Animal & Plant Health Agency

APHA

Annual Science Review 2015

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2. INTRODUCTION

This review of APHA's science for 2015 was originally pulled together in order to help our external science advisory board assess the quality of the agency's science, which was one of our key performance indicators in 2015-16.

It proved to be such a useful document that I thought – with a little editing – that we should publish it so that all our stakeholders could see our many scientific achievements during the year.
I hope you enjoy reading it and please get in touch if you want any further information on any of our work.

Dr Kath Webster
APHA Director of Scientific Services
June 2016

3. OVERVIEW

In October 2015 we published our refreshed APHA Science Strategy 2015-2020, which reflects our much broader remit although still remains focussed on threat awareness, threat identification and threat mitigation. The strategy is underpinned by revised portfolio strategies in our key science areas and discipline capability plans, both of which ensure that we have the most appropriate scientific capability to deliver our customer requirements.

150 years of state veterinary medicine was celebrated in October 2015 and APHA (and its precursors) have played a pivotal role throughout this time. The event was marked by a number of events and seminars at Defra HQ and APHA Weybridge. An article showcasing the history was published in the October 2015 issue of Science in Parliament.

We responded to the Government call for experienced scientists to help the humanitarian crisis in West Africa caused by the outbreak of Ebola. Between October 2014 and April 2015 six members of staff each spent five weeks in Sierra Leone working in a Public Health England (PHE) Ebola diagnostic laboratory near Makeni run by the International Medical Corps. A further four members of staff went out on 18th October 2015 to work at the Port Loko lab run by the charity GOAL. In recognition Her Majesty the Queen awarded each volunteer a new medal – this is the first time a medal has been created specifically to recognise the responders to a humanitarian crisis.

As part of Ebola contingency work we also put in place arrangements for the safe transport and quarantine of family pets of anyone in the UK showing signs of infection.

A wide range of disease emergency situations have been tackled throughout 2015. Details on these are included in the respective disease portfolio highlights.

4. PORTFOLIO HIGHLIGHTS

Animal and Zoonotic Viral Diseases

This portfolio provides surveillance and policy-relevant research on viral diseases of significance, in terms of their impact on animal welfare, the economy, the food chain and human health. Our strategy is targeted to enable effective control of exotic viral disease threats to livestock, companion animals and wildlife, also to protect public health through the control of zoonotic viral diseases. Our research portfolio is highly collaborative and of a size that aligns with the minimum core capability for emergency laboratory response.

Avian and Mammalian Influenza

We successfully responded to two avian influenza (AI) outbreaks in 2015. In February, a low pathogenic H7N7 was detected in a farm in Hampshire. The response was not challenging and readily coped with. An outbreak of HPAI H7N7 virus in Lancashire in June tested our laboratory contingencies and our research data were pivotal in understanding how the virus was introduced and the downstream epidemiological investigation. A Ministerial/Chief Veterinary Officer plaudit was received.

In April, we detected an avirulent Newcastle disease virus (NDV), following receipt of samples from central England under the Notifiable Avian Disease (NAD) Testing-To-Exclude (TTE) scheme; PCR-positive results for APMV-1 (possible NDV) were received from swabs. The case was raised to the NAD-report case status, AI was excluded and APMV-1 confirmed as an avirulent NDV and as such NAD was ruled out.

In September, we detected a second avirulent NDV in broiler chicken premises. Our analysis revealed it to be of low virulence in vivo and to be genetically similar to a number of wild type avirulent viruses and vaccine strains.

In November, we detected a more virulent APMV-1, following a NAD investigation in a flock of ducks in

southern England where there had been 40-50% drop in egg production over the preceding three days and respiratory signs. Phylogenetic analysis showed two isolates to be lineage 1 clustering with isolates from the UK and Denmark in 2005 and 2003, respectively. These viruses were quite different from APMV-1 Queensland-like lineage 1 viruses detected in April following an NAD testing to exclude submission.

As part of our services to CITES (Convention on International Trade in Endangered Species) and conservation, we received a plaudit for the role played by the Avian Virus Investigation Unit in the conservation movement of threatened bird species. We have been involved in a project to reintroduce the Great Bustard to UK and certified freedom-from-infection from AI and NDV among 12 Javan Green Magpies and seven Sumatran Laughing Thrushes imported into the United Kingdom from Indonesia.

The EU Reference Laboratory for AI has maintained a high profile on the international scene, dealing with crises as they emerge such as the global spread of the sub-family of H5 HPAI both to the Americas and Europe; we have worked through strong international networks to rapidly characterise these viruses and inform risk assessment and preparedness at EU level for further incursion. We are continuing to provide timely genetic analysis on emerging viruses that might have implications for public health.

The EU Reference Laboratory, in conjunction with the National Reference Laboratories (NRLs) have undertaken an H5 'birdflu' serology tool assessment – ELISA versus HAIT for poultry surveillance. The study revealed that the commercial ELISAs trialled are satisfactory, but the HAIT with optimised antigens (e.g. H5N8) was more robust and remains the OIE/EU "gold standard".

We produced a surveillance report for AI in poultry and wild birds in 2014 in all EU Member States, which was delivered to the European Commission on 31st July as part of the its responsibility as the EU Reference Laboratory. We analysed patterns in results of serological surveillance for exposure to H5 and H7 influenza viruses in poultry across Europe, in addition to detection of AI in wild birds. The report is now available on the Commission's website
http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/eu_resp_surveillance_en.htm

During the last quarter of 2015, we were closely involved in working with the EU Commission and UK government to monitor and understand the developing outbreak with H5 HPAI viruses in a large area of south western France. We used in-house research and knowledge of the epidemiology of the infection to inform specific UK risk assessments but also to guide the EU Commission and Member States on the potential risk pathways and approaches for preparedness should these viruses spread beyond France, providing a preliminary report to the European Commission on 27 November, concerning epidemiology and surveillance relevant to H5N1 HPAI reported in France and updated reports on 18 and 23 December 2015.

We continue to use our knowledge and expertise for the benefit of all UK stakeholders; specifically we have been practically engaged with the poultry industry in providing the scientific evidence base for developing proportionate but robust protocols for completion of secondary C&D necessary for UK industry to return to "business as usual".

Dr Andrew Breed was invited to join the EFSA Animal Health and Welfare Network, and also a working group on AI. The working group will provide an opinion on aspects of risk, biosecurity, surveillance and epidemiology of Highly Pathogenic AI in 2016.

A proposal for EFSA funding on the epidemiology and modelling of AI involving a consortium of scientists from Erasmus MC (lead partner), CVI, APHA, FLI, IZSVe and Linnaeus University was successful.

In a Defra funded project, H7N9 LPAIV had previously been detected in China in spring 2013 by virtue of its clearly apparent morbidity and mortality in humans and was it shown that contact with poultry at live bird markets was a major risk factor for acquiring zoonotic infection. Novel *in vivo* experiments were carried out at APHA, which revealed that H7N9 LPAIV can also be spread among turkeys, together with some unexpected pathogenesis that has led to mortality in several instances. It also appears that this mortality in

turkeys does not involve mutation to the highly pathogenic form of AI. By contrast, this H7N9 LPAIV did not spread between chickens in our experiments.

While turkeys are not a poultry production species in China, any future insidious spread of this H7N9 LPAIV beyond China towards the borders of Europe would represent a threat to the commercial turkey sector which is significant in the UK and other EU member states. Further understanding of the potential for H7N9 spread and disease in European / UK poultry is required. In addition, it is crucial that diagnostic tools (molecular and serological) used at APHA and EU NRLs for disease confirmation and surveillance remain fit-for-purpose regarding this H7N9 virus.

Full-genome sequencing of chicken and turkey progeny viruses revealed evidence of reversion from mammalian-adapted to avian-adapted genetic change in the haemagglutinin gene in both turkeys and chickens. Observation of this genetic change in chickens is interesting, particularly as this reverted H7N9 LPAIV did not transmit efficiently to contact chickens, whereas dissemination was observed in the turkeys.

Final deliverables for the RISKSUR EU project have now been submitted. These included modelling and economic outputs evaluating the probability and timeliness of detection for existing passive surveillance and a novel surveillance component investigating the use of mortality/production thresholds which would automatically trigger testing to exclude AI. Novel tools for designing, documenting and evaluating surveillance developed during the RISKSUR project are being disseminated across APHA. Our epidemiologists are currently considering application of these tools to a number of initiatives looking to enhance surveillance.

An APHA driven CoVetLab project was recently completed for the development and validation of molecular tools for sub-typing swine influenza viruses (swIAVs). From this project, PCR protocols have now been established for detection and differentiation of human and avian lineages of H1 and H3 strains of influenza, also simultaneous detection of N1 and N2. The protocols have now been used to test clinical material from the field in addition to amplified virus. Promising results have been achieved following the identification and classification of influenzas viruses in previously virus isolation-negative, untyped influenza A-PCR positive (matrix gene) swabs and tissues (n=37). These new tools will be key to sub-typing swIAVs that are not amenable to virus isolation and traditional typing techniques (HAIT) and provide quicker turnaround times if required. Further validation and accreditation of these assays for clinical material will follow, including application for a new test code.

A vaccine pilot study was completed for the swine influenza dynamics BBSRC sLoLa project (OR1032). Trials included a commercial trivalent swine influenza vaccine and two laboratory-formulated vaccines that were homologous with or heterologous to the challenge strain. The data clearly showed that, in order for vaccines to prevent virus replication and shedding, the vaccine and challenge strains must be closely matched. Antibodies elicited by the different vaccines also displayed distinct strain reactivity. The results indicate that available commercial vaccines may not provide ideal protection against currently circulating swine influenza virus strains or prevent spread through “sterile” immunity. Further analysis is ongoing to dissect the specificity of the immune responses to the different vaccines.

Mammalian Virology

An outbreak of porcine epidemic diarrhoea (PED) occurred in the summer of 2014 in Ukraine, severely affecting piglets less than 10 days of age; the mortality rate approached 100%. We investigated this outbreak and full genome sequencing showed the virus to be closely related to strains reported from North America, showing a sequence identity of up to 99.8%. The presence of such a PEDV strain in Ukraine highlighted the threat to neighbouring countries and those in the European Union where PEDV has not been detected (e.g., Scandinavia) or has not caused disease in recent decades (e.g., the United Kingdom) and where pig herds are considered largely naive to PEDV.

Accordingly, on December 18th 2015 PED was made a notifiable disease in England and suspect or

confirmed cases need to be reported to APHA.

As part of an Agriculture and Horticulture Development Board Pork funded project, repeated attempts to orally infect pigs with PEDV were made. All attempts failed despite the use of multiple strains of virus from several sources, including the highly pathogenic US virus, using weaned pigs of varying ages. These findings indicate that there was insufficient infectious virus left to rescue in-faecal material as PEDV are enveloped viruses, which tend to have a low resistance to environmental or chemical challenges such as the proteases present in faeces. This phenomenon, also reported by other European and US groups, supports the view that strict biosecurity measures can control PEDV outbreaks. In addition, our results explain why repeated back-feeding of faecal material to sows often fails to deliver a strong immunity. These results also highlight that good farm biosecurity practice could be very effective in controlling the local spread of infection following a possible incursion into the UK.

We also successfully executed a PEDV RT-PCR ring trial, organised with members of CoVetLab. The ring trial comprised 10 samples tested using a commercial and an in-house PCR. The two PCRs delivered comparable sensitivity and were among the most sensitive PCRs used by the participants in the ring trial. This exercise provides confidence that incursions of high path PEDV into UK and key trading partners would be rapidly and accurately diagnosed. Subsequent to this we were asked for assistance from Cyprus regarding a suspect outbreak of PED which successfully tested our procedures and full genome sequence was available on the 4th day. This was done during working hours without diverting resources or staff from other routine activities

We participated in the Bovine Leukaemia Virus (BLV) realtime PCR ring trial. The trial consisted of 58 clinical samples of different genotypes and varying viral loads, collected from around the world. Of the six laboratories that took part, APHA was the first lab to return results and the only lab to correctly identify all samples. Since each participating laboratory used their own detection methods, this confirms our ability with regards to the molecular detection of BLV. APHA is an OIE Reference Laboratory for this disease.

We have successfully executed the bovine herpesvirus 1/infectious bovine rhinotracheitis/infectious vulvovaginitis ring trial. The trial included 16 laboratories globally and samples for serological and molecular assays. All the tests (iELISA, cELISA, SNT and PCR) performed as expected providing further assurances for our quality and customer support.

A classical swine fever challenge study to examine the role of CD8 T cells in vaccine protection against CSFV was successfully completed. The data indicated there may potentially be some protection conferred by the transfer of CD8 cells from vaccinated pigs to naïve recipients. However, differences are not statistically significant and further studies, which will require further funding, are likely to be needed to confirm the hypothesis.

Our mathematical modellers played a vital role during Exercise Rowan, a foot and mouth disease (FMD) outbreak preparedness exercise, in November. Modelling with Exodis-FMD was a key aspect of the exercise as it provided both the outbreak ‘story-line’ and was also used to provide evidence-support at key decision meetings such as the CVO stock take, the National Experts Group, and the Animal Disease Policy Group.

Two National Emergency Epidemiology Group (NEEG) training workshops have also been delivered this year, on the different outbreak data systems that are used during a disease outbreak and the principles of risk assessment (February 2015) as well as an overview of NEEG processes, outputs and customer requirements (September 2015). This outbreak preparedness training is vital to ensure that our staff are equipped to participate in notifiable disease outbreaks.

DSTL have agreed to set up a contract to develop an immunohistochemical (IHC) method for the detection of Western and Eastern equine encephalomyelitis virus (EEV) in murine tissue, with the intention of applying it in the histopathology study of the infection in mice and the effect of intervention strategies. The IHC method could be employed subsequently to support the investigation of exotic equine cases in the UK.

Another contract for Venezuelan EEV is under discussion.

Additional funding has been secured from Chester Zoo and ZSL for research into elephant endotheliotropic herpesviruses. Sufficient money has now been secured to allow the funding of a PhD student, which will be advertised shortly.

Wildlife Zoonoses and Vector Borne Diseases

As part of our work on surveillance of wildlife for zoonotic viral diseases, we detected European bat lyssavirus type-2 in a Daubenton's bat from Wales.

UK bat workers are considered to be at risk of exposure to rabies and therefore receive a primary course of rabies vaccine (three intramuscular injections) with a single reinforcing dose one year later, followed by subsequent boosting every three to five years. The WHO recommends boosters should be administered when rabies virus-neutralising antibody (VNA) titre falls below 0.5IU/ml. We performed a study, in collaboration with PHE, to improve our understanding of the serological antibody response to rabies vaccination in order to develop more specific advice on the timing of rabies booster vaccinations for bat handlers and to record the rate of adverse reactions due to booster vaccinations.

Bat workers recruited to the study were due second or subsequent rabies booster vaccinations between August 2010 and August 2013. Blood samples taken before boosting, were tested for rabies VNA. Bespoke questionnaires were used to collect information about adverse reactions. A total of 150 bat workers participated in the study. Only one batworker had a titre below the minimum VNA recommended by WHO. No associations were observed between titre and age, sex, number of previous boosters or time since previous vaccination. A range of mild adverse reactions, including redness and swelling at the site, were reported by 65 (43.3%) participants. The results of this study suggest there is little evidence to recommend a change to the current approach and schedule. Further investigation is required in order to establish if individual monitoring of rabies VNA may be preferable to boosters.

