented. Serotype O (genetically highly related isolates within the O/ME-SA/Ind-2001e clade) continues to be the most dominant FMD viral strain in the country; however, three viruses collected during 2017 were from the O/ME-SA/Ind-2001d sub-lineage. In addition to serotype O, there were also three cases due to serotype Asia 1, but none for serotype A.

Pool 3: Turkey (SAP Institute, Turkey) -Presented by Don King on behalf of the laboratory.

Samples received from Turkey (n=491) during 2018 are predominantly serotype O (O/ME-SA/PanAsia-2^{QOM-15}). Only a single serotype A FMD virus (A/ASIA/G-VII^{BAN-12}) has been detected this year (in January 2018). No cases due to A/ASIA/Iran-05 were reported, and the last case of due to serotype Asia 1 was in 2015. From a wider perspective, it is interesting to note that the A/ASIA/G-VII lineage appears not to have spread into all countries in the region, nor replaced the A/ASIA/Iran-05 lineage in countries such as Iran. Looking forward, it is possible that the A/ASIA/Iran-05 lineage will re-emerge into Turkey, and vaccine selection needs to consider this in view of the antigenic differences between A/ASIA/Iran-05 and A/ASIA/G-VII.

Pool 4: Kenya and East Africa (Embakasi, Kenya)

The FMD Reference laboratory has recently received samples from Kenya (n=77), South Sudan (n=38) and Tanzania (n=150, as part of an on-going project). FMD virus lineages detected in the Kenyan samples were O/EA-2, A/AFRICA/G-1, SAT 1/I and SAT 2/IV, where O/EA-2 appears to account for 85% of FMD outbreaks. Samples from South Sudan have also been sent to WRLFMD, where O/EA-3 was detected by real-time RT-PCR (see above). Vaccine matching testing has been performed using VNT showing that only 1/4 FMD viruses collected in Kenya during 2017 were matched against O K77/78.

Pool 4: Ethiopia and East Africa (NAHDIC, Ethiopia)

Samples (n=105) from Ethiopia have been received for testing during 2018. Serotypes reported were serotype O (most common, O/EA-3), A (A/AFRICA/G-IV and A/AFRICA/G-I) and SAT 2 (V-II^{ALX-12}). For the first time SAT 2 has been recorded in the far north of the country. The reports of A/AFRICA/G-I represent a new viral introduction into Ethiopia, since this lineage has not recently been detected in the country. NSP sero-surveillance indicates that 67% of cattle (n=2151) were antibody positive while less than 1% of small ruminants (n=1232) were seropositive.

Pool 5: West Africa (Sciensano, Belgium - including presentation on behalf of NVRI, Nigeria)

During 2017, there were FMD outbreaks due to serotype O (EA-3), A (G-VI) and SAT 2 (VII); however, in 2018 only serotype O (in three states) and SAT 2 (in one state) have been detected. During this period, O/WA and SAT 1 X have not been detected which was unexpected. NVRI also received four samples from Sierra Leone, where serum samples were positive for NSP; and the swabs were all negative by rRT-PCR. Importation of animals into Nigeria (particularly from Niger and Chad) appears to be an important risk-factor for FMD in the country. The trans-Saharan highway now links Algeria, Mali, Niger and Nigeria, and traffic on this new road may increase opportunities for the spread of FMD in the region (and beyond).

Pool 5: West Africa (ANSES, France)

Samples (n=53) have been collected (from FMD cases that occurred in 2017 and 2018) from Guinea (O/EA-3), Mauritania (O/EA-3 and A/G-VI) and Tunisia (A/AFRICA/G-IV). In addition, a small number of serum samples from Guinea (n=13 – 30.8% positive) and Mauritania (n=47 – 27.7% positive) have been tested. Sequences shared with WRLFMD indicate that the O/EA-3 topotype is spreading at a rapid pace (as described above in the headline summary). One point raised during the presentation is that recent FMD cases in West Africa may be associated with higher mortality in small ruminants which may be the reason why samples have been collected for these cases. Training carried out by ANSES includes Madagascar, Senegal, Mauritania, Guinea, Tunisia and Cameroon.

