Project number: 6520
Funding source: Teagasc
Date: Jan 2020
Project dates: Jan 2014 – Dec 2018

Schmallenberg virus – Irish dairy studies

Key external stakeholders:
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Vets, farmers, advisors.

Practical implications for stakeholders:
The outcome/technology or information/recommendation is that SBV infection will recur cyclically in the Irish national dairy herd therefore a prospective, national monitoring programme is recommended.

- National monitoring will alert stakeholders of recent SBV incursion and likely risk to animal health

Main results:

1. The midges which are the vectors for SBV are ubiquitous on Irish dairy farms sampled

2. Within-herd seroprevalence ranged widely from 8.5%-84.1% indicating natural infection does not ensure an even distribution of herd-level immunity

3. BTM-ELISA results were highly correlated (r =0.807, p < 0.0001) with, and predictive (R^2 =0.832, p <0.0001) of within-herd seroprevalence, indicating that BTM (bulk tank milk) samples are an inexpensive method of monitoring farm SBV status at the herd level.

Opportunity / Benefit:
Results available through 6 scientific publications and others and as summarized hereunder.

Collaborating Institutions:
UCD,
AHI,
ICBF,
DAFM,
The Pirbright, UK,
Pulawy Institute, Poland,
Elizabeth MacArthur Institute, Australia.

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1. Project background:

Schmallenberg virus (SBV) was first identified in Ireland in Cork in October, 2012 and the highest level of sero-prevalence has occurred since in Munster. When the index incursion occurred there was considerable interest amongst Irish dairy, beef and sheep farmers and their veterinary practitioners in what will happen with this virus infection in our food animal ruminant populations in the future. As this is a novel virus internationally (first isolated in 2011) there were no longitudinal studies on the progression of natural infection in herds or flocks, in particular in seasonally-calved herds as in Ireland.

2. Questions addressed by the project:

Hence, the questions to be answered in this project were: (1) establish the presence and prevalence of the midges that might spread the virus on Irish dairy farms (2) establish the prevalence of viral infection in Irish dairy herds (3) determine how useful bulk tank milk sampling might be to screen herds for exposure to infection (4) monitor changes in herd and animal-level infection over time (5) develop a novel experimental model to test for the virus in chicken embryos instead of in cattle.

3. The experimental studies:

To answer these questions the following approaches were taken:

(1) the presence and prevalence of the midges that might spread the virus on Irish dairy farms – Ultraviolet-light trapping for *Culicoides* was carried out on 10 sentinel farms. Each site was sampled fortnightly over 16 weeks (21st July to 5th November). One Onderstepoort Veterinary Institute UV light trap was run overnight at each site and catches were transferred immediately into 70% ethanol. *Culicoides* were morphologically identified to species level. Collection site habitats were characterised using the Phase 1 habitat survey technique (Joint Nature Conservation Committee).

(2) establish the prevalence of viral infection in Irish dairy herds - Whole-herd SBV serosurveillance was conducted in 26 herds before (spring) and following the 2014 vector-season (winter), and following the 2015 vector-season (winter). In spring 2014, 5,531 blood samples were collected from 4,070 cows and 1,461 heifers. In winter 2014, 2,483 blood samples were collected from 1,550 youngstock (8-10 months old) and a subsample (n=933; 288 cows, 645 heifers) of the seronegative animals identified in the spring. Youngstock were resampled in winter 2015. *Culicoides* spp. were collected in 10 herds during the 2014 vector-season and analysed for SBV; a total of 138 pools (3,048 *Culicoides*) from 6 SBV vector species were tested for SBV RNA using real-time PCR.

(3) determine how useful bulk tank milk (BTM) sampling might be to screen herds for exposure to infection - BTM samples (n=24) and blood samples (n=4,019) collected from all lactating cows contributing to the BTM in 26 Irish dairy herds (58-444 cows/herd) located in a region exposed to SBV in 2012/2013, were analysed for SBV-specific antibodies using IDVet® ELISA kits. The correlation between BTM-ELISA results and within-herd seroprevalence was determined by calculating Pearson's correlation coefficient. Linear regression models were used to assess the ability of BTM-ELISA results to predict within-herd seroprevalence. The distributions of individual animal serology results were explored by determining the empirical distribution functions (EDF) of the individual animal serum ELISA results in each herd. EDFs were compared pairwise across herds, using the Kolmogorov-Smirnov statistical test. Herds with similar BTM-ELISA results, herds with similar within-herd seroprevalence and herds with similar mean-herd serology ELISA results were stratified in order to explore their respective paired-herd EDF comparisons. Statistical significance was set at p <0.05.

(4) monitor changes in herd and animal-level infection over time - A population of 1,550 spring-2014-born animals, which had been monitored for SBV infection in 2014 and 2015 as part of a previous SBV surveillance study, were re-sampled for evidence of SBV infection during 2016. A total of 366 blood samples was collected in the 25 study herds (15 samples per herd) between 3-10 March 2017 (before the 2017 vector-active season) and analysed for SBV antibodies using a competitive ELISA kit (IDVet).