We have been involved in a collaboration with St George's University of London and the Institut Pasteur in an ambitious project to examine ways to facilitate the transport of immunoglobulin across the blood-brain barrier, as a possible treatment for clinical rabies. Rabies post-exposure prophylaxis (PEP), which is a combination of wound cleansing and administration of rabies vaccine and rabies immunoglobulins, is highly effective if it is correctly administered promptly after a potential exposure. However, in the case of delayed treatment and the onset of symptoms, PEP is ineffective. Rabies antibodies are unlikely to offer therapeutic benefits once RABV has entered the central nervous system, as they are large molecules and cannot cross the blood-brain barrier (BBB).

In this study, we devised a mechanism of using a peptide derived from the sequence of rabies virus, which had previously shown the ability to deliver molecules to the CNS, linked to an antibody fragment of a rabies neutralising monoclonal antibody. We chose two strategies to link the peptide to the antibody fragment: chemically and via co-expression in, and purification from *Nicotiana benthamiana* leaves. We were able to demonstrate that the peptide does not affect rabies virus neutralization or protection against infection, but did facilitate transport of a rabies ScFv in our in vitro model. Our results are promising because they indicate a potential strategy to deliver neutralizing monoclonal antibody fragments across the blood brain barrier and into the CNS. This may lead to a new approach to post-exposure management of patients at risk of rabies, but further *in vivo* animal studies are planned, to assess pharmacokinetics of antibody fragments linked to this rabies peptide and efficacy of late post-exposure prophylaxis in the murine model.

Working with PHE, we participated in a study of suspect hantavirus in a pet / 'fancy' rat colony in Wales. The investigation was initiated following the diagnosis of hantavirus infection by PHE Wales, in three patients, all of whom had association with rat breeding. PHE Porton Down provided the initial diagnostic testing. Epidemiological links had been established between index case (i), his father (ii) & a breeder-feeder worker (iii)). We obtained a number of rats from the suspect colony and successfully detected the

hantavirus Seoul virus (SEOV) in a high proportion of the rats. Further prevalence studies of SEOV in domestic pet rat colonies are continuing in order to further understand the risks posed to humans and other domestic pet animals by recreational or occupational contact of pet rats. It is likely that, with the enhanced awareness of SEOV in pet rats in the UK, additional cases in humans and rats will be reported in the future.

In a retrospective study of a significant outbreak of disease in sheep in the Kent Marshes in 2005 we have finally diagnosed an infection with *Theileria luwenshuni* – a blood-borne protozoan parasite transmitted by ticks. The disease has never been previously described anywhere in Europe. The tick vector, *Haemaphysalis punctata* is also an introduction to UK and the source of both the parasite and vector are postulated to have been brought in with imported Asian deer.

In collaboration with PHE and the University of Liverpool, as part of our preparedness and developing competency in deep sequencing, we have recently succeeded in sequencing the full genome of Rift Valley fever virus strain Lunyo.

Bacterial Diseases and Food Safety

Our strategy is aimed at providing scientific evidence, assurance and biosecurity advice to Government; to enable effective control of exotic bacterial disease threats to livestock, and to protect public health through the control of zoonotic diseases, chemical toxicity and antimicrobial resistance in livestock and the food chain.

Exotic Bacterial Diseases

We provide a unique national capability familiar with potentially devastating notifiable exotic bacterial diseases of livestock. Scientific effort is focused on assurance of disease freedom, contingency planning and emergency response capability. Research is focused around our International Reference Centre functions and includes development of diagnostics, pathogen characterisation and international partnership to sustain skills, and support control efforts in other countries thereby reducing global threat.

We provided laboratory training and technical expertise to support implementation of locally adapted, sustainable programs to understand and reduce the prevalence of brucellosis in West Africa, Tanzania and India as part of BBSRC led initiatives including the Zoonoses in Emerging Livestock Systems programme. This followed completion of similar laboratory development in Afghanistan as part of an OIE twinning project on *Mycoplasma* and *Brucella* which was recognised by EU Animal Health Development Programme as a great success.

In support of improved diagnostics; a new disaccharide test for brucellosis developed by APHA in collaboration with the University of Alberta was reported in the scientific press as the *'first major breakthrough in decades towards avoiding false positive serological reactions'*; a GB priority since eradication.

A new serodiagnostic assay for the detection of porcine brucellosis using the rough (r) LPS developed through APHA research is being run alongside conventional serology as an additional tool to assess cases and validate the performance of the test in field conditions. The test will be used to assist resolution of false positive serological reactions in swine and will transfer to operational and commercial use in 2016.

We developed a monoperosamine glycoconjugate ELISA for detection of bovine anti-*Brucella* antibodies. The finding that the monosaccharide antigen reacts well to *Brucella* specific antibodies is of relevance to development of *Brucella* DIVA (differentiating infected from vaccinated animals) vaccines as it identifies a new avenue for exploitation.

Working in conjunction with PHE the first cases of anthrax in animals since 2006 were identified and contained. This follows transfer of confirmatory testing to PHE under the APHA/PHE strategy to improve efficiency by sharing services. Our risk analysts reviewed two veterinary risk assessments; on cattle movements and the use of silage which helped provide the evidence and recommendations for operational and policy decisions.

An investigation of suspect notifiable disease, contagious agalactia (CA), in imported sheep confirmed that animals were negative following further testing and restrictions were promptly lifted. It is unusual to get cross reactions in the diagnostic tests and sequencing identified the presence of enterococcus and this is being investigated as a potential cause of the test reactions. The threat of disease incursion from sub-clinical CA infection, following an incident in Wales in 2014 and increasing reports of asympomatic carriers of CA in the literature, was raised to the Veterinary Risk Group. APHA confirmed the occurrence of sub-clinical infection in studies carried out with a sub-contractor in Sicily, where CA is present.

An investigation of suspect contagious bovine pleuropneumonia negated the disease within a week using specialist confirmatory tests, despite a positive complement fixation test.

Following an unexpected and unexplained case of glanders in Germany APHA published an awareness raising article in the veterinary press.

APHA were co-organisers of the European Mycoplasma Meeting and provided an invited expert to the FAO/EMPRES/OIE/AU—IBAR/IAEA and FAO technical consultation meeting on contagious bovine pleuropneumonia.

Endemic Zoonoses and Food Safety

This area is focused on control of public health risks with a major role in the national control of *Salmonella* in livestock. The research programme and National Control Programme (NCP) has successfully controlled serovars of high public health impact in chickens and turkeys and resulted in a large reduction in human disease but continued effort is needed to contain potentially epidemic drug resistant non-resident strains and control *Salmonella* in other livestock.

Salmonella Enteritidis is now rarely reported in chickens following the success of the NCP. However, a large outbreak of *Salmonella* Enteritidis (SE) PT21 and related PTs, predominantly PT35, was detected in broiler flocks in 2015. A detailed epidemiological investigation was undertaken and, although all broiler farms were linked to a single broiler hatchery, a definitive source was not identified and the origin of infection is likely to lie outside the UK. There was some evidence of spread to turkey flocks and the organism was also detected in raw dog food containing chicken. Our experts advised on actions to take to prevent carry-over of SE on farms, which has been successful with all premises testing clear of infection. Whole Genome Sequencing (WGS) demonstrated the poultry strains were similar to human strains and were part of a single outbreak affecting UK and RoI. This unusual outbreak reinforced the essential role of APHA science in outbreak response. Prompt detection and action taken to exclude potentially contaminated meat from the food chain minimised foodborne risk.

There were a number of other high priority *Salmonella* incident investigations, including a case of ciprofloxacin resistant *S*. Kentucky strains in cattle (first report of ST198 in GB livestock, a highly resistant strain spreading epidemically in poultry in many European countries), cephalosporin resistant monophasic *S.* Typhimurium in pigs, colistin resistant *S*. Typhimurium in pigs (see AMR section) and multi drug resistant *S*. Infantis in a layer flock. Follow-up studies have so far revealed elimination or significant reductions in infection following withdrawal of selective pressure and improved farm hygiene measures. The threat posed by exotic, non- regulated *Salmonella* serovars was highlighted at a UK CVO/CMO meeting and these emerging threats were discussed in cross government risk assessment groups.

Whilst *Salmonella* control programmes exist in the chicken and turkey sectors, no regulatory programme is

currently in place for the duck sector. A research study of the epidemiology of *Salmonella* in ducks flocks was undertaken in response to human outbreaks in 2010 linked to a specific phage type of *Salmonella* Typhimurium (DT8), associated with ducks, and increased sales of duck eggs. The research developed sensitive methods for monitoring flocks and hatcheries and, through longitudinal studies and targeted advice supported the industry to effectively control *Salmonella*; including *S*. Typhimurium DT8 in a large integrated company, previously linked to human cases. This correlated with a decline in human cases demonstrating the direct public health benefit of the research. The benefit of effective disinfection programmes was demonstrated both on farm and *in vitro* and washing of duck eggs with chlorine based products was confirmed as a suitable public health intervention for eggs produced by *Salmonella*-positive flocks. Recommendations were provided to the duck industry via a proposal for an update of the British Poultry Council Duck Assurance Scheme and advisory leaflets on *Salmonella* control. Molecular investigations demonstrated DT8/30 strains causing human outbreaks in 2010 represented a newly emerged clonal grouping of non UK origin. WGS also suggested an absence of a major gene associated with survival in egg albumen, which was consistent with the low rate of internal egg contamination found and indicated human infection from duck eggs may predominantly result from contamination from the shells of unwashed duck eggs.

We have similar needs to PHE for sequencing and bioinformatics and a strategic objective is to unify approaches and pipelines for analysis of WGS data and work towards replacing *Salmonella* and *E. coli* typing with high throughput WGS and development of national and EU wide cross sector (animal, human, food) databases and platforms for outbreak detection, containment and mitigation. During 2015, access was established to the PHE SnipperDB whole genome sequencing analysis pipeline via the CLIMB (Cloud Infrastructure for Microbial Bioinformatics), the MRC Enterobase project led by Birmingham University. CLIMB has a large amount of memory and RAM to analyse hundreds of sequences at the same time and allows data sharing and a searchable database of analysed sequences. The SnipperDB pipeline is mostly for *Salmonella* sequencing in real time but can also be used for other organisms. The system was tested with the *S*. Enteritidis outbreak sequences. This is a good step forward towards integration with PHE and the wider scientific community and a step towards sequencing in real time. APHA was also successful in attracting EFSA funding to establish collaboration between the public health, food and veterinary sectors across the EU for building and enhancing the use of real-time WGS and analysis in food safety and public health protection.

WGS was used for the first time in UK Poultry *Salmonella* surveillance to confirm an atypical live vaccine strain which could not be confirmed by conventional means. This avoided a laying hen flock from being restricted and the associated cost of confirmatory sampling.. The vaccine company confirmed APHA findings and a multiplex PCR has been developed to facilitate improved differentiation of live vaccine strains, including rare biochemically atypical ones.

Campylobacter control in chickens is a high priority due to the human disease burden and APHA provides national monitoring of broiler chicken and is seeking to apply similar research approaches to develop practical on-farm controls that have proved successful for *Salmonella*.

We are carrying out quantitative *Campylobacter* monitoring in broiler caeca and carcases at abattoirs on behalf of FSA to measure change in *Campylobacter* load in response to implementation of interventions and progress towards a voluntary target to reduce highly contaminated carcases from 27% to 10% by 2015. To date, the data demonstrate the target has not been achieved although a reduction in level of contamination was observed (results are reported by FSA; https://www.food.gov.uk/science/microbiology/campylobacterevidenceprogramme/campy-monitoringresults). Our epidemiological analyses identified a number of independent risk factors providing evidence for intervention. We have been awarded further funding from FSA to continue monitoring in 2016 and develop an integrated risk framework and real time data analyses and reporting system to provide enhanced information for industry control.

We are part of a consortium working on molecular-based surveillance and source attribution of *Campylobacter* infections in the UK led by PHE and Oxford University in collaboration with many other institutes and research groups in the UK. This collaboration will focus on the translation of genetic sequencing technology and population biology techniques to public health benefit. An extensive dataset of WGS of *Campylobacter* from broilers and other sources will be established for future analyses.

We keep a watching brief on a wide range of other zoonoses with a focus on risk assessment and outbreak control and integrated delivery, particularly with public health bodies. We provide a national veterinary capability to investigate and respond to potential public health threats linked to animal infections or toxins, led by specialists and supported by epidemiologists.

We continue to support PHE&W in investigations of zoonoses outbreaks in people epidemiologically linked to animals. This includes the on-going investigation of cases of VTEC O55, never previously linked to human illness in the UK, where a source has not been established. With PHE we are reviewing the animal diagnostics offered to ensure suitable animal tests are available for incident investigation for emerging non O157 strains of *E. coli* and, as a priority, we are developing a sensitive customised test for *E. coli* O55, based on the IMS method used for detection of *E. coli* O157. We were involved in a BBC documentary (broadcast on 2nd November) detailing the cross government efforts being made to investigate the source of this serious outbreak.

We assisted PHE in a farm-to-fork investigation into an outbreak of 15 cases of VTEC O157 PT21/28 in people who purchased ready to eat food from two branches of a local butcher outlet in North East England. It is concluded that it was highly likely that there was cross-contamination from raw meat within both butcher shops. Customer purchase records and details of the cattle supply chain were used to identify a number of farms which supplied cattle to the butchers. WGS provided strong microbiological evidence (single or double SNP differences only) to link the human cases to isolates from raw meat in the butcher shops, and also to isolates yielded from animals on one farm supplying cattle the butchers for slaughter.

A Lancashire farm shop admitted health and safety breaches following a serious VTEC O157 outbreak linked to a lambing live event. APHA input included the veterinary investigation, laboratory testing, and witness statements. This successful prosecution reinforces the message about the importance of zoonotic disease precautions in animal handling events.

We revised a previously developed risk assessment model for risk of human illness with VTEC O157 given consumption of burgers, particularly rare burgers, to inform policy. Collaborating with RIVM (The Netherlands) a thermodynamic model for the cooking module of the risk assessment was incorporated. A joint APHA/RIVM report on the updated assessment was published on the FSA website and was used to inform an FSA Board Paper on developing controls on burgers served rare in catering outlets.

Nationally our experts contributed to the cross government Zoonoses summary report UK 2014 (https://www.gov.uk/government/publications/zoonoses-summary-report-uk-2014) and APHA data was used by the ACMSF ad hoc group report of foodborne viral infections in production of an update on viruses in the food chain (http://www.food.gov.uk/sites/default/files/acmsf-virus-report.pdf).

Internationally, our experts completed analyses for the EFSA Community Summary Report (CSR) on *Salmonella* serovars and their trends (http://ecdc.europa.eu/en/publications/Publications/zoonoses-trends-sources-EU-summary-report-2014.pdf). APHA publications and data also contributed to a number of EFSA scientific opinions including public health risks associated with Enteroaggregative *E. coli* as a foodborne pathogen (http://www.efsa.europa.eu/en/efsajournal/pub/4330); public health risks related to the consumption of raw drinking milk (http://www.efsa.europa.eu/en/efsajournal/pub/3940); and risk to public and/or animal health of the treatment of dead-in-shell chicks (Category 2 material) to be used as raw material for the production of biogas or compost with Category 3 approved method (http://www.efsa.europa.eu/en/efsajournal/pub/4306). Our experts also contributed to an OIE review of the scientific literature on the control of *Salmonella* in pigs and cattle (OIE bulletin 2015-1,64-71).