North Africa: (IZSLER, Italy)

No clinical samples have been received during 2018 for laboratory confirmation or characterisation. However, sero-surveillance studies in cattle and sheep have been performed for the Maghreb (Algeria, Morocco and Tunisia) in support of the vaccination campaigns that are underway. To date, work has been completed for sera received from Morocco testing SP responses (by VNT and ELISA) to vaccination in naïve and previously vaccinated animals. Training has also been provided to Botswana and Libya and there is an increasing demand for ELISA kits where 2002 kits have been sent to 48 countries during 2018.

Pools 4-6: Sub Saharan Africa (OVI, South Africa)

Virology tests have detected the presence of SAT 2 (topotype I) and SAT 3 in samples from South Africa and Mozambique, respectively. The SAT 2 FMDV was detected in the vaccination zone (Gyiani, Halahala, Khakala and Matiyane) and is considered to have been circulating for some time (most likely a number of years). Most outbreaks can be traced back to contact with buffalo as a source (based on sequence analyses), but more recently the potential role of small ruminants (such as goats) is receiving more attention. OVI-ARC has developed 5 new vaccine strains and are preparing for commercial release within 2 years. The presentation also summarised work that has been undertaken in Uganda (2014-18) where the FMD viruses detected were: A (AFRICA/G-I), SAT 1 (I, IV topotypes), SAT 2 (VII, IV and X topotypes) and O (EA-1 and EA-2). There is also evidence for SAT 3 circulation but only using serological approaches and work is currently underway to assess whether this might be due to the cross-reactivity of the ELISAs.

Pools: 4-6: Sub Saharan Africa (BVI, Botswana)

Five countries have submitted samples (n=55): Mozambique (no virus detection), Zambia (SAT 3 and O), Botswana (SAT 2), Malawi (SAT 2), Zimbabwe (SAT 2). Across the region the circulating FMDV lineages are: SAT 3/I, O/EA-2, SAT 2/III and SAT 2/II. BVI are planning to initiate training on collecting samples to decrease the number of negative samples that are submitted. A small number of sera for surveillance purposes were also received from Botswana, Malawi, Mozambique and Zambia. In addition, Botswana and Zimbabwe are carrying out post-vaccination studies with LPBE, which highlighted that vaccine coverage was low.

Pool 7: South America (PANAFTOSA, Brazil)

PANAFTOSA has received outbreak samples from Colombia for field outbreaks (n=5) that occurred between August and October 2018. Sequence analyses place these viruses within the O/EURO-SA/cluster 6 (described in 2011) and are 90% identical to outbreaks in Andean Region of South America. The affected species appear to be cattle and swine; evidence indicates that partially immunized cattle (mainly 1-2 years old) have been infected and moved into the affected region via

illegal trade from Venezuela. EPP for the Colombia strain is 79.03% (with O1 Campos Br/58); however, further vaccine matching is still being undertaken. The vaccine strain C_3 Indaial will be withdrawn from the vaccines in Brazil, Paraguay and Bolivia in 2019. Brazil is currently in a transitional period with all vaccination stopping by 2023. Suriname is free without vaccination from 2018. Samples were also received from Trinidad and Tobago which were negative for FMDV, but positive for Orfvirus (contagious ecthyma).

Pool 7: South America (SENASA, Argentina)

Since 2006, there have been no FMD outbreaks in Argentina and no clinical samples have been received for FMDV testing. Samples have been received for differential diagnosis including bovine herpesvirus 1, contagious ecthyma, and BVD. During 2018, 14,000 sera have been received for epidemiological surveillance purposes to demonstrate absence of FMD virus circulation. Training in laboratory diagnostic methods has been provided to Paraguay.

