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Example of response to epidemics: the impact of two health emergencies (the emergence of the Bluetongue and Schmallenberg viruses) on a research and reference laboratory

S. Zientara, UMR 1161 Virologie ANSES-INRA-ENVA, Maisons-Alfort, France

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Two viruses vectored by midges (the bluetongue virus and the Schmallenberg virus) emerged in northern Europe in 2006 and 2011 respectively. The Joint research unit (JRU) for Virology at ANSES's Maisons Alfort laboratory was confronted with the emergence of these two viruses in France. In just a few weeks, it was necessary to develop and validate serological and molecular diagnostic tools and to set up and coordinate a network of laboratories capable of processing thousands of samples while researching into the physiopathogenic mechanisms by which these infections operate. The structure of a JRU, where research teams work alongside the National Research Laboratory, enables it to meet these various requirements in a very short time.

Presentation of the Maisons Alfort UMR for Virology

The ANSES, INRA and ENVA Joint Research Unit (UMR) for Virology No.1161 (on the campus of the French National Veterinary School of Alfort - ENVA) focuses its activity on animal viral diseases representing a zoonotic and/or emerging risk. The UMR has a staff of about 40 people, working on: i) developing the most appropriate diagnostic tools for bio-monitoring and phylogenetics; ii) studying the physiopathology of some of these diseases, concentrating on the risks of inter-species transmission, especially from animals to humans; and iii) pursuing new approaches to vaccination, favouring those that can be administered orally. Apart from this applied and fundamental research, the UMR also houses reference laboratories at global level (OIE Reference Laboratory for epizootic haemorrhagic disease in deer), European level (EU Reference Laboratory for equine diseases) or national level (National Reference Laboratories – NLRs – for Bluetongue, foot-&-mouth disease, vesicular stomatitis, swine vesicular disease, African horse sickness and West Nile fever). This unusual association of ANSES-controlled reference activities and research activities helps the UMR confront emerging threats such as those that occurred in 2006 (with the emergence of bluetongue) or in 2011 (with the emergence of the Schmallenberg virus).

Brief history of the emergence of two vectored diseases in France and Europe

In August 2006, the European Commission gave official notification of the presence of bluetongue virus (BTV), a major pathogen for domestic and wild ruminants, in the Netherlands, Belgium and Germany. Although BTV had been circulating in the Mediterranean basin for several years, this was the first epizootic episode of the disease documented in northern Europe and the first time serotype 8 had been identified on the continent. At the end of 2006, six outbreaks were reported in the Grand-Duchy of Luxembourg and in France. Unexpectedly, the virus, which is vectored by midges of the *Culicoides* genus, survived over the winter period and spread rapidly over a large part of northern Europe in 2007 and 2008. Cattle were soon found to be heavily affected, while mortality in sheep reached 30% [3], in regions where the usual vector, *Culicoides imicola* (known to be responsible for the transmission of BTV in the

Mediterranean basin), has never been found. In France, more than 50,000 outbreaks were reported between 2007 and 2008. However, the prophylactic measures that were rapidly taken (a massive vaccination programme) successfully controlled the epidemic and most of the countries concerned have now recovered their BTV-free status [5].

The sudden and unexpected emergence of serotype 8 of BTV (BTV-8) was a major animal health event for Europe and just a few years later history seemed to be repeating itself with the emergence of a new arbovirus affecting ruminants in northern Europe.

During the summer of 2011, several cases of febrile diarrhoea together with loss of appetite and a significant drop in milk production were reported in adult cattle in Germany (North Rhine-Westphalia), sometimes with clinical symptoms similar to those for BTV, giving rise to fears of a return of bluetongue. These symptoms were transitory and generally disappeared in a few days. The search for numerous pathogens in samples taken from affected cattle proved negative, despite the use of innovative techniques such as the Epizone Biochip 5.1, which contains more than 2000 virus primers. After many investigations had been carried out, in November 2011 the Friedrich-Loëffler-Institut (FLI) in Germany used high-speed sequencing without prior knowledge on blood samples from diseased cattle to identify nucleotide sequences belonging to a new virus that was given the name of the town the samples came from – the Schmallenberg virus (SBV) [1]. The implication of SBV in the clinical symptoms observed was later confirmed by the experimental infection of 9-month-old cattle, which showed that the viremia caused by the SBV seemed to be transitory (4 days) [1]. Analysis of the virus's gene sequence showed similarities with the Akabane, Aino and Shamonda viruses, which belong to the *Orthobunyavirus* genus in the *Bunyaviridae* family.