Farm Disinfection

Research on efficacy of disinfectants against *Campylobacter* in simulated field conditions of broiler farms demonstrated differences in product performance, particularly for short contact times. The current Defra General Orders test with its 30 minute contact time may not be stringent enough to allow farmers to select the best products for specific applications where a short contact is predicted e.g. boot-dips or vehicle wheel-washes. The method we developed may be a step towards a standard test for these scenarios. The findings demonstrated that organic matter must be carefully controlled in boot-dips to maximise efficacy and that some products should be avoided in this situation.

Our work on disinfection of *Salmonella* in pig production identified deficiencies in the activity of several commonly used disinfectants in the presence of organic matter or biofilm, although this was less marked than in similar studies involving poultry faecal contamination. Aldehyde products were best for surface disinfection, but there was variability between different commercial formulations. Chlorocresol disinfectants showed the greatest activity in biofilm and boot dip models, although boot dips without a boot change are unlikely to be reliable.

Farmer Engagement and Knowledge Transfer

We hosted a roadshow in conjunction with British Quality Pigs (BQP), Agriculture and Horticulture Development Board (AHDB) and ADAS to update farmers on current pig health and biosecurity issues, to feedback on *Salmonella* research we are conducting and to discuss the development of a new biosecurity auditing tool. The event was attended by more than 40 participants, along with industry experts, who gained advice on control and provided valuable input to the development of an auditing tool. Positive feedback was received from British Quality Pigs, AHDB-Pork and the German manufacturers of the Salmonella vaccine (IDT).

We hosted an annual modelling symposium, 'Modelling transboundary diseases: A one-health approach' in February 2015 with presentations from APHA, Defra, Public Health England and academia highlighting the synergies of the use of modelling across different government departments, especially in emergency response and with emerging diseases. The yearly meeting brought together policy makers, scientists and modellers to communicate face to face and a review of the meeting published in the Veterinary Record ensured dissemination of the discussions to a wider audience.

Antimicrobial Resistance

Antimicrobial resistance (AMR) is recognised as an emerging issue in the National Risk Register for Civil Emergencies. We are at the frontline of AMR surveillance in GB livestock and key priorities are to maintain this monitoring evidence stream and provide statutory data for EU requirements.

Through scanning surveillance, we identified an unusual antibiotic resistance in enteric bacteria recovered from clinically ill pigs in September. We have been investigating the mechanism of the identified resistance to colistin (a last line human antibiotic). The incident was discussed with policy stakeholders, FSA and medical colleagues, including PHE, as part of the VMD Resistance Alert system on 13/11/2015. Co-incidentally, a paper was published[1] in the Lancet (18/11/15) which reported that a specific gene, *mcr-1*, has been identified in China which confers resistance to colistin and is located on a plasmid, which means it is able to pass its resistance genes to other bacteria**.** This is a major new development and the finding was widely reported. Resistant organisms were widely found in Chinese pigs and on pig and poultry meat in

[1] Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Liu et al, published on line in the Lancet 18/11/2015 http://dx.doi.org/10/1016/S1473-3099(15)00424-7

China. We immediately performed a PCR on isolates from this incident, using information from the Chinese paper, and have found that the same gene is present in isolates from the GB pig herd. We provided rapid evidence to VMD, Defra, devolved administrations and FSA to support decisions on incident response, antibiotic policy and communications for this novel finding. This has included large scale screening of archived material for evidence of the gene and transferable plasmid to put the finding in context. Another potential case from surveillance of pigs is currently under investigation and PHE have reported the presence of the gene in a small number of archived isolates from humans. We have prioritised this investigation to ensure that the evidence required for a considered and proportionate response is available to policy leads. This has been a very large effort from all our staff and VMD CEO expressed his thanks for the rapid and high quality of the evidence provided. A paper has been submitted for publication on the results of our work taken to characterize the novel resistant bacteria from this case and understand the mechanisms for dissemination of resistance. The results suggest dissemination of resistance through different horizontally transferrable elements, which may account for its rapid global dissemination.

A research study found that oral dosing of poultry with a combination of enrofloxacin with apramycin was more effective than either antibiotic alone and resulted in reduced resistance to fluoroquinolones in target organisms (Campylobacter, *E. coli* and *Salmonella*). This demonstrated that combination therapy may limit selection of antibiotic resistance, but ideally this should involve non-antibiotic co-treatments, such as essential oils or bacteriocins.

As part of a collaboration with PHE and Oxford University, a WGS AMR gene analysis pipeline which includes >2000 genes is being developed at APHA and will be harmonised across all three organisations. Following training, APHA now also has access to Danish pipelines for AMR analysis of WGS data.

We provided expert advice to a number of scientific reviews addressing the growing threat of antimicrobial resistance, including the Independent Review commissioned by the UK Prime Minister on Antimicrobial Resistance (AMR), the Defra Science Advisory Council on AMR surveillance and an EFSA meeting on AMR monitoring.

Nationally, we contributed to the production of the national annual surveillance report on the antimicrobial susceptibility of veterinary bacteria (https://www.gov.uk/government/publications/veterinary-antimicrobial-resistance-and-sales-surveillance-2014). Internationally, our experts contributed to the scientific analyses of the EU Summary Report on antimicrobial resistance in zoonotic and indicator bacteria (http://www.efsa.europa.eu/en/efsajournal/pub/4036) and the ECDC/EFSA/EMA first joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals (http://www.efsa.europa.eu/en/efsajournal/pub/4006). Our experts also participated in an OIE working group on antimicrobial resistance and usage/sales of antimicrobials in animals, including drafting reporting guidance. This important initiative to develop a global system to collect antimicrobial sales / usage/ consumption data from all OIE member countries will be presented at the OIE General Session in 2016.

We hosted Japanese AMR experts as part of an FCO inward mission (veterinary networks for surveillance and stewardship) to discuss possible collaborations to address antibiotic resistance.

We successfully organised the veterinary part of a Diagnostics and Antimicrobial Resistance Stakeholder Workshop, held at the Churchill War Rooms in London. The Workshop explored how we can make better use of existing diagnostic techniques to have an impact on AMR and make best use of new technologies on the horizon, identifying gaps. The Chief Medical Officer and NHS Chief Scientist both spoke at the meeting, as did APHA.

Toxicology and Chemical Food Safety

We identify report and investigate potential on-farm chemical food safety incidents on behalf of FSA and provide advice on toxicology and chemical hazards in relation to animal health.

A multi-agency investigation of arsenic poisoning of beef cattle on a moor in Lancashire suggested it seemed most likely to be associated with historic coal mining in the area and exposure to cattle to contaminated soils. Low levels of lead and cadmium were detected suggesting the source of arsenic was most likely to be of geochemical origin. Cattle were moved and a voluntary restriction placed on the group to allow arsenic time to be excreted and therefore no risk to the food chain. Advice to remove offal at slaughter also prevented contamination of the food chain. The Local Authority ensured there was no public health risk from recreational activities.

We provided several private analytical laboratories and the Veterinary Poisons Information Unit with information to ensure the correct advice goes to private veterinary surgeons alerting them to food safety concerns and how best to follow these up. This approach is aimed at maintaining surveillance inputs to detect chemical contamination incidents following changes in APHA surveillance structure and external engagement.

Advice on toxicology and chemicals was provided to Local Authorities and the Environment Agency when there were or could be animal health implications associated with a wide variety of chemical hazards e.g. PCB and dioxins from landfills, flooding, and tanker spillages. A report on the potential risk of shale gas extraction to animal health was produced for the Veterinary Risk Group.

Plant and Bee Health

We protect the country's plants and bees by providing world class inspection, quarantine and certification services working in many areas to ISO 17020. An EU FVO fact finding visit in 2015 which looked at the coordination of plant health across the UK reported that the UK had developed an excellent system for the control of new and emerging risks. In order to help achieve this our bee, plant health and seeds inspectorates, genetic modification inspectorate and plant varieties and seeds undertake a range of activities in addition to their regulatory roles. We provide surveillance, testing, training and outreach awareness campaigns for existing, new and emerging EU quarantine pests and diseases and assess any GMO events and issues. The aim of the diagnostic research, in partnership with Fera for example, is to improve capability of front line staff, using new technologies to provide rapid and effective field identification (such as having lateral flow detection devices in the field and Genie Lamp technology at Heathrow which is unique to APHA) and to further understand epidemiological and biological aspects of plant and bee pests and disease. The work also helps contingency planning for future outbreaks by raising awareness of biosecurity and communicationing science to UK stakeholders, the general public, government and non-government organisations and inspectorate services throughout Europe. The latter includes active involvement in the EU Better Training for Safer Food programme.
The numbers below show key changes and trends in the last year in trade (imports, exports, plant passporting activity) and surveillance and action activities throughout England and Wales to highlight current threats and eradication or containment of pest and pathogens.

Imports

We have carried out a total of 38,464 inspections on controlled plant material entering the UK (a 26% increase on 2014) and failed 505 consignments as a result of the plant health check (1.3% failure rate of those inspected) and 59 (0.6%) during the document checks. The most frequent pest notifications were for tobacco whitefly (*Bemisia tabaci*) and false codling moth (*Thaumatotibia leucotreta*).

Certification

As a result of changes to the EU marketing directive, a new seed potato certification scheme was introduced (the first in 30 years). 1032 stocks were entered for certification on 3335 Ha (an increase of 3%). Health of stocks was good with a marked reduction in stocks affected by bacterial blackleg.

Exports

13,243 phytosanitary certificates were issued for England & Wales (similar to 2014 figures).There has been an increase in rejections of potatoes to the Canary islands (4.3 %) primarily due to excessive skin disease tolerance levels. This has resulted in significant industry costs in the region of £4-5K per rejected consignment. Negotiations are ongoing with Spain to find a way of solving this problem.

Plant Passporting

The number of registered plant passporters in the UK increased from 660 to 750 in 2015. There has been a significant rise in the number of new hosts (36) added to the scheme due to the impact of *Xylella fastidiosa* in Europe. Two editions of a new newsletter for plant passporters were circulated in 2015.

Surveillance and Action

All EU commissioned surveys, general quarantine surveillance and Plant Health Risk Group commissioned surveys have been carried out. This includes 6,760 inspections for *Phytophthora ramorum* and *P. kernoviae;* with 18 new *P. ramorum* outbreak sites identified. Of note is a finding of *P. ramorum* on larch in Yorkshire. This site is some distance from any previously known larch infection.

P. austrocedri has been found at nine different nurseries on *Juniper* and a new host *Chamaecyparis* in the UK. In the wider environment a further two new sites have been identified as infected with this pathogen.

A new pest to science, the Agapanthus gall midge (Diptera: Cecidomyiidae) was found in a UK nursery in August 2015 and the outbreak was escalated to the Chief Plant Health Office (CPHO) and run via an APHA incident management team. A subsequent survey revealed the midge was widespread across the south of England and the pest was found at a range of nurseries, public and private gardens.

A total of 2,519 inspections were carried out at 1,328 premises looking at c. 5,640,000 plants/trees of *Prunus* for the bacterial plant pathogen *Xanthomonas arboricola pv pruni* and tested positive at nine premises.

An outbreak of *Xanthomonas fragariae* quarantine bacterial pathogen was found at a site in close proximity to UK high grade propagating stock. This was escalated to the CPHO and has been run via an APHA incident management team. Two additional findings of strawberry red core (*Phytophthora fragariae*) were found within the propagating stock. This will have an impact on releasing some stock butthe trade have options within the scheme to maintain supply through the system.

The quarantine nematode *Meloidogyne fallax* was detected on sports pitches in the north of England). The outbreaks were escalated to the CPHO which were then run by an APHA incident management team. Statutory notices were issued on all sites requiring safe disposal of any infected material.

In 2015 surveys for Asian long horn beetle, following an outbreak in 2012 in Kent, revealed no further findings using pheromone traps and sentinel trees.

There has been a significant rise in *Bemisia tabaci* (tobacco whitefly) findings from UK surveys. We made 117 interceptions in 2015 which is a significant increase compared with the previous year. Each interception required eradication and trace back. The high number of findings led to the recent Defra consultation on *B. tabaci* on extending the host range to *Nerium oleander*, *Mandevilla and Dipladenia*.

2015 was the first year that imported potato tubers affected by *Eptrix* flea beetle have been found, with seven interceptions from the European continent.

Engagement

We attended five targeted public engagement events to raise awareness of plant pests and diseases and the work the UK government is doing alongside the Plant Health and Seeds Inspectorate. This included the Chelsea Flower Show where the prestigious Gold medal was awarded for the APHA "Beyond our Borders" garden.

Working across government with the Ministries of Justice and Defence we have organised training workshops in plant biosecurity good working practice. In doing so we have enhanced understanding of plant and tree pest and disease pathways and risks, encouraged behavioural change and improved biosecurity including implementation of plant biosecurity into Ministry of Defence policy (2016).

We have exhibited at eight trade shows each targeting a specific sector of the industry including landscape, horticulture, agriculture, forestry and aquatics.

Importantly, we have raised awareness of plant passporting, the tree notification scheme and the responsibility of growers. We have communicated the need for general plant health awareness by publishing in the Plant Heritage magazine and through publication of new leaflets, postcards and quarantine inspection cards for use with engagement activities.

Another important area of engagement has been with retailers with professional training events delivered to Asda and to Marks and Spencer. Over 25 trainees from across the businesses attended. All of the attendees reported an uplift in biosecurity and plant pest and disease knowledge. They all felt able to support others within their organisation by sharing this knowledge.

We have successfully launched a new MSc. developed through collaboration with Fera and Harper Adams university. Two of our students were successful in applying for the course and development of a plant health biosecurity module is in progress.

Future Proofing Plant Health Project

The "Origins of Asian long-horned beetles (isotype analysis)" pilot study highlighted differences in isotope ratios between specimens from different locations although the results showed significant sources of variation. Further work is needed to investigate the sources of this variation, including evaluation of the different host species upon which the larvae could be feeding.

The quality testing of wood under ISPM15 (assurance of treatment) review indicated no methods are currently available for monitoring the treatment of wood to kill wood boring insects. Therefore the risk remains of fraudulent activity where wood is marked as treated when it has not been.

Two joint dissemination workshops were organised with the European and Mediterranean Plant Protection Organisation (EPPO) on improved detection methods and attended by inspectors from the EU. A range of techniques for detection of plant pests and pathogens were discussed. A LAMP (loop mediated isothermal amplification) assay using the Genie platform was favoured and assays were selected for ring testing (*Phytophthora ramorum*, *Clavibacter michiganesnsis* subsp. *Sepedonicus*, *Liriomyza sativae*, *L. trifolii* and *L. huidobrensis.)*

Genetic Modification Inspectorate

Members of the GM Inspectorate attended meetings and contributed to best practice documents for the coexistence of GM and conventional crops in the EU. These documents synthesised the scientific evidence base on the cultivation and behaviour of GM crops and their potential interaction with conventional agriculture into practical recommendations for maintaining GM presence in conventional crops below acceptable thresholds. They are used by EU Member States to help them transcribe coexistence regulations into national legislation. The Inspectorate has contributed to work on three GM crops during the year:

potatoes (document in preparation); cotton (document under consultation with Member States) and stakeholders; and soya (recently published: http://ecob.jrc.ec.europa.eu/documents/BPDSoybean.pdf)

The European Enforcement Project (EEP) is a network of GM Inspectors across the EU who come together at an annual meeting to share experience and develop best practice. The annual meeting in 2015 was held in Austria and the GM Inspectorate presented a paper on the UK approach to contingency planning, as part of the aim to develop an EU-wide approach to dealing with incidents of unauthorised release of GMOs.