Update from Winnipeg, Canada

Twenty-one samples from Canada were received; of which fifteen were positive for Seneca Valley Virus (SVV). Vesicular cases due to SVV now appear to be decreasing in Canada. Samples (23 clinical samples and 603 sera) were also received from Ghana. Testing is still ongoing; however, initial results from sero-surveillance support the circulation of serotypes A, O and SAT 2 (testing done by VNT).

Update from FADDL, USA

NBAF in Kansas is currently being constructed to replace the facility at Plum Island. The USA is still reporting a large number of SVV outbreaks, which clinical signs that are very similar to FMD; however, the number of outbreaks appears to be stabilising. Since 2014, SVV has increased from 2% to 98% of all vesicular diagnostic cases. National laboratories are carrying out rRT-PCR for SVV; therefore only inconclusive or suspect FMD cases are now being sent to Plum Island. Complete FMDV genome sequencing have been undertaken for samples from Colombia (2017), Uganda and from the FADDL repository. Additional samples are tested from FMD endemic countries as part of on-going research project undertaken by ARS, providing data that would be helpful to include in reports from the global laboratory network (point discussed at recent GFRA meeting in Argentina).

Action O1-18 – FADDL to investigate whether a summary of ARS data can be included in reports

Update from CSIRO, Australia

One of the roles of CSIRO is to ensure that vaccine in the Australian bank continue to be efficacious. Recent vaccine matching carried (by LPBE) suggest that O-3039 and A/MAY/97 may protect against the majority of circulating FMDV field isolates in Southeast Asia. No vaccine efficacy studies have been performed during 2018, but future studies may consider the extent to which the new A/G-VII vaccines provide protection for A/ASIA/Iran-05 viruses, and whether the A/G-VII can replace A/Iran-05 in vaccine banks. Research at CSIRO is focussing on the role of goats in the epidemiology of FMD, testing new vaccine adjuvants, intradermal vaccination in pigs (using IDAL from MSD), and inactivation of FMDV using lysis buffers (RNAShield and RNAlater). A collaborative project with (Alejandra Capozzo, INTA, Argentina) has evaluated new approaches that might be used for vaccine matching.

8th November 2018

Review FMD status in endemic pools and significant epidemiological events (and gaps) and risks during 2018

Updates (with annotated maps) were provided from Pool 1, Pool 3, Pool 4, Pool 5 and Pool 6. Perhaps the most significant risk identified is the possibility that serotype SAT 2 (topotype VII) will enter North Africa in a pattern similar to A/AFRICA/G-IV and O/EA-3.

Discussion – How do we better link the phylogenetic trees to the field reports to show the viral pools are connected? In order to help visualise this links, the WRLFMD has a draft image that could be used (and improved).

Action O2-18 – Don King to send around a draft figure for comment

Review of vaccine-matching work currently undertaken by Network partners – Anna Ludi

A summary of the vaccine matching that is currently being undertaken by the Network partners was described including the limitations of the currently used tests. Vaccine matching results generated by FMD reference laboratories typically only includes vaccines from one or two commercial vaccine suppliers (for instance WRLFMD data only evaluates MSD and Boehringer-Ingelheim vaccines). Clearly there is more work for the Network to do to (i) harmonise existing vaccine matching methods, (ii) expand the range of FMDV vaccine sera available that can be provided to FMD Reference Laboratories and (iii) continue to evaluate new methods to assess vaccine homologous potency and cross-protection.

Summary of topics that were discussed:

- Is there a better way to share data throughout the year? New WRLFMD website now includes vaccine matching reports.
- In addition to r_1-values, should reports also include heterologous titres?
- Reference sera: partners were reminded of previous discussions where we agreed to standardise the use of monovalent $6PD_{50}$ post vaccine sera collected from cattle at 21 or 28dpv.
- Gaps in serum panels and methods exist for pigs which are increasingly important in Asian countries (where there is sometimes uncertainty about the performance of an FMDV vaccine).