The FLI rapidly developed a test to detect the genome of the SBV by RT-PCR in real time, and the protocol was shared with several European partners. At the same time, a bio-surveillance scheme was set up across Europe.

In December 2011, the Netherlands for the first time reported SBV having a teratogenic effect on sheep, with similar characteristics to the effects observed with the Akabane and Aino viruses [1]. Female sheep, goats and cattle infected at the start of gestation were capable of transmitting the virus to their foetuses which then developed atypical malformations,



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most often leading to stillbirths or death of the offspring shortly after birth.

On 25 January 2012, the virus's genome was detected for the first time in France by our laboratory, in the brains of stillborn lambs from two farms in Moselle and Meurthe-et-Moselle (north-east France).

On 1 July 2012, 5234 outbreaks of SBV had been reported in Europe, 2865 in cattle, 2491 in sheep and 78 in goats (source: www.survepi.org).

Initial diagnosis of BTV and SBV viruses

In France, both in August 2006 and in January 2012, the NRL would have come under considerable strain if it had received all the biological samples taken from suspected cases of BTV or SBV. For example, for BTV in 2007, the Italian veterinary authorities insisted that some 100,000 cattle (grass-fed calves) exported from the centre of France to the Po Valley be tested individually by RT-PCR which the three people staffing the NRL could never have handled alone. The structure of the UMR, which combines research with reference activities, enabled us at least in the first few weeks of the two crises, to redeploy certain personnel (technicians from other teams interrupted their research projects and switched to helping their colleagues). We also worked in collaboration with various companies specialising in veterinary diagnosis (AES-ADIAGENE, LSI, IDvet, IDEXX and others) and asked them to develop molecular virology diagnosis kits that had to be sensitive, specific, inexpensive and automatable. For BTV, LSI and AES-ADIAGENE first used our in-house PCR test [2, 4] before developing their own methods. The same approach was later applied for the SBV crisis.

The same procedure was followed for the development of serology kits. We worked with IdVet to validate an ELISA diagnosis kit at the end of February 2012 [6]. It was in fact the first ELISA test developed anywhere in the world for detecting SBV antibodies.

In parallel with this, and with the support of the Directorate General for Food of the French Ministry of Agriculture, we set up, trained and organised a network of 66 departmental veterinary laboratories which were able to process several thousand biological samples a day (for BTV and SBV). The network first used the real-time RT-PCR kits created by LSI and AES ADIAGENE, developed and validated with help from our laboratory. (As a result, all samples found to be positive by RT-PCR were sent to the UMR for viral isolation, for both BTV and SBV). For SBV, the network was able to use the indirect ELISA serology test developed by IDvET for detecting antibodies to the nucleoprotein of the Schmallenberg virus.

We can therefore see that in each of these two health crises we were able to set up in only six weeks a large-scale diagnosis system consisting of an NRL and a network of over 60 laboratories capable of using real-time RT-PCR for the molecular diagnosis of infection by BTV or SBV, plus serodiagnosis for SBV.

Conclusion

The mutual respect and confidence that has been established between the different National Reference Laboratories on the subject of BTV since 2000 (when the virus first emerged in the Mediterranean basin) enabled us to exchange protocols and reagents rapidly and efficiently in order to start screening for BTV and SBV in the different countries of Europe. During the BTV crisis, the RT-PCR method that we had developed and validated [2] was transferred rapidly to our counterparts

in the other European NRLs. Equally, for the case of SBV in France, it was during a meeting in Brussels in November 2011 that our German opposite numbers told us they had identified this new virus. By mid-December 2011, the specific RT-PCR for detecting SBV had been made available to the French NRL at Maisons-Alfort. From this time on, numerous scientific and technical exchanges have taken place between the national laboratories.

It can therefore be said that the BTV crisis had the effect of fostering scientific and professional relationships between the various national laboratories of the Member States of the European Union, which now share the information they hold, almost in real time. This is a considerable weapon in the event of emerging threats.

The Schmallenberg virus was detected in Germany by FLI using metagenomics. Considering the cost and highly specialised nature of this type of technology, cooperation clearly allows different laboratories to benefit from this type of method without investing the considerable sums that would otherwise be necessary, for what may prove to be only occasional use.

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