The GM Inspectorate led a work package under GMO European Research Area-Network (PreSto), which is a two-year FP7 EU/Defra-funded project. Expert elicitation and a multi-criteria decision analysis model were used to develop and prioritise a set of research gaps on the health, environmental and techno-economic impacts of GMOs. The work has now been submitted to the Commission along with a strategic implementation plan for the development of a GMO ERA-Net. Further information can be found at http://www.presto-gmo-era-net.eu/.

National Bee Unit

Under the programme of exotic pest surveillance (EPS) 2,500 apiaries were inspected (an additional 1,500 apiaries than in 2014). The network of sentinel apiaries at risk points and also at other sites was expanded to increase likelihood of detecting introductions at non-risk sites.

There has been an increased level of checks on the imports of bees from Italy following the confirmation of the Small Hive Beetle in the south west of Italy in 2014. Two field based two-day contingency exercises for the Small Hive Beetle (South West and in Wales) were carried out together with a desk top exercise for Asian Hornet.

MLST (multilocus sequence typing) of each American and European bacterial foul brood infection detected has been completed. This knowledge of the different profiles will help with the management of future outbreaks of this infection.

A comprehensive range of public and specialist national engagement events have been attended.

Surveillance Intelligence

APHA's mission is to develop and manage integrated veterinary scanning surveillance activities, and evaluate the effectiveness of the surveillance system, to enable the timely detection, investigation, characterisation, assessment and management of animal-related new and re-emerging threats and associated risks in livestock and wildlife in Great Britain[2]. This work is mainly led and coordinated by the APHA Surveillance Intelligence Unit (SIU).

Over recent years, veterinary scanning surveillance in England and Wales has been subject to several reviews, including the independent Surveillance Advisory Group (2012)[3] and 'Surveillance 2014'[4]. This has resulted in the development of a new surveillance model, placing greater emphasis on shared responsibility

[2] APHA Vet Gateway – Scanning surveillance: http://ahvla.defra.gov.uk/vet-gateway/surveillance/index.htm

[3] Surveillance Advisory Group Final Report, March 2012: http://webarchive.nationalarchives.gov.uk/20140707135733/http://www.defra.gov.uk/ahvla-en/files/pub-sag-final-report.pdf

[4] Surveillance 2014 - Changes to the delivery of Veterinary Scanning Surveillance in England and Wales, December 2013: http://webarchive.nationalarchives.gov.uk/20140707141417/http://www.defra.gov.uk/ahvla-en/files/surv-changes-delivery.pdf

and partnership working between government, universities, vets in practice and the livestock industries. This includes partnerships between APHA and non-APHA providers for post-mortem examination (PME) services and sources of animal health information and data.

Our strategic objectives:

- To coordinate activities enabling the timely detection, investigation and characterisation of animal-related new & re-emerging threats (NRTs) to agri-food supply chains, providing actionable intelligence for our customers and stakeholders across Great Britain (GB).

- To develop and maintain an integrated and sustainable 'early warning' scanning surveillance system for animal-related NRTs that delivers required outcomes through partnership between government and industry.

- For APHA to provide impartial, high quality veterinary scientific evidence, expertise and consultancy that supports and informs assessment and decision-making, and adds value to the activities of a wide range of stakeholders within and outwith Government, supported by different levels of expertise.

- To enable the management and mitigation of the risks and impacts arising from animal-related NRTs, by Government and/or the livestock industries.

A large amount of the work of the SIU during 2015 has also been directed to implementing and developing the scanning surveillance network in England and Wales, and associated activities and requirements following on from 'Surveillance 2014'.

Outcomes & Impact during 2015

During 2015, the work of the SIU and all those working in the physical and virtual networks of expertise and delivery that make up the scanning surveillance system in England and Wales has resulted in the identification, investigation and characterisation of 111 NRTs, of which 64 were present in GB. For the first time we have developed a systematic and standardised method of categorising these NRTs for each of the main livestock species and wildlife (Table 1). Information from surveillance partners, horizon-scanning or non-submission data sources detected 54 of the 111 threats (~50%). Of the 64 threats present in GB, a total of 43 (67%) were detected by post-mortem examination (PME) submissions to APHA, SAC-CVS or one of the five non-APHA PME providers. A further 14 threats were detected from analysis of scanning surveillance submissions data or non-PME submissions. Approximately half of all threats detected (56/111) were raised with the Veterinary Risk Group[5] as either Threats or Points for Information. Summary information that describes the identified threats by species is provided in Table 2. This approach will enable comparative analyses to be performed in future years using this baseline of threat metrics as and indicator of the key outcomes of scanning surveillance and Species Expert Group (SEG) activities.

The activities and outputs of the SIU, SEGs and APHA scanning surveillance activities encompass a very broad range of people and organisations that exist in the surveillance value chain[6]. Each SEG acts as a focus for veterinary investigation, collaborative surveillance intelligence gathering and analysis in their species, and for disseminating findings within APHA[7], with SAC, UK government policy officials and CVOs, vets in

[5] Anon (2016) Identifying new and re-emerging animal-related threats to the UK. *Veterinary Record* 2016;178:41, available online: http://veterinaryrecord.bmj.com/content/178/2/41.full.pdf+html

[6] Irvine, R.M. (2015) A conceptual study of value chain analysis as a tool for assessing a veterinary surveillance system for poultry in Great Britain. *Agricultural Systems*, Vol.135, pp143-158.

[7] Within APHA the work of the Veterinary Investigation Centres (VICs) and the Surveillance Laboratory Services

practice, BVA species divisions, universities, the livestock sector groups, AHDB and farmers. This leads to action at a range of scales, from industry-wide initiatives to individual farmers and vets adopting health planning and/or disease prevention, treatment or control measures on-farm. During the year this information has been communicated by a number of different channels to a range of different audiences, including:

- Fortnightly surveillance digest to APHA veterinary and scientific staff, non-APHA PME service partners, SAC-CVS veterinarians and GB policy customers.
- Monthly Threats report to the government's Veterinary Risk Group (VRG).
- Veterinary Record monthly report, of which a new format was developed[8] and published: http://veterinaryrecord.bmj.com/content/178/2.toc#Surveillance.
- Quarterly GB Emerging Threats reports, by species: http://ahvla.defra.gov.uk/vet-gateway/surveillance/reports.htm.
- Information notes and disease alerts on specific issues to farmers, vets, AHDB and livestock industry sector groups.
- Letters and other items in the Veterinary Record.
- Presentations at BVA specialist division meetings, livestock industry/sector group and other agri-food stakeholder conferences and meetings.
- Peer-reviewed publications and non-peer review articles.
- National and international veterinary and scientific conferences and symposia: presentations & posters.
- Training and CPD meetings – internal and external to APHA.
- The 2014/15 population reports from the Livestock Demographics and Data Groups (LDDG) for cattle, sheep, pigs and horses were published internally and externally. These reports help to inform policy and enhance understanding of livestock demographics and data interpretation.
- The first Surveillance Intelligence Forum was held in November 2015 which brought together colleagues involved in surveillance from across APHA to discuss and learn about existing and new surveillance initiatives and methodologies. This cross-cutting initiative aims to improve communication about surveillance across the agency and will be further developed to include a shared knowledge bank for publications and project reports.

In addition to the identified NRTs, the outputs of the scanning surveillance network provide an improved understanding of production-limiting or endemic diseases. Taking actions to control these diseases also helps protect herds and flocks from exotic and notifiable diseases. Examples include new approaches to the prevention and control of diseases like PED, Johne's Disease and BVD that are being driven forward by vets and industry. The APHA SEGs continues to work closely with the livestock industry sector groups and BVA specialist divisions to support these efforts. Most recently this has led to the government-assisted, but industry-led approach, resulting in PED being made a notifiable disease in England on 18th December 2015[9]. The work of the Pig Expert Group was instrumental in achieving this outcome after initially raising the threat of PED to government, pig vets and the pig industry following the emergence and spread of virulent PED from China to the USA.

Department (SLSD) is integral to NRT detection and investigation through its veterinary diagnostic and laboratory testing roles. Links with other areas of scientific expertise within APHA have been essential in investigating and characterising identified NRTs, specifically: Virology, Bacteriology, Sequencing, Pathology, Antimicrobial Resistance, Toxicology/Food safety, Non-statutory zoonoses, International Diseases Monitoring and Exotic Diseases Teams. Expertise, knowledge and skills from outwith APHA, including vets in practice, academia, policy officials, SAC and industry sector/stakeholder groups also contribute to and benefit from scanning surveillance and NRT detection and investigation.

[8] Irvine and others (2016) Changes to the monthly APHA disease surveillance report in *Veterinary Record. Veterinary Record;*178:50, available online: http://veterinaryrecord.bmj.com/content/178/2/50.1.full.pdf+html

[9] Gibbens, N. (2015) Porcine epidemic diarrhoea: Obligation to notify in England. *Veterinary Record*;177,24, pp628-629, available online: http://veterinaryrecord.bmj.com/content/177/24/628.3.full

Table 1: Animal-related threats summary from APHA Scanning Surveillance and Species Expert Group activities during 2015

APHA SIU THREATS SUMMARY: JANUARY- DECEMBER 2015 (Cattle, Small Ruminants, Pigs, Avian, Wildlife) [1]SS = scanning surveillance						
Type of threat	**Number of Threats detected and described**	**Threats present in GB**	**Threats detected from [1]SS data analysis or non PME submissions**	**Threats detected from [1]SS PME submissions**	**Threats detected by horizon scanning, other data source or surveillance partner**	**Number raised to Veterinary Risk Group (VRG)**
Notifiable disease-related	20	3	0	1	19	9
New disease or pathogen	12	6	2	3	7	11
New pathogen variant	8	3	0	2	6	5
New, rare or emerging AMR	5	5	1	2	2	3
Public health-related	9	5	0	3	6	9
Changes in endemic disease trends	23	20	7	9	7	7
Unusual diagnoses or presentations	23	14	1	19	3	7
Other	11	8	3	4	4	5
TOTALS 2015	**111**	**64**	**14**	**43**	**54**	**56**

Table 2: Summary description of animal-related threats detected by APHA Scanning Surveillance and Species Expert Group activities during 2015

Summary Description of Threat				
Avian	**Cattle**	**Pigs**	**Small Ruminant**	**Wildlife**
H7N7 HPAI confirmed in Lancashire (layer hens)	BTV4: Ongoing spread in Europe	Rise in African Swine Fever (ASF), wild boar, E. Europe	Sheep Scab (and new serological test at Moredun)	H5N2 HPAI epizootic in USA and migratory wild bird source
Direct transmission of Histomonas in turkeys	BTV8: Re-emerging in central France	ASF persistence in wild boar in Eastern Europe	Suspect Contagious Agalactia cases in imported sheep	New chytrid pathogen (Bsal) in captive amphibian site, England
H7N7 HPAI in Germany	First identification of bovine astrovirus in UK	Seneca A virus and associated disease in USA	Contagious Agalactia: current GB surveillance appropriate?	New amphibian CMTV like ranavirus: mortality in ESP/NL.
Mycoplasmosis in game birds increasing in GB	Besnoitiosis: First case in Irish Republic	New neonatal porcine diarrhoea syndrome report	BTV8: Re-emerging in central France & vaccine availability	Bornavirus in captive squirrels variegated & fatal zoonosis, DE
Transmissible diseases in backyard flocks, risks, GB	Haplotype cholesterol deficiency in Holsteins	Pandemic H1N1 2009 virus, India: HA gene mutations	Sheep & Goat Pox: risk to UK, illegal imports from Turkey	Hedgehogs in GB: 3x potential zoonotic pathogens detected
Green livers in backyard chickens	Interstitial pneumonia cases rise in Scotland	Pathogenic orthoreovirus from diarrheic pigs, USA	Peste de Petite Ruminants (PPR) outbreaks in Morocco	Threats to health from toxic blue-green algae, N. England
Change *M.gallisepticum* vaccine vaccine strain	USA: Bovine leukaemia virus, link to breast cancer	Virulent PEDV entry to USA: reusable tote bags?	Neospora causing deformity in aborted lambs	Tularemia risk of incursion into GB (cases in NL & Sweden)
LPAI: Cases of H5N2 (NL) & H7N7 (DE)	Jejunal Haemorrhage syndrome investigation	Virulent PEDV strain similar to US OH-851, INDEL in EU	*Coxiella burnetti* in large milking goat herd (raw milk)	'Vector risks to the UK' by PHE Threats to UK human health
S. Enteritidis in several broiler premises in GB	Idiopathic necrotic enteritis investigation	*Klebsiella pneumoniae* (Kpp) septicaemia: 1st case, Yorkshire	*C. burnetti* in second goat herd in England	Asian hornet incursion in continental Europe
Inclusion body hepatitis in broilers	*Chlamydophila abortus*: Cattle in Cumbria	Evolution of Eurasian H1N1 swine influenza virus strains	Schmallenberg: Cases in NL suggest virus is circulating	False positive point-of-care influenza A virus tests in seals
'Spotty liver' layer hens: new *Campylobacter* spp	Hereditary Neuraxial oedema in Herefords	Rapid global increase of variant PCV2b/d & PCVAD	OIE listed diseases: reporting & surveillance data risks	False positive West Nile Virus report for England on ProMED
Pullorum disease: fancy fowl & pheasant chicks	JH1 disease in Jerseys (China, stock from USA)	New cluster of PCV type 2b strains in pigs in Germany	BVD in neonatal lamb: risks to cattle control programmes	Mass mortality of reptiles and amphibians after heath fires
HPAI in EU: H5N8, H5N2, H5N1 (classical)	*Mycoplasma wenyonii* and *candidatus M.haemobos* in dairy herds in Cumbria	Antimicrobial resistance: Colistin, plasmid-mediated	Ethylene Glycol poisoning	DDT product residues detected in Scottish wildlife food chain
LPAI: H5N2, H7N2 & H7N7 in EU poultry		Rise in Ampicillin-resistant *A. pleuropneumoniae*	Autoimmune anaemia from cow colostrum in lambs	Possible nitrite/nitrate/fertiliser toxicity in swans
H7N7 LPAI in Hants, England		Penicillin sensitivity change in clinical *Strep suis* isolates	Oak processionary moth: SE England, horses/animal risk	*Alaria alata:* trematode, wild mammals, EU & zoonotic risks
Changes in colibacillosis incidence in chicks		Coccidiosis in older pigs in England	Bilaterial microphthalmia in Texel lambs (SE England)	Migration of British bats to and from EU (Nathusius' pipistrelle)
Broiler enterococcal & streptococcal infections		Increased diagnosis rate of *Haemophilus parasuis*, GB	Border disease (BD): neonatal lambs, with hydronephrosis	
Histomonosis increased incidence in chickens		Changes in monophasic *S.* Typhimurium-like variants, GB	GB: BD 'Hairy shakers' & BVD abortion storm, sheep flocks	
Tetratrichomonosis: GB, new partridge disease		Unusual bacterial causes of abortion in pigs in GB	GB: Serological evidence of Schmallenberg virus in lambs	
Histomonas meleagridis genotype 2 in Germany		Severe reproductive disease due to porcine circovirus 2, GB	Weather impacts in GB due to strong El Niño event	
HPAI H5N1, H5N2 and H5N9 in France		1st swine dysentery case in East Anglia since May 2014	Zwartbles breed: Hereditary Neuraxonal Dystrophy, GB	
H7N7 LPAI in broiler breeders in Scotland		Metabolic bone disease, GB: growing/lactating pigs	Nitroxynil toxicity in lambs: 4x overdose by wrong dosing route	
Fowl cholera (*P. multocida*) in geese		Continuing upward trend of disease due to *E coli* in GB		
		Acute salmonellosis in finishers resembling PED		
		Risks from rise in wild boar population, Forest of Dean		

Threats that are shown as shaded in blue are those identified as being outside the UK at the time of the report.