FMD vaccine matching: inter laboratory study for improved understanding of r_1 values - David Lefebvre

This presentations summarised a previous inter-laboratory exercise undertaken by the Network to assess the repeatability of r_1 values within and between laboratories as well as the comparability of r_1 values generated using VNT and LPBE methods. For all laboratories, individual and mean r_1-values by VNT were below 0.3 and this matched with experimental challenge data and field data. The LPBE gave a much more complicated picture and it appears that laboratories could/should adjust their cut-off to fit with the data.

A new model for independent FMDV vaccine QA/QC via an OIE twinning project with AU-PANVAC – David Paton

During 2019, a new project will start between WRLFMD and AU-PANVAC (in Ethiopia). This is a new opportunity to look at the way that QA/QC is undertaken for FMDV vaccines. The project will address

two connected priorities for the use of FMDV vaccines in Africa: vaccine QA/QC (homologous protection) and vaccine performance in the field (heterologous responses). Considering the recommendations of the OIE, the project will produce new reference materials including panels of representative field viruses (involving work of the Network), BVS and recombinant antigens for use in ELISA formats (including new avidity and IgG1/IgG2 isotype ELISAs). The project will focus on approaches to evaluate homologous potency and heterologous match.

From our Industry partners: Industry perspective on vaccine QA/QC: who does what? And how can the Reference Laboratory Network contribute? – presented by Alasdair King

How does a country decide on whether a vaccine is "fit for purpose"? A lot of attention is placed on vaccine matching data, but vaccine performance is also dependent upon vaccine potency, vaccine quality, the adjuvant and vaccination regime used. The difficulty is that methods are complicated and there is no prospect of harmonisation across the Network laboratories (as well as those used by the different vaccine companies). Furthermore, most data generated by FMD reference laboratories only considers monovalent vaccines – and not the final formulated product that may contain complementary FMD virus strains to cover antigenic diversity. An important message from this talk was that vaccine matching data is often used incorrectly by customers, and that perhaps a priority for the Network should be education and standardisation of these methods. New reliable tests and harmonised reagents are certainly required, and also more work should be undertaken to better understand the relationship between antibodies and protection. The presentation also considered vaccine batch QA and minimum qualification of vaccines for use in different endemic settings (focussing on homologous responses). It was suggested that some products could be pre-qualified (subject to meeting GMP requirements); alternatively, each batch would need testing at national/pan-national level. Standardised sera could be provided by the manufacturers to assist the Network to define post-vaccination responses (using standardised viruses and methods to focus on heterologous responses), but if funding from the commercial sector is required for these activities, there must be a benefit to the producers.

Three level of fitness:

1. Quality reliable – each batch consistent
    a. External audits (manufacture w/ accredited source of documentation), GMP
2. Does the strain/adjuvant have good immunogenicity
    a. Homologous challenge studies (manufacturer through independent testing facilities)
3. Does the strain give field specific protection
    a. Heterologous data (laboratory network).

Discussion - Batch testing should not be complicated, but it may be difficult due to producing sera at the cut-off of protection. However, the network would benefit from having this cut-off sera for it would give laboratories an understanding of how the vaccine behaved before it left the factory. If there is a difference after vaccination in the field, then the problem is with vaccination not the vaccine. Currently the batch cut-off is based on what potency they can expect.