The total number of threats in Table 2 will not be the same as in Table 1 as some threats continue to be assessed by the VRG.

Tuberculosis

This year we refreshed our TB science strategy, which is focused around supporting the eradication of bovine tuberculosis (bTB) from GB. A summary of this strategy is given below.

We perform surveillance forbTB in order to meet legal obligations, demonstrate freedom from disease, and permit international trade. We support the government's statutory obligations via reference laboratory activities, also maintaining our expertise and the appropriateness of our tests. We provide expert advice and consultancy on bovine TB, allied tests and their interpretation, in support of government policy. We also provide data for epidemiological analysis, including the geographical location of *Mycobacterium bovis* and identify gaps in diagnostic capability and develop new tests where necessary.

Under the TB Surveillance Contract funded by the governments of England, Wales and Scotland we conduct the following main activities:

- Undertake diagnosis on skin test positive animals, slaughter house cases as well as non-bovine samples
- Produce the bTB surveillance reports for GB, England and Wales.
- Contribute to the production of the annual EU co-funding claim report and TB Eradication Strategy
- Respond to *ad hoc* requests for data and reports.
- Provide post-mortem capacity for investigation of all suspect animals and laboratory capacity for gamma interferon testing of cattle.
- Generate quarterly statistics on pre-movement testing monitoring & compliance.
- Generate regular reports, including statistics on all non-bovine TB cases, interferon-gamma test usage, TB culture and slaughterhouse submissions.
- Advise on the management, supply, and performance of tuberculin.
- Hold the Marketing Authorisation for BadgerBCG injectable vaccine.

We maintain a strong and innovative research portfolio on bovine TB of importance to GB government, in support of policy and in order to maintain competency and up to date knowledge of the disease. In line with the APHA Science Strategy our research is predominantly applied, translational and customer focussed. The key research activities are applied in nature and focussed around early and accurate detection of infection or the pathogen, the most effective deployment of existing diagnostic methods and the development of new ones, data collection to track the spread of disease and to feed into mathematical models to help monitor progress of disease control, epidemiological understanding of the disease from the molecular to the macro level, and development of intervention tools such as vaccines. The applied science is underpinned by more fundamental research of host-pathogen interactions, pathogenesis and disease transmission.

Epidemology, Risk Analysis and Data Sciences

Information bTB (ibTB www.ibtb.co.uk) is our interactive tool mapping bTB outbreaks in England over the previous five years. It was launched in July 2015 as part of the Government's 25-year strategy to eradicate bTB and is a response to the farming industry's wish to be better informed with regard to local TB risks. The tool allows farmers, vets and the general public to access information on local bTB incidents ('breakdowns') and their status.

The national badger sett survey performed by APHA (Judge et al 2014 Nature Scientific Reports 4, 3809) and the Social Group Size estimation project produced estimates of the mean number of social groups and numbers of badgers per social group respectively per Land Class Group for England and Wales. Monte

Carlo resampling procedures using the raw data from the Badger Sett Survey and Social Groups Size project were carried out to produce estimates of population size for various areas of GB.

The TB Surveillance Reports for England, Wales and GB are published on our website https://www.gov.uk/government/publications/bovine-tb-surveillance-in-great-britain-2014 and http://gov.wales/topics/environmentcountryside/ahw/disease/bovinetuberculosis/bovinetberadication/?lang=en. We also helped develop a TB dashboard for Wales which is used by policy holders, the CVO Wales and Ministers to track the progress of TB Eradication in Wales.

The development of a risk-based trading farm scoring system may assist with the control of bovine tuberculosis in cattle in England and Wales. Identifying and ranking cattle herds with a higher risk of being or becoming infected on known risk factors can help target farm biosecurity, surveillance schemes and reduce spread through animal trading. A quantitative approach was used to develop risk scores, based on the probability of infection in a herd with bTB, for use in a risk-based trading (RBT) scheme in England and Wales. To produce a practical scoring system the risk factors included need to be simple and quick to understand, sufficiently informative and derived from centralised national databases to enable verification and assess compliance. A logistic regression identified herd history of bTB, local bTB prevalence, herd size and movements of animals onto farms in batches from high risk areas as being significantly associated with the probability of bTB infection on farm. Risk factors were assigned points using the estimated odds ratios to weight them. The farm risk score was defined as the sum of these individual points yielding a range from 1 to 5 and was calculated for each cattle farm that was trading animals in England and Wales at the start of a year. Within 12 months, of those farms tested, 30.3% of score 5 farms had a breakdown (sensitivity). Of farms scoring 1-4 only 5.4% incurred a breakdown (1-specificity). The use of this risk scoring system within RBT has the potential to reduce infected cattle movements; however, there are cost implications in ensuring that the information underpinning any system is accurate and up to date. A pilot trial of the use of these risk scores is underway as part of the provision of farm level data packs to farms within badger cull areas to help farmers manage risk pathways of TB infection into their herds.

A mathematical method for estimating the endemic status of bTB in cattle in England and Wales was developed. 6.25km(2) hexagonal cells were used as the base resolution. Maps were produced for overlapping two-year periods spanning 2001/03 to 2009/11. Distance from a farm to the ten nearest 'Officially Tuberculosis Free status - Withdrawn' incidents within the same time period was measured. Endemic areas were defined as those hexagons containing farms where the 3rd nearest incident occurred within 7km. Temporal spread of endemic bTB was estimated by creating a contour map displaying the spread of endemic bTB over the two-year periods, and using boundary displacement to calculate the rate of spread across each hexagon. A rate was obtained for ~2300 cells and varied between 0.04km and 15.9km per year (median=3.3km per year). This work will enable further analysis of the factors associated with this expansion and has helped target increased interventions in within the 'Edge' areas of England where TB is spreading rapidly.

Laboratory Testing

APHA is the national and OIE Reference Laboratory for bTB. A number of tests are carried out in support of TB control.

Gamma Interferon Testing Delivery. The number of tests carried out in TB breakdown herds in GB during 2015 totalled 104,402. In June alone 10,449 tests were carried out, the highest monthly figure recorded. Of the total samples tested, 71% were from English holdings, 1% from Scottish herds and almost 28% from Welsh holdings, approximately half of which were tested at the new Carmarthen gamma laboratory which had been set up following a request from the Welsh Government. Carmarthen will be steadily increasing their intake until all Welsh submissions are tested at Carmarthen. The expansion of laboratory capacity will also help underpin increased use of gamma interferon testing in the high risk areas of England in support of Defra's TB Eradication strategy for England.

Culture of *Mycobacterium bovis* - over 2,500 *M. bovis* isolates were obtained from over 10,000 tissues submitted from animals suspected of being infected with *M. bovis*.

Camelid serology tests - these were established at our laboratory in Starcross for private and statutory TB testing and approximately 2,000 tests were performed. The major antigen used in these assays, MPB83, was discovered by our TB Research Group in the 1990s. It is one of the major antigen used internationally for serodiagnosis of TB in a wide range of species including badgers, camelids, cattle and man.

***M. bovis* Genotyping** - over 2,500 *M. bovis* isolates were genotyped in 2015 in order to support field epidemiological investigations into the likely sources of bTB herd breakdowns . This work underpinned the detailed analysis of all lesion- or culture-positive (OTF status withdrawn) herd breakdowns in the Low Risk Area of England (LRA) in 2015 and helped demonstrate that more than half of those breakdowns were caused by introductions of infected cattle from higher risk areas of GB without further dissemination to other herds. The evidence generated by these analyses will be used to support Defra's application to the European Commission for official recognition of the LRA as a new TB Free region of the UK in 2018.

We collaborated with the Belgian *M. bovis* Reference Laboratory to genotype and identify the origin of a strain of the bacterium isolated from tuberculous alpacas exported to Belgium from Devon. This was the first documented (and bacteriologically confirmed) example of spread of *M. bovis* through international trade of live South Amercian camelids.

We performed diagnosis and genotyping of a cluster of three closely linked, but independent, cases of *M. bovis* infection in cats in Malvern, Worcestershire.

We have developed methods to perform whole genome sequencing (WGS) of strains of *M.bovis* directly from stored samples. This innovation will make WGS of historical samples much cheaper and simpler. We have also continued to apply WGS to specific incidents of epidemiological significance such as human cases, cattle dispersals and TB in cats.

Seminal Paper

A paper published by our TB researchers in collaboration with colleagues at the Institut Pasteur crossed the coveted academic milestone of 1000 citations according to Google Scholar. The paper entitled 'A new evolutionary scenario for the *Mycobacterium tuberculosis* complex' was published in 2002 in the Proceedings of the National Academy of Sciences of the United States of America (PNAS) and was underpinned by information that emerged from the *M. bovis* genome sequencing project that we led. It had always been claimed that TB was a disease of zoonotic origin and that humans had contracted it from infection with a cattle form of the bacillus – form *M. bovis.* In this paper we identified a series of genetic markers which had been deleted from the genome of some species—such as *M. bovis* or BCG (Bacille Calmette-Guérin), the strain used in vaccines – and found that these deletions had occurred after *M. bovis* and *M. tuberculosis* had separated. Hence the ancestral strain was more like *M. tuberculosis* and *M. bovis* was descended from it. This finding suggested that the theory in the literature was wrong and that humans could not have acquired the disease from cattle.

This work also resulted in the molecular definition of each member of the *M. tuberculosis*-complex and has helped in supporting revisions to the OIE Trade Chapter (see below) to include other members of the *M. tb*-complex. This knowledge has been exploited to develop *M. bovis*-specific tests including PCR based assays. Also, by identifying that pieces of genetic code were lost from the genome in a stepwise fashion as the *M. tuberculosis* complex evolved, our researchers and elsewhere have been able to exploit these differences to develop diagnostic tests for animals and humans that differentiate between TB infected and BCG vaccinated individuals (DIVA tests).

Cattle Vaccines and Diagnostics

A novel method for estimating specificity of the Single Intradermal Comparative Cervical Tuberculin (SICCT) test for bTB using surveillance tests results was developed in collaboration with the University of Cambridge. The specificity of the SICCT test at three cut-offs was estimated from the dates, locations and skinfold measurements of all routine tests carried out in Officially TB Free (OTF) cattle herds in Great Britain (GB) between 2002 and 2008, according to their separation (by distance and time) from known infected (OTF-withdrawn) herds. The proportion of animals that tested positive was constant (P>0.20) when the distance between tested herds and nearest infected herd exceeded 8 km. For standard cut-off, calculated specificity was 99.98 per cent (95 per cent confidence interval ±0.004 per cent), equating to one false positive result per 5000 uninfected animals tested. For severe cut-off it was 99.91 per cent (±0.013 per cent) and for ultrasevere cut-off (selecting all reactors and inconclusive reactors) it was 99.87 per cent (±0.017 per cent). The estimated positive predictive value of the test averaged 91 per cent and varied by regional prevalence. This study provides further evidence of the high specificity of the SICCT test under GB conditions, suggests that over 90 per cent of cattle currently culled using this test in GB were infected, and endorses slaughter of at least these cattle for bTB control.

In collaboration with Ag Research, New Zealand we are working on the development of defined skin test reagents. The single intradermal tuberculin skin test (using bovin tuberculin only) is the primary screening test for the diagnosis of TB (outside the UK and Ireland, and use of this test has been very valuable in the control of this disease in many countries. However, the test lacks specificity when cattle have been exposed to environmental mycobacteria or vaccinated with *M.bovis* bacille Calmette-Guérin (BCG). Recent studies at APHA showed that the use of three or four recombinant mycobacterial proteins or a peptide cocktail derived from those proteins, in the skin test greatly enhanced test specificity, with minimal loss of test sensitivity. The proteins are present in members of the pathogenic M. tb-complex but are absent in or not expressed by the majority of environmental mycobacteria and the BCG vaccine strain. To produce a low-cost skin test reagent, the proteins were displayed at high density on polyester beads through translational fusion to a polyhydroxyalkanoate synthase that mediates the formation of antigen-displaying inclusions in recombinant *E. coli*. Display of the proteins on the polyester beads greatly increased their immunogenicity, allowing for the use of very low concentrations of proteins (0.1 to 3 µg of mycobacterial protein/inoculum) in the skin test. Polyester beads simultaneously displaying all four proteins were produced in a single fermentation process. The polyester beads displaying three or four mycobacterial proteins were shown to have high sensitivity for detection of *M. bovis*-infected cattle and induced minimal responses in animals exposed to environmental mycobacteria or vaccinated with BCG.

In collaboration with the National Animal Disease Centre, Ames Iowa and the Albert Einstein College of Medicine, New York we are working on the identification of biomarkers for bTB. T helper (Th) 17-associated cytokines are integral in the immune response to tuberculosis, initiating both protective and harmful inflammatory responses. The aim of this study was to evaluate applied aspects of IL-17 biology in the context of *M. bovis* infection of cattle. Using RNA-seq, numerous Th17-associated cytokine genes were up-regulated > 9 fold in response to purified protein derivative stimulation of peripheral blood mononuclear cells from experimentally *M. bovis*-infected cattle. Protective vaccines elicited IL-17A, IL-17F, IL-22, and IL-27 responses. As compared to non-vaccinated animals, reduced IL-17A responses by vaccinate recipients at 2.5 weeks after *M. bovis* challenge correlated with reduced disease burden. Additionally, IL-17A and IFN-γ responses were highly correlated and exhibited similar diagnostic capacity. Present findings support the use of Th17-associated cytokines as biomarkers of infection and protection in the immune response to bovine tuberculosis.

In collaboration with Imperial College, London and McMaster Immunology Research Centre, Canada we are developing improved cattle vaccines against bTB. Much experimental data indicates that pulmonary local immunity is important for protection against respiratory infections including *M.tb* and that pulmonary immunisation is highly effective. We therefore evaluated protection against *M. bovis*, the main causative agent of bTB, conferred by BCG delivered subcutaneously, endobronchially or by the new strategy of simultaneous immunisation by both routes. We also tested simultaneous subcutaneous immunisation with

BCG and endobronchial delivery of a recombinant type 5 adenovirus expressing mycobacterial antigen 85A. There was significantly reduced visible pathology in animals receiving the simultaneous BCG/BCG or BCG/Ad85 treatment compared to naïve controls. Furthermore, there were significantly fewer advanced microscopic granulomata in animals receiving BCG/Ad85A compared to naive controls. Thus, combining local and systemic immunisation limits the development of pathology, which in turn could decrease bTB transmission.

In collaboration with University of Cambridge Vet School we are modelling potential benefits of cattle vaccination as a supplementary control for bTB. Vaccination for the control in cattle is not currently used within any international control program, and is illegal within the EU. Candidate vaccines, based upon *M. bovis* bacillus Calmette-Guérin (BCG) all interfere with the action of the tuberculin skin test, which is used to determine if animals, herds and countries are officially bTB-free. New diagnostic tests that Differentiate Infected from Vaccinated Animals (DIVA) offer the potential to introduce vaccination within existing eradication programs. We used within-herd transmission models estimated from historical data from GB to explore the feasibility of such supplemental use of vaccination. Results showed that the economic impact of bTB for farmers is dominated by the costs associated with testing, and associated restrictions on animal movements. Farmers' willingness to adopt vaccination will require vaccination to not only reduce the burden of infection, but also the risk of restrictions being imposed. We found that, under the intensive sequence of testing in GB, it is the specificity of the DIVA test, rather than the sensitivity, that is the greatest barrier to see a herd level benefit of vaccination. The potential negative effects of vaccination could be mitigated through relaxation of testing. However, this could potentially increase the hidden burden of infection within Officially TB Free herds. Using our models, we explored the range of the DIVA test characteristics necessary to see a protective herd level benefit of vaccination. We estimate that a DIVA specificity of at least 99.85% and sensitivity of >40% is required to see a protective benefit of vaccination with no increase in the risk of missed infection. Data from experimentally infected animals suggest that this target specificity could be achieved in vaccinates using a cocktail of three DIVA antigens while maintaining a sensitivity of 73.3% (95%CI: 61.9, 82.9%) relative to post-mortem detection.

In collaboration with Leibniz Centre for Medicine and Biosciences, Borstel, Germany we are working on the identification of novel mycobacterial lipid antigens. Mycobacterial lipids play an important role in the modulation of the immune response upon contact with the host. Using novel methods, we isolated highly purified phosphatidylinositol mannoside (PIM) molecules from virulent *M. tuberculosis* to assess their potential to stimulate peripheral blood mononuclear cell (PBMC) responses in *M. bovis*-infected cattle. Of these molecules, one ($AcPIM_6$) induced significant levels of gamma interferon (IFN-γ) in bovine PBMCs. Three PIM molecules ($AcPIM_6$, Ac_2PIM_2, and Ac_2PIM_6) were shown to drive significant proliferation in bovine PBMCs. $AcPIM_6$ was subsequently used to phenotype the proliferating cells by flow cytometry. This analysis demonstrated that $AcPIM_6$ was predominantly recognized by $CD3^+$ $CD335^+$ NKT cells. In conclusion, we identified PIM lipid molecules that interact with bovine lymphocyte populations, and these lipids may be useful as future subunit vaccines or diagnostic reagents. Further, these data demonstrated, for the first time, lipid-specific NKT activation in cattle.

Genetic Resistance of Cattle to bTB

Our comprehensive database on cattle TB test results was supplied to the Roslin Institute who used it to identify a number of genetic signatures associated with a reduced susceptibility to TB in the offspring of certain dairy bulls. This work led to the launch of the 'TB Advantage' trait at the beginning of 2016. This is a new genetic index published by AHDB Dairy, which can help dairy farmers make informed decisions when selecting specific bulls to breed cattle with an improved likelihood of resistance to bTB.

The index follows extensive research into the genetics of bTB, undertaken jointly by the University of Edinburgh, Roslin Institute and Scotland's Rural College (SRUC), and which was supported by Defra and the Welsh Government. Their work showed genetic variation between animals, and formed the basis of the TB Advantage; the first genetic index of its kind in the world. Using data on over 650,000 Holstein cows who have bTB data recorded by APHA, breeding patterns have been established and more resistant bloodlines

identified. Initially, the TB Advantage will only be available for the Holstein breed, but work is under way to establish if the index can be extended to other dairy and beef breeds, in the longer term.

In a Wellcome Trust funded collaboration with Trinity College Dublin, the Amauer Hansen Research Institute and Institute of Pathobiology Ethiopia we are working on the admixture mapping of tuberculosis resistance in an African-European hybrid cattle population**.** Admixture mapping affords a powerful approach to genetic mapping of complex traits and may be particularly suited to investigation in cattle where many breeds and populations are hybrids of the two divergent ancestral genomes, derived from *Bos taurus* and *Bos indicus*. In this study we designed a minimal genome wide SNP panel for tracking ancestry in recent hybrids of Holstein-Friesian and local Arsi zebu in a field sample from a region of high bTB endemicity in the central Ethiopian highlands. We first demonstrate the utility of this approach by mapping the red coat color phenotype, uncovering a highly significant peak over the MC1R gene and a second peak with no previously known candidate gene. Secondly, we exploited the described differential susceptibility to BTB between the ancestral strains to identify a region in which *Bos taurus* ancestry associates, at suggestive significance, with skin test positivity. Interestingly, this association peak contains the toll-like receptor gene cluster on chromosome 6. With this work we showed the potential of admixture mapping in hybrid domestic animals with divergent ancestral genomes, a recurring condition in domesticated species.

Evaluation of Badger Diagnostic Tests

PCR - detection of *M. bovis* by qPCR allows the presence of faecal shedding and hence infectious badgers to be established non-invasively and raises the possibility of identifying infectious social groups. In collaboration with the University of Warwick, we developed a noninvasive diagnostic procedure and sampling regimen using field sampling of latrines and detection of *M. bovis* with quantitative PCR tests, the results of which strongly correlate with the results of immunoassays in the field at the social group level. This method allows *M. bovis* infections in badger populations to be monitored without trapping and provides additional information on the quantities of bacterial DNA shed and the opportunity to assess spatio-temporal variations in the environmental distribution of this potential source of infection for cattle, badgers and other wild mammals. One likely route of transmission to cattle is through exposure to infected badger urine and faeces. The relative importance of the environment in transmission remains unknown, in part due to the lack of information on the distribution and magnitude of environmental reservoirs. In a further collaborative study with the University of Warwick, we identified potential infection hotspots in the badger population at Woodchester Park and quantified the heterogeneity in bacterial load; with infected badgers shedding between 1×10^3- 4×10^5 *M. bovis* cells g^{-1} of faeces, creating a substantial and seasonally variable environmental reservoir.

Badger gamma Interferon assay -using data collected from longitudinal studies of naturally infected wild badgers, we found that that the magnitude of the IFN-γ response to *M. bovis* antigens at the disclosing test event was positively correlated with subsequent progression of disease to a seropositive or excreting state. In addition, we show that the magnitude of the IFN-γ response, despite fluctuation, declined with time after the disclosing event for all badgers, but remained significantly higher in those animals with evidence of disease progression.

Understanding *M. bovis* Zoonosis in Developing Countries

We have been collaborating with researchers from Ethiopia and the UK to understand the risk pathways for *M. bovis* infection in cattle and humans in Ethiopia, a high tuberculosis burden country, which reports one of the highest incidence rates of extra-pulmonary TB dominated by cervical lymphadenitis (TBLN) and to provide evidence to the Ethiopian Government to develop effective interventions for TB control. This work has been ongoing for 13 years and has been funded by the Wellcome Trust, DIFD, The Bill and Melinda Gates Foundation and the BBSRC and DIFD through the recent ZELS initiative. The latter proposal is a collaborative project lead by Cambridge University and builds on our previous work.

Our previous work has shown that infection with *M. bovis* is not the main reason for the high rate of

extrapulmonary human TB in Ethiopia, has detected natural *M. tuberculosis* infection in cattle demonstrating the potential for reverse zoonosis (which provided useful supporting evidence for the inclusion of *M. tuberculosis* infection of cattle in the OIE Trade Manual), has provided Defra with evidence on the efficacy of BCG vaccination in cattle under natural transmission conditions and highlighted the risks associated with intensification of cattle farming.

The project has also provided insights into the origin and spread of human tuberculosis in Africa which were published this year in Current Biology. In summary, although a detailed medical history of Ethiopia supports the view that human TB was rare until the 20th century, over the last century Ethiopia has become a high-burden TB country. Our results provided further support for an African origin for human TB, with some genotypes already present on the continent well before European contact. Phylogenetic analyses revealed a pattern of serial introductions of multiple genotypes into Ethiopia in association with human migration and trade. In place of a "virgin soil" fostering the spread of TB in a previously naive population, we proposed that increased TB mortality in Africa was driven by the introduction of European strains of *M. tuberculosis* alongside expansion of selected indigenous strains having biological characteristics that carry a fitness benefit in the urbanized settings of post-colonial Africa.

Stakeholder Engagement and Knowledge Transfer

We were involved in producing training videos for Official Veterinarians (OVs) in support of the Welsh Government's Cymorth TB initiative; providing CPD on bovine TB at OV Conferences organised by Improve International; presenting an overview of TB vaccination at a conference celebrating 150 years of veterinary medicine; and providing a TB Science Day to inform OVs from Wales on the latest scientific developments on bTB. One of our scientists also took part in a Q and A session with the CVO Wales at the Hay Festival.

We were involved in two OIE *ad hoc* expert Groups (Co-Chair and Rapporteur). These groups revised the OIE Trade Manual for bTB and produced recommendations to produce a new International Standard for bovine tuberculin.

Two of our scientists co-organised a Bill and Melinda Gates Foundation-Sponsored Workshop on 'Accelerating bTB Control in Developing Countries'.

APHA scientists led an international initiative to produce a gap analysis for bTB through two EU Funded initiatives – DISCONTOOLS and the Global Research Alliance for Bovine Tuberculosis (GRAbTB).

TSE and Animal By-Products

During 2015, Defra and the devolved administrations re-iterated their commitment to APHA and its TSE and Animal by-product programme of work, and provided the following guidance on its priorities in the short, medium and long term:

- **Short Term** - maintain risk-based BSE controls and surveillance, in line with EU requirements, to continue to reduce the annual number of new BSE cases and protect our OIE 'controlled risk' status. To also maintain and improve our current diagnostic capability for use in TSE surveillance.

- **Medium Term** - To continue to support UK statutory obligations and maintain minimum capability and capacity to detect and respond to new and emerging TSEs, inform cost-proportionate changes to regulations relating to existing TSEs and support NRL/EURL function. The R&D programme builds on work carried out in the surveillance programmes. Also evidence in support of OIE 'negligible risk' status for BSE in the UK.

- **Medium / Longer Term** - Although classical BSE has been virtually eradicated and scrapie persists only at very low levels, there is some uncertainty around atypical BSE and it is important that we have the capacity and expertise to support future policy development. Therefore we will collaborate with FSA, DH and APHA to develop a research protocol for further studies aiming to gain additional data on atypical BSE.

Fundamentally, these priorities are linked to maintaining and developing an understanding of TSEs in Europe and worldwide. APHA is both the EU reference laboratory and an OIE reference laboratory for BSE and scrapie, and continues to wield considerable influence in the area of TSEs via its publications,(24 peer-reviewed publications in 2015) and its contributions to the EFSA Risk Assessment forum which represents the key advisory fulcrum for EU food and feed legislation. APHA contributes actively to the various national committees which advise Defra, Scottish and Welsh Governments, the FSA and Departments of Health, including the Advisory Committee for Dangerous Pathogens TSE Sub Group.

APHA Impacts via the EFSA forum and ACDP TSE SG

APHA staff were founder members of the EFSA BioHaz and Animal Health and Welfare panels in 2003 and the Agency continues to maintain its contribution to the work of EFSA with four members of staff, including one TSE specialist, on the current Panels. There has been *ad hoc* involvement of various staff on TSE working groups;"Protocol for further laboratory investigations into the distribution of infectivity of atypical BSE" (EFSA-Q-2013-01015), "Concerning the zoonotic potential of ovine scrapie prions" (EFSA –Q-2015-00048) and the "Annual report of the Scientific Network of the BSE-TSE 2015" (EFSA-Q-2015-00738) . In particular, the EFSA scientific opinion on "The situation of scrapie in the EU after 10 years of monitoring and control in sheep and goats" (EFSA Journal, 2014;12(7):3781, 155 pp) that was published in 2014 made substantial use of recent outputs from APHA research and surveillance projects funded by Defra, Scottish and Welsh Governments and by the EU via the EURL - a clear example of research outputs influencing international bodies, and eventually European policy.

In 2014/15, our Epidemiology and Risk Assessment group also built and developed under contract to EFSA the iTSE infectivity model which is used to evaluate changes in TSE surveillance approaches Europe-wide and whose outputs provided the scientific rationale for the latest changes in the list of specified bovine risk material such as continued exclusions of sections of intestine and mesentery from the food chain.

On the domestic front in 2015, our staff briefed FSA and Defra colleagues, and the ACDP TSE SG on a broad range of issues including the iTSE model and the basis for European law changes relating to bovine SRM, and the use of whole genome sequencing of confirmed BSE cases to search for a possible causative mutation and test the Ferguson-Smith hypothesis. In particular APHA contributed to discussions regarding the ongoing five-year project in which APHA and the Institute of Neurology are carrying out the immunohistochemical screening of 40K human appendix tissues, collected both pre- and post the BSE epidemic, to generate control data for the previous study of human appendices collected during the BSE epidemic (Gill et al, BMJ. 2013 Oct 15;347).

OIE/EURL/NRL Activities and Impact

Subject	Historical	2015	Overall Impact
Storage and release of reference tissues from UK field suspects	This is managed through the TSE Archive. Many NRLs – including the UK NRL – have been supplied with positive control tissue	Reducing number of requests. Mostly internal to support reference panels and batch testing	Supply of positive material to countries with low or zero prevalence. Tissues also supplied in support of test evaluation exercises, and troubleshooting activities
Experimental production of atypical scrapie atypical BSE, classical ovine BSE and classical caprine BSE.	21 ovine BSE 18 atypical scrapie 9 caprine BSE 6 bovine L-BSE 6 bovine H-BSE. Two goats challenged with atypical scrapie did not succumb	One sheep challenged ic with BSE in 2005 with a 'resistant' genotype (T112M) succumbed more than 10 years after challenge	Used as above, in addition to natural cases, to provide full panel of agent strain/host combinations. Provides information on genetic effects on susceptibility in small ruminants, and data on stability of agents on subpassage – supplements data from research projects and provides material for collaborative studies. Material collected from the bovine challenges was included in the hypothetical experimental plan published by EFSA in 2014
Strain typing Expert Group - Bioassay of unusual isolates	Group has met 13 times since its inception to advise on specific cases. In total there have been 16 referrals (10 sheep and 6 goats), from UK, France, Cyprus and Poland.	Two caprine isolates (one French and one Polish) are currently under investigation. Independently, as an OIE ref lab, we also strain-typed the two Brazilian BSE cases, confirming them as H-BSE.	The remit of STEG is to discuss the approach to classification of unusual small ruminant samples in which statutory testing cannot rule out BSE. The COM will only confirm small ruminant BSE if this classification is ratified by STEG. The group was also asked to provide formal guidelines for the classification of H-BSE and L-BSE, which were based on a publication by one of the STEG members, and is hosted on the TSE-LAB-NET website. The group ratified the data supporting the confirmation of naturally-occurring caprine BSE in a French goat and (retrospectively) a Scottish one. Such referrals provide excellent 'unusual cases' with which to establish the diagnostic and classification strengths and limitations of currently available methods, and indicate test research and development options. They also help to maintain and strengthen our diagnostic interpretation skills base, and provide a very strong panel of positive controls against which we can compare any UK cases.
Annual workshop, and communication functions, external quality assurance, training	This has grown as the EU membership has grown, and is now attended by over 50 people from all 27 MS plus the EEA. These workshops are 'closed' and are only for NRL representatives	This year was the 14th annual workshop. The webpages are open access, and facilitate the provision of advice and consultancy globally	In addition to representation from all of the MS, Switzerland, Norway and Iceland also send regular representation helping to build a very established communication network with a high degree of trust and openness. The COM also attends annually, and the rapid test manufacturers and EFSA have also actively contributed to past meetings. It is highly valued by those who attend

Science Highlights from Defra-funded TSE R & D Projects (ongoing or recently completed)

We have a portfolio of projects that address a range of questions relating to disease pathology, pathogenesis and epidemiology, the molecular and biological phenotypes of TSE, the transmissibility of strains, and their stability following their passage through a range of hosts. The following highlights give examples of the relevance of this data to the current policy questions.