Implementing PVM in endemic countries – Kees van Maanen

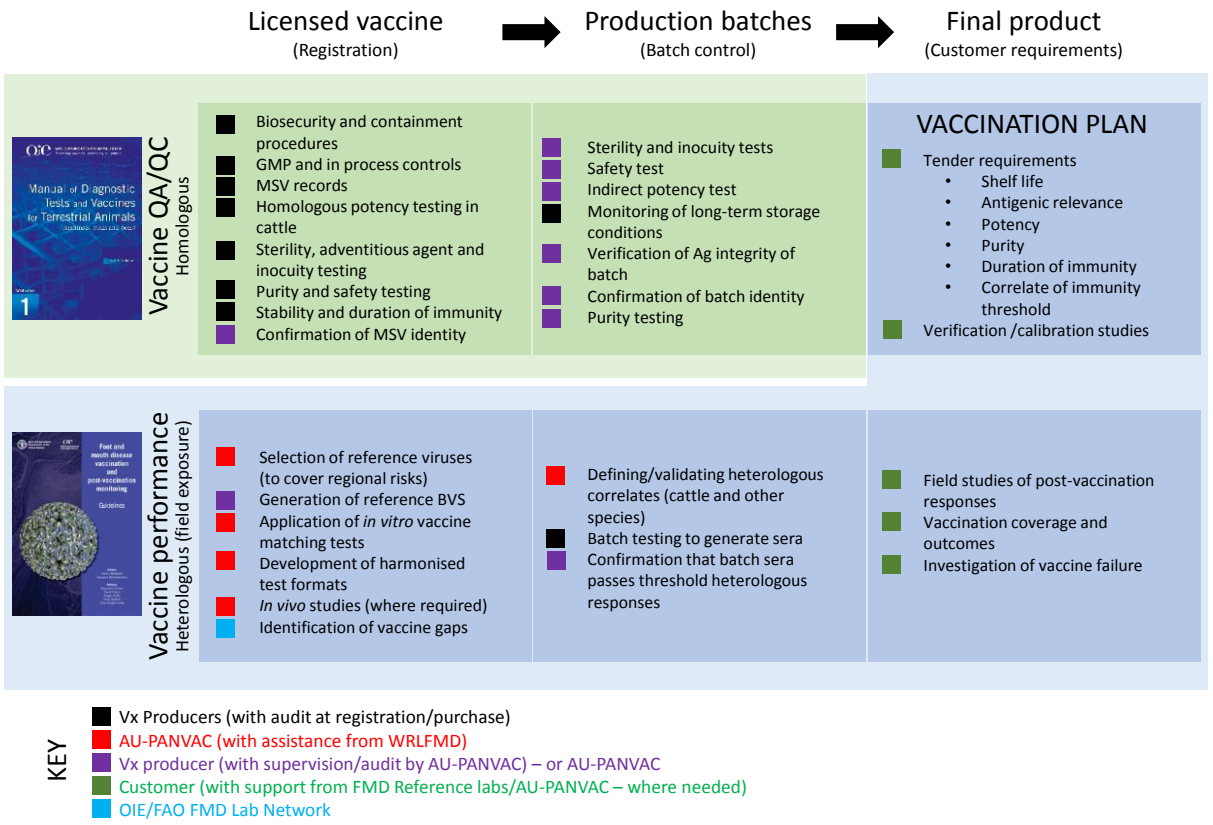
The relationship between probability of protection and serological results is sometimes not clear. There are different protective cut-offs for different serotypes and different assays. The talk discussed what the different options are for vaccine batch testing and a description of the various trials that can be done was presented.

Vaccine assessment: Actions and Recommendation to take forward:

1. For vaccine matching reports:
    a. Include how the r1-values are produced on each report (i.e. VNT, LBPE) and the reagents used including details of BVS.
    b. Make a note on each report with any limitations. This could include if the assay is based on a single test and whether the data should be interpreted by considering other isolates of the same lineage.
2. Limited data (including potency testing and *in-vitro* vaccine matching) is available for swine. This is particular a problem for Pool 1 and delegates may want to consider writing to the OIE for assistance.
3. How do we make reference sera available for post-vaccination study? Could there be a link between industry and reference laboratories?

    Action O3-18 – Anna Ludi to draft a document containing the BVS currently available at The Pirbright Institute. Other institute including industry could then add to this list (large quantities would only be included). This could include a reference panel.

4. Action O4-18 - Invite vaccine manufacturer from China to next year's meeting
5. Action O5-18 – Delegates to provide feedback on the figure (see below) developed from ideas raised at last year's Network meeting regarding who takes responsibility for Vaccine QA/QC



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