BSE - Towards a worldwide market for British beef

Britain is classified by the OIE as "Controlled Risk" with respect to its risk of BSE and is working towards OIE Negligible BSE Risk (NBR) status, a situation where all BSE-related restrictions in the trade of British cattle and cattle products worldwide would be lifted. Two BSE cases were confirmed in Britain in 2015 and this will delay application for OIE NBR status until 2020. In addition to the APHA Operational Delivery Network identification and removal from the food and feed chain of any cohort animal related to the index case or

which had shared feed rations with the case, APHA also follow up each new case with a detailed epidemiological investigation and strain-typing in mice to determine its likely origin and phenotype. This all helps to provide assurance that our surveillance system and feed controls are functioning effectively, and that all the information necessary for full OIE classification is available.

BSE - Atypical BSE

Amongst the more than 200,000 cases of classical BSE confirmed worldwide, some 90 atypical cases, dubbed H- and L-type on the basis of their molecular features, have been detected through active surveillance. The OIE has been asked to consider declassifying atypical BSEs as TSEs on the basis that they do notappear to have an infectious aetiology and that countries reporting atypical case should retain negligible BSE risk status and not be restricted in trade. Currently atypical and classical cases of BSE are treated as the same disease. There would be a particular impact of this change of classification in the area of animal by-products and the worldwide trade of bovine meat and animal feed.The EU commissioned EFSA to review knowledge of atypical BSE, and to support this, asked member states to re-type historical cases of BSE (from 2003 onwards). APHA recently completed this review of more than 1000 BSE cases, confirming that the prevalence of atypical BSE has been very low throughout.

The sporadic occurrence of atypical BSE has been interpreted by some as evidence for an epigenetic or genetic origin of prions within an affected animal. EU-funded transmission studies to cattle of these atypical forms, at APHA have helped define the incubation period and clinical characteristics of these diseases when inoculated intra-cerebrally (ic) and confirmed that the differences from classical BSE seen in atypical field cases are maintained on serial passage. These experimental studies have also provided a range of tissues and body fluids from infected animals which could be used to define the tissue distribution of infectivity in these ic-atypical cases, as outlined by EFSA; data which would be relevant to any future revision of food and feed controls.

Concurrent studies at APHA on the heat stability of atypical BSE agents during standard autoclaving conditions have proved that these decontamination measures are as effective at inactivating H- and L-type BSE prions as they are at destroying those causing classical BSE and this has given confidence to risk managers concerned about the persistence of infectivity in animal by-products after heat treatment.

Scrapie - Low risk of prion exposure from consumption or handling of peripheral tissues from sheep with atypical scrapie

Scrapie in sheep occurs in one of two main forms; a classical form and an atypical form (also known as Nor98) that was first recognised almost 20 years ago following large-scale biochemical testing of small ruminants in the EU. Nothing was known at that time of the transmissibility, pathogenesis or tissue distribution of infectivity in sheep incubating atypical scrapie. We have recently concluded a ten-year series of experimental, intra-cerebral challenge studies in sheep of various genotypes, including those highly resistant to classical scrapie, and found that all genotypes appear susceptible experimentally, and that the overall pathology and the neuroanatomical location of prion protein (as determined by immunohistochemistry) in terminally-affected animals is consistent, regardless of genotype. Interestingly, the micro-architecture of prion protein deposits in brains of valine 136 sheep, in which naturally-occurring atypical scrapie has never been seen, is highly aggregated compared to the fine particulate deposits seen in other genotypes. These animals also remained clinically normal, despite substantial accumulation of prion protein in the brain. Oral challenge was also successful in a small number of animals. Critically, from the viewpoint of food safety, atypical prion infectivity was rarely detected by transgenic mouse bioassay in peripheral, edible tissues and if it was, levels were much lower than those found in the central nervous system of the same infected sheep. These semi-quantitative data indicate a low risk of prion exposure from consumption or handling of peripheral tissues from sheep with atypical scrapie.

In vitro assay can detect BSE in small ruminant samples

EC regulations require the discriminatory testing of all scrapie-positive small ruminant samples to classify the causal TSE agent strain as BSE or non-BSE because of the risk of the zoonotic agent (BSE) occurring in the small ruminant population. Since this regulation was implemented in 2005, naturally-occurring cases of BSE have been identified in two goats. It has also been demonstrated that more than one distinct strain can co-infect a single animal in natural field situations. We conducted a comparative study, collaboratively with ADAS and Nottingham University, to investigate how well the current validated methods for discriminating BSE from scrapie in sheep samples, and the *in vitro* assay, serial protein mis-folding cyclic amplification (sPMCA) could detect evidence of BSE within mixed samples from scrapie- and ovine BSE-affected animals.. In this comparative study, ELISA and Western blot methods did not perform particularly well, but both mouse bioassay and sPMCA were able to detect evidence of BSE prions at much lower concentrations within mixtures. Therefore, the PMCA assay appears to offer a fast and cost-effective alternative to bioassay for the screening of unknown isolates for the presence of BSE prions.

BSE phenotype is maintained upon transmission in sheep under natural husbandry conditions

APHA conducted a transmission study in an experimental sheep flock comprising around 400 animals to investigate whether ovine BSE could be transmitted under natural husbandry conditions, and whether the BSE characteristics were maintained following transmission. Following oral challenge over 100 sheep with BSE inoculum, BSE was confirmed in 118 sheep including 14 lambs that were naturally born offspring of orally infected dams. This work was delivered through a series of consecutive research projects, and took almost 15 years to complete. These studies demonstrated that natural transmission of disease could occur under commercial husbandry conditions, and did so without a change in disease phenotype in the offspring. The final conclusions from these studies were published this year (Jeffrey et al Vet Res. 2015; 46: 126).

Scrapie – persistence of contamination in the environment

Prions can persist and remain potentially infectious in the environment for many years and thus pose a risk of infecting animals after re-stocking. In vitro studies using serial protein misfolding cyclic amplification (sPMCA) have suggested that objects on a scrapie-affected sheep farm could contribute to disease transmission. We conducted an in vivo study within the APHA 'dirty flock' premises, aiming to determine the role of field furniture (water troughs, feeding troughs, fencing, and other objects that sheep may rub against) as a vector for disease transmission. When field furniture (which had been shown to harbor prions using PMCA detection methods) was placed in clean accommodation, 'clean' sheep from the APHA NZ-derived flock could become infected when exposed to it. A high proportion of clean sheep also became infected through exposure to contaminated field furniture placed within pasture not used by scrapie-infected sheep for 40 months, even though swabs from this furniture tested negative by PMCA. This infection rate decreased on the same paddock after replacement with clean field furniture. The findings of this study highlight the role of field furniture used by scrapie-infected sheep to act as a reservoir for disease re-introduction although infectivity declines considerably if the field furniture has not been in contact with scrapie-infected sheep for several months. It also highlights that in vitro detection methods such as PMCA may not be as sensitive as susceptible sheep when testing for residual environmental contamination in the context of decontamination and re-stocking of depopulated premises, for example.

Wildlife

The National Wildlife Management Centre (NWMC) provides evidence, primarily to Defra, to support policy and decision making, together with services to assist delivery. It provides scientific support and advice to help resolve human-wildlife conflicts, drawing on expertise in wildlife disease; wildlife control methods; invasive non-native species, population modelling, and welfare. The team has also provided assistance to

other parts of the agency during disease outbreaks. The NWMC includes the work done at Woodchester Park on badgers and bTB (covered in the TB section).

Invasive Species Actions

We successfully captured all adult beavers living free on the River Otter in Devon. In an operation lasting five weeks and involving a range of stakeholders, four adult and one yearling beaver were trapped for health screening. The presence of the beavers was of concern to Defra for two reasons. First, their presence was contrary to the Wildlife and Countryside Act 1981, which makes it illegal to release species not present in England without a licence from Natural England. Second, with their origin unknown, there was a risk that the beavers carried a zoonotic parasite *Echinoccocus multilocularis* which can pose a risk to human health. Although *E. multilocularis* is present on mainland Europe, the UK is presently free of the parasite. The beaver is a secondary host for *E. multilocularis* with no transmission between beavers or into the environment. The parasite can only enter the environment via a primary host (a carnivore such as a fox or dog) predating or scavenging an infected beaver; within the carnivore the parasite develops into the reproductive and egg-laying stage. It was only adult beavers, therefore, that posed a risk and required capture.

At the same time that Defra stated their intention to have the beavers removed from the river, the Devon Wildlife Trust (DWT) applied for a licence to undertake a Natural England licensed trial re-introduction of beavers into the River Otter. It was agreed that those beavers currently present would form part of that re-introduction on the condition that they were proven to be parasite-free following their capture and health screening.

We undertook the recovery of the beavers. The exercise involved a series of sequential tasks – surveying the river to locate signs of recent beaver activity, the provision of bait (the beavers had a predilection for apples) and the installation of remote cameras at identified sites, the placement of traps at those sites at which beaver presence was confirmed and active trapping.

The operation had to be undertaken within a very tight schedule. Approval for fieldwork to commence was only sanctioned at the beginning of February and had to be completed by the end of March – the capture of animals after this date increased the risk of any pregnant females giving birth in captivity, severely complicating the timing of any re-release.

Surveillance with remote cameras and surveys along 20 miles of waterway indicated two separate social groups with a total of nine individual beavers. In the event, the trapping exercise was extremely successful with all known adult beavers (and one yearling) captured by the 3rd March, well ahead of schedule.

The adults were subsequently screened by beaver experts form the Royal Zoological Society of Scotland (RZSS) and concluded to be clear of *E. multilocularis*. On confirmation that the animals were free of other diseases (screening organised by DWT) they were released back into the River Otter by DWT under licence from Natural England.

This work received plaudits from Devon Wildlife Trust and Defra, including recognition from both Parliamentary Under Secretary and the Secretary of State.

Research

During 2015 the Woodchester Park team continued to produce high quality scientific outputs and to provide advice to Defra TB policy on the subject of badgers and bovine tuberculosis. Several Defra funded projects came to an end in spring 2015. This included a project to develop and demonstrate the use of a restraint cage to allow blood samples to be taken from wild unanaesthetised badgers for the purposes of real-time field diagnosis of *M.bovis* infection.

With collaborators at the Royal Vet College we completed a study to assess the performance of several PCR

tests to identify *M. bovis* in badger faecal samples collected from the field. We attended an 'expert review meeting' and helped Defra reach conclusions regarding the performance of these tests. Both projects demonstrated the value of the Woodchester Park study population for developing and testing techniques with the potential to influence policy on TB control in badgers. In collaboration with Exeter University the team also successfully initialised fieldwork in Northumberland to explore badger contact behaviour at a fine scale and its implications for disease transmission as part of a NERC funded research project.

The NRL function for *Trichinella* and *Echinococcus* has recently been moved to the wildlife portfolio to capitalise on the expertise and technical capability of the York Parasitology group, who have been carrying out Trichinella and *Echinococcus* testing of wildlife for >10 years. This consolidates all wildlife parasitology within the NWMC team who form a key part of the parasitology virtual group. The move also brings a cost benefit as *Trichinella* & *Echinococcus* testing of wildlife is ongoing and NRL function testing can integrate smoothly with this. Transfer of testing from Bury to York is underway subject to accreditation status transfer.

The congregation of the St. Hilda's church, Ellerburn, North Yorkshire have had a long-standing problem with bats fouling the interior of their church. Legislation protecting bats and their roosts appeared to prevent the easy resolution of the conflict, and their initial licenced attempts exclude the bats were unsuccessful. We bid and secured work from Natural England and used a novel conceptual model of bat social and spatial dynamics (developed to support the prediction of disease epidemiology in wild bats), to understand how the bats used the church and develop a solution that satisfied all stakeholders. The successful resolution of the case has been welcomed in subsequent parliamentary debates and answers about the ongoing bats in churches issue

Non-Native Species

The non-native Species Secretariat (NNSS) provided key expertise on the new EU IAS Regulation, including providing the permanent UK representation at the EU Scientific Forum, attending all, and leading the UK delegation at one, of the Committee meetings in Brussels.

The Secretariat attends the Monthly Biosecurity Meetings with Defra Ministers - leading on technical matters related to invasive species. As part of the MBM process NNSS has also established and runs a monthly risk identification process which is analogous to what exists in Plant and Animal Health.

NNSS continues to run the GB Non-native Risk Analysis Mechanism – a scheme unique in Europe. Our risk assessments underpinned over half of the 37 species proposed for listing under the EU Regulation. Several of these will be relevant to APHA as we need the capacity to rapidly respond to new arrivals in the UK that are on this EU list (Asian hornet, raccoon, coati, Siberian chipmunk, pallas and fox squirrels, sacred ibis, ruddy duck). Most other member states are also using the UK scheme for their risk assessments because it is one of the few that meets the European Commission's minimum standards. Our risk analysis panel has also reviewed several risk assessments from other EU member states.

NNSS has also developed one of the first invasive species risk management schemes in Europe to assess the feasibility of eradicating species that are already established as well as those likely to invade. When combined with risk assessment this will help Policy/Ministers prioritise species for management. The results of the workshop are currently being written up as a paper and will be used to support the development of contingency plans – which NNSS are developing for over 40 invasive species.

Other Research and Associated Work

A short report has been completed for the Welsh Government on the effects of not having BCG available for badgers this coming summer (2016). This report was well received and presentations to the Welsh Deputy Food and Farming Minister, Assembly Members and Stakeholders occurred in early February 16.

Six members of our team are teaching for the second year of the Wildlife Management MSc run at Newcastle University. Last year three of the MSc students performed projects with NWMC and two scientific papers are in preparation from this work. Two members of the team are teaching occasional sessions/workshops at Hull University.

We continue to run the Home Office approved 'Wild mammal and bird training course'. The 11 delegates who attended the October workshop represented four universities, a wildlife trust and ZSL. All delegates passed and we received excellent feedback.

Following on from a number of visits to Kosovo we have a completed a short contract to investigate the occurrence of wild mammals (principally foxes as carries or rabies and wild boar as potential carries of CSF) using local staff and camera traps. This will be the first quantitative assessment of wild mammal densities in the Balkans.

5. DISCIPLINE HIGHLIGHTS

APHA have a number of Discipline Champions who promote development of their discipline across the agency , sharing best practice, promoting training and ensuring that we have the right capability. As part of their role they have reviewed current capability across the agency and developed capability plans following challenge by the Portfolio Lead Scientists. The plans are being implemented. Given that the disciplines cross cut the science portfolios many highlights will be picked up elsewhere but some specific highlights are:

Animal Sciences

- The agency became a signatory to the Concordat on Openness on Animal Research, which has the support of Professor Sir Mark Walport, Government Chief Scientific Advisor. Signing the Concordat demonstrates a commitment to improve openness and transparency about the ways in which animals are used in scientific, medical and veterinary research in the UK.

- The APHA Ethics Committee was appointed a Regional Ethics Hub by the Animals in Science Committee (under the Animals (Scientific Procedures) Act 1986.

- We held an RSPCA/APHA meeting on the welfare of wild animals used in regulated procedures (third in a series of joint workshops dealing with various species/topics).

Biomathematics

- APHA's ethical review process (ERP) was modified early in 2015 to improve statistical review. Now applications are expected to include evidence of statistical advice or review from statistics experts, with only a few, clearly defined exceptions. This has increased planned use of statistical consultation before experiments involving animals, as well as dialog between scientists and statisticians, which is raising scientific standards. Increasing the standard of applications has also allowed more thorough statistical scrutiny, because fewer applications require revision. Engagement within the ERP committee has also benefited from witnessing careful examination of applications. These changes were timely, because awareness of shortcomings in the statistical standards of designs for biological studies is increasing nationally and globally, which has been reflected by updated guidance on information required about animal experiments for funding applications to the UK research councils.

- Estimating parameters for models from real world data can be a challenge. To help with this, we have introduced a recently developed approach (Approximate Bayesian computation) and implemented it in the APHA Scientific Computing Environment (which means it can be run on many processors simultaneously for computational speed). We have applied this method to mortality and egg production data from low pathogenic avian influenza outbreaks, and found that we are able to

effectively estimate key parameters (such as rate of transmission and the impact of LPAI on mortality and egg production) from it using this approach. In addition, the method is very effective at estimating uncertainty of the parameters, a key element in the communication of results to policymakers.

Epidemiology

- Professor Dirk Pfeiffer,Head of the Veterinary Epidemiology, Economics and Public Health Group at the Royal Veterinary College (RVC) joined us on a part-time secondment on 1 June 2015. He takes on the role of Chief Epidemiologist located in the Department of Epidemiological Sciences (DES) in the Science Directorate . Dirk will work with people across APHA to challenge, support and develop epidemiological capability, modernise epidemiology at APHA and set up governance for the discipline, strengthening APHA's work in this area. This secondment will also build and strengthen APHA's strategic partnership with RVC.

- To build capability and improve international networking opportunities, epidemiologists have attended several international meetings, including the final RISKSUR project meeting, the annual Society for Veterinary Epidemiology and Preventive Medicine (SVEPM) and International Symposium on Veterinary Epidemiology and Economics (ISVEE) conferences. Meeting reviews have been published internally to help disseminate the key messages from these meetings and several initiatives are being taken forward from the RISKSUR project to determine how the tools developed in RISKSUR can be applied to APHA surveillance and how the RISKSUR training can be best utilised.

Molecular Biology

- APHA is a partner in two large EU H2020 consortium grants: COMPARE and EVAg, both of which will develop molecular biology techniques for pathogen characterization. COMPARE also aims to better integrate molecular biology data with risk analysis and epidemiology.

- State-of-the-art molecular biology techniques have been used to provide vital data in support of a number of outbreak and other high profile pathogen investigations.

    - Whole genome sequences generated on our high-throughput sequencing platforms are now a key part of the rapid response to both bacterial and viral pathogens
    - In 2015; MRSA in January, Influenza outbreaks in February and July; *Salmonella* in March, Colistin resistant *Escherichia* and *Salmonella* in December

- We are working closely with PHE to ensure a unified approach to molecular biology, particularly in the areas of high-throughput sequencing and bioinformatics.

    - High-level meeting to ensure aligned strategies
    - Specialist knowledge exchange of best practice to ensure that data processing is identical at both institutions
    - Cross Agency attendance and presentations at internal workshops.
    - In particular we are working closely on the implementation of WGS for routine characterization of *M.bovis* and *Salmonella*.

- Three 'Bio-Linux' servers have been set up for general use at Weybridge; these are primarily for compute-intensive data analysis, such as bioinformatics for high throughput sequence data.

    - The availability will enhance bioinformatics skills of a larger number of staff and has been supported by initial training for 15 people.

- These servers include the analysis pipelines for bacterial WGS analysis that are currently in use at PHE (see above)

- Testing and early implementation of Nanopore Sequencing technology.

  - Participating in Oxford Nanopore's MinION Access Programme and are investigating various applications. This sequencing technology is both fast and portable. The portability means that it is likely to lend itself to rapid diagnostics in the field.

  - Represented at Oxford Nanopore's first user group meeting and are developing further contacts made there.

- We are involved in four grant applications to the NERC-led UKRC 'AMR in the Real World' call. These have involved building collaborations with SRUC, University of Oxford, Centre for Ecology and Hydrology, Cardiff University, King's College London, RVC and Nottingham Trent University. The application of genomics and metagenomics to understanding the persistence and dissemination of antimicrobial resistance are important components of these proposals.

Parasitology

- We have written information notes on *Besnoitia besnoti* for the APHA cattle expert group following its detection in Ireland. The notes were forwarded to private veterinary surgeons via regional laboratories.

- Presentations and posters were presented at the World Association for the Advancement of Veterinary Parasitology (WAAVP) meeting at Liverpool.

- Various presentations/seminars were given to state and private veterinary surgeons and farming industry on:

  - *Schmallenberg (SBV) and Blue tongue (BT) update (vector borne diseases)*
  - *Animal health and welfare* at two 'women in agriculture' meetings
  - *Besnoitia besnoiti*- an introduction.

- A survey was initiated for veterinary surgeons, Suitably Qualified Persons (animal medicines advisors) and others carrying out or advising on parasitological diseases, to investigate interest in being part of a parasitology network, providing information to extend surveillance activities and having added quality assurance. The survey will run until the end of January.

Pathology

- We have initiated a successful strategic partnership with the RVC for development of the next generation of specialist veterinary pathologists. This initiative, originally supported through Seedcorn investment funding, was designed to help address the UK skills gap in farm animal pathology expertise. There are currently two Fellows, who spend 50% of their time at APHA, progressing successfully towards formal pathology board qualifications over three or four years (ACVP, ECVP & FRCPath). A third fellowship in avian pathology is under negotiation however is dependent upon external funding sources. For existing and new permanent veterinary pathologists,

training opportunities at RVC [10] have been secured to enable progression towards board qualifications on a part time, longer term basis.

- Mammalian surveillance pathology has been consolidated to Weybridge, following retirement/resignation of pathologists at Lasswade. This has enabled recruitment of two new pathologists at Weybridge to increase critical mass and develop flexibility, specialist expertise and resilience across the range of portfolios, diseases and species required. This, in conjunction with the training initiative helps to address previous fragmentation and succession planning concerns.
- A subcontract has been secured with Finn Pathology, to assure surveillance and diagnostic resilience and contingency is in place for mammalian and avian diagnostic caseload. This utilises expertise previously developed within APHA and is currently supporting transition of surveillance pathology to Weybridge and the training of new and existing APHA pathologists by retrospective assessment of cases and microscopy meetings. This will be extended to short term placements.
- Cross disciplinary integration of pathology into research and surveillance portfolios has improved, with veterinary pathologists funded into, and embedded across all areas. Viral Diseases portfolio continues to be an exemplar in this respect.
- The avian pathology team at Lasswade has been strengthened to three veterinary pathologist posts, and training of the recent recruit (transferred from a field role) has begun.
- Discussions with Edinburgh University (Royal Dick School of Veterinary Medicine) and SAC-Consulting are in progress to develop a strategic partnership in Edinburgh to mirror that of APHA-RVC and a training partnership to build capability in specialist avian pathology.
- APHA Pathology Weybridge is the preferred pathology partner for Dstl, with a number of successful projects delivered over recent years, and further project funding for UK defence research involving animal studies.
- Pathology Weybridge have a successful collaboration with PHE and the Institute of Neurology to complete the high profile, large scale vCJD retrospective prevalence study in human appendix samples over the past five years.
- We have consolidation and centralisation previously fragmented TSE expertise and specialist containment laboratories into Pathology at Weybridge, centred around building resilience and staff flexibility across key core skills required for TSE NRL and EURL functions. The team responded very well to recent cases of TSE.

[10] The co-funded APHA-RVC Fellowship programme provided two new specialist farm animal pathology gross and histopathology training residencies. Each Fellow, selected from the high calibre intercalated pathology degree post graduate students with farm animal practice experience, spends 50% of their time with APHA Pathology and the remainder at RVC over a three or four year period. The residency programme in anatomic farm animal pathology provides detailed instruction in gross and histopathology as well as the use of modern pathology research and diagnostic tools, plus disease investigation and biocontainment. It focusses on farm animal pathology while ensuring a broad exposure to other veterinary species. Students sit the Board Exams of ACVP or ECVP and/or examinations leading to FRCPath and complete MSc in veterinary medicine with a Defra policy relevant research project based at APHA. Our first Fellow, Sam Beck has now passed FRCPath part 1 and 2 and the initial exams for ACVP. Our second Fellow Alex Civello has completed FRCPath Part 1.

Virology

- We have been working closely with colleagues from The Pirbright Institute (TPI) as part of ongoing cross government review of future options for delivery of Virology and high containment laboratory work. Our senior virologists continue to work in scientific partnership with colleagues from TPI and in year this has realised two new initiatives in the area of vector borne diseases and avian influenza with joint funded projects.

- Cross disciplinary working opportunities have continued to be maintained with several members of the Pathology Department and DES embedded and well-funded from Virology led projects. Further initiatives are being developed for closer working with PHE through the Joint Board for Emerging Infectious Zoonoses (JBEIZ).

- We maintained strong international network connectivity, but in year in particular across many disease areas we used our contacts to enhance knowledge and capabilities to respond to the emerging threat from PED.

- Agreement has been reached with the CVI Lelystad, Netherlands for agreement to support emergency response in the event of calamity affecting our ability to deliver. This provides contingency for our key functionality on major diseases in our portfolio.

- Direct assistance has been provided to industry to enable early return to business as usual following AI, involving scientific data to support refinement of C&D processes.

- The 'Jenner' Fellow has produced some excellent work on defining correlates of vaccine protection in swine influenza challenge models as part of a collaborative project with TPI and has received plaudits from the Jenner Institute for their work.

- There has been a significant consolidation of resilience around our SAPO4/ACDP3 containment facilities; this has specifically been applied to contingency for rabies, vector borne diseases and avian notifiable disease in both our modular SAPO 4 labs.

- Four new assays have been added to scope under our ISO 17025 accreditation, forming part of our continuous improvement programme.

6. QUALITY

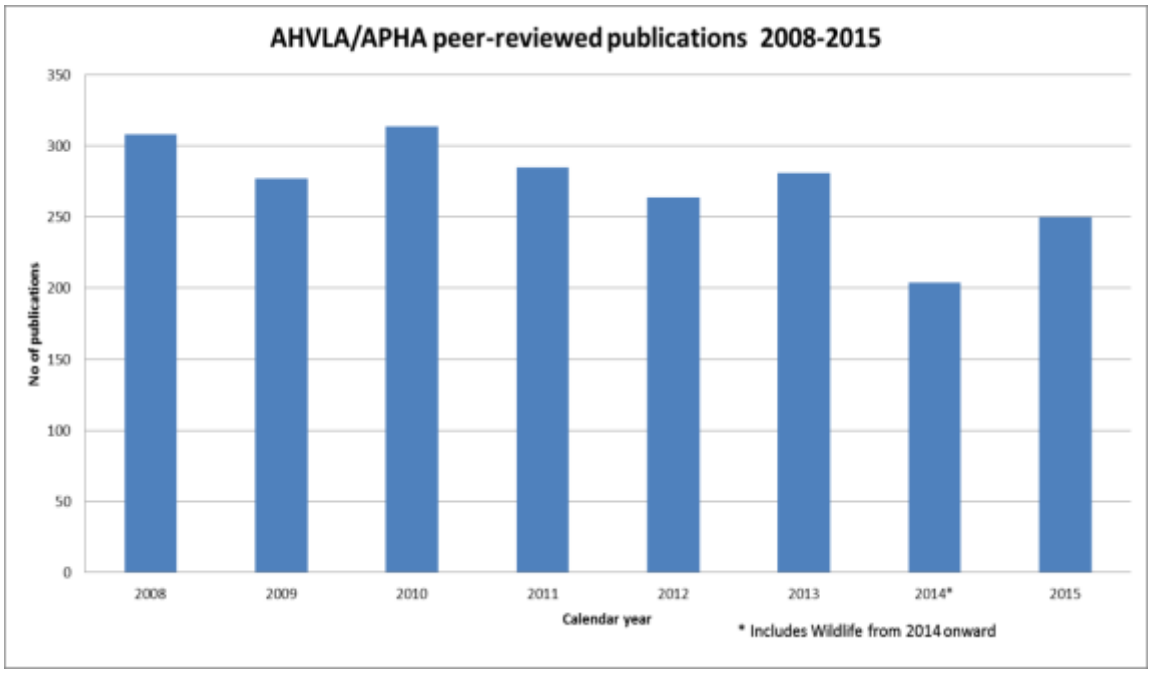
We maintain several Quality Management Systems (QMS) for which we have accreditation, certification or compliance assessed by Third Parties. They are recognised internationally. These are ISO17025 (testing), ISO17020 (inspection activities), ISO17043 (proficiency testing), Good Laboratory Practice (vaccine safety tests), Good Manufacturing Practice (quality control of veterinary vaccines), Good Distribution Practice (for the distribution of vaccines) and Good Clinical Practice vet (efficacy testing of veterinary vaccines). In most cases these schemes are mandatory if we want to continue to provide services, such as Salmonella testing, compost testing and regulatory GLP and GMP vaccine tests. We are also responsible for undertaking pharmacovigilance of vaccines produced by APHA as well as aid the manufacturer of Tuberculins supplied to the UK if need be. All the quality management systems support the quality of the evidence supplied by APHA, add value, enhance our reputation and give customers confidence in our services.

The QMSs are subject to inspection by external awarding organisations. We had major inspections by UKAS for ISO17025, LRQA for ISO9001, MHRA for GLP and VMD for GMP, GDP and Pharmacovigilance. We had no serious findings for the ISOs and Pharmacovigilance. A number of findings were found for GMP and GDP in a recent inspection by VMD, which are being addressed. We had a routine surveillance visit from UKAS for ISO17043 with no major findings. A number of audits were also undertaken by customers as part of their own internal requirements, which again did not raise any findings of concern.

Major milestones were reached with the successful assimilation of ISO17020 into APHA when the PHSI were transferred from FERA and a comprehensive due diligence audit of the seven VDPs under the new contract run by APHA to provide veterinary services to Defra. The Plant Varieties and Seed Inspectorate is being integrated into APHA ISO9001.

7. PUBLICATIONS

The table below shows the trend of APHA (and former AHVLA) publications since 2008.



Publications in 2015:

- Total Number of Publications: **235**
- Number of Publications with APHA lead author: **136** (**57.87%** of total)
- Number of Publications without APHA lead author: **99** (**42.13%** of total)
- Mean Impact Factor (Total): **2.906**
- Mean Impact Factor (APHA lead author): **2.607**
- Mean Impact Factor (APHA not lead author): **3.283**

For a full list of publications go to Gov.UK.

8. REPRESENTATION

APHA is represented on 147 national committees with 203 individual members; and 132 international committees with 154 individual members. We have 17 visiting professors with national and international universities and two tenured professors.