(5) develop a novel experimental model to test for the virus in chicken embryos instead of in cattle - Two studies were conducted. In Study A, SBV ($10^{6.4}$ $TCID_{50}$) was inoculated into the yolk-sac of embryonated chick embryos (ECE) at 6-days (n = 43) and 8-days (n = 41) of incubation. In Study B, SBV and Akabane (AKAV) viruses were inoculated into ECE at 7-days of incubation at doses ranging between $10^{2.0}$ -$10^{6.0}$ $TCID_{50}$ (approximately 40 ECE per virus dose). ECE were incubated at 37°C until day 19, when they were submitted for pathological and virological examination.

4. Main results:

(1) Midge study - A total of 23,929 individual *Culicoides* from 21 species was identified, including two species identified in Ireland for the first time; *C. cameroni* and *C. clastrieri.* The most abundant species identified were members of the *Culicoides obsoletus* (*C. obsoletus/scoticus*; 38%, *C. dewulfi*; 36% and *C. chiopterus*; 5%) and *Culicoides pulicaris* groups (*C. pulicaris*; 9% and *C.*

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punctatus; 5%) comprising 93% of all *Culicoides* identified. Collection site habitats were dominated by improved grassland with a mixture of broadleaf woodland and native woody species. The most abundant species of *Culicoides* identified are the putative vectors of Bluetongue virus (BTV) and SBV in northern Europe. Their presence and abundance on Irish farms demonstrates the potential for future transmission of arboviruses among livestock in Ireland.

(2) Seroprevalence study - In spring 2014, animal-level seroprevalence was 62.5% (cows=84.7%; heifers=0.6%). Within-herd seroprevalence ranged widely from 8.5%-84.1% in the 26 herds. In winter 2014, 22 animals (0.9%; 10 cows, 5 heifers, 7 youngstock) originating in 17 herds (range 1-4 animals/herd) tested seropositive. In winter 2015 all youngstock, including the 7 seropositive animals in winter 2014, tested seronegative suggesting their initial positive result was due to persistence of maternal antibodies. All of the *Culicoides* pools examined tested negative for SBV-RNA. SBV appears to have circulated at a very low level in these herds during 2013 and 2014, while there was no evidence of SBV infection in naïve youngstock during 2015. A large population of naïve animals were identified and may be at risk of infection in future years should SBV re-emerge and re-circulate as it has done in continental Europe.

(3) Herd BTM screening test study - Twenty-two herds were BTM-ELISA-positive (within-herd seroprevalence 30.6-100%) and two herds were BTM-ELISA-negative (within-herd seroprevalence 10.7 and 16.2%) indicating BTM-ELISA-negative herds can have seropositive animals present. BTM-ELISA results were highly correlated (r =0.807, p < 0.0001) with, and predictive (R^2 =0.832, p <0.0001) of within-herd seroprevalence. Predictions were most accurate for upper-range BTM-ELISA antibody titres, while they were less accurate at higher and lower antibody titres. This is likely a result of overall high within-herd seroprevalence. In herds with similar BTM-ELISA results 82% of the paired-herd EDF comparisons were significantly different. In herds with similar within-herd seroprevalence and in herds with similar mean-herd serology ELISA results, 46% and 47% of the paired-herd EDF comparisons were significantly different, respectively. These results demonstrate that BTM antibody titres are highly predictive of within-herd seroprevalence in an SBV exposed region. Furthermore, exploring the serum EDFs revealed that the variation observed in the predicted within-herd seroprevalence in the regression models is likely a result of individual animal variation in serum antibody titres in these herds.

(4) Changes in SBV infection over time - A total of 256 animals tested seropositive; an AP of 69.9% (95% CI: 65.1-74.4) and TP of 77.7% (95% CI: 72.3-82.8%) when correcting for imperfect test characteristics. These results demonstrate that a new epidemic of SBV circulation occurred in these previously exposed herds in Ireland in 2016.

(5) Alternative SBV infection model - Mortality was greater in embryos inoculated with SBV at 8-days (76%) compared to 6-days (47%), (P < 0.01), while the prevalence of stunted growth (6-days: 37%; 8-days: 51%) and musculoskeletal malformations (6-days: 42%; 8-days: 41%) (arthrogryposis, skeletal muscle atrophy, contracted toes, distorted and twisted legs) did not differ between days (P > 0.05). Mortality was greater in embryos inoculated with SBV (31%) compared to AKAV (19%), (P < 0.05), suggesting that SBV was more embryo-lethal. However, embryos infected with AKAV had a significantly higher prevalence of stunted growth (SBV: 46%; AKAV: 76%; P < 0.05) and musculoskeletal malformations (SBV: 18%; AKAV: 42%; P < 0.01), suggesting that AKAV was more teratogenic in this model. These studies demonstrate for the first time that the ECE model is a suitable *in vivo* small animal model to study SBV. Furthermore, these results are consistent with the clinico-pathological findings of natural SBV and AKAV infection in ruminants

5. Opportunity/Benefit:

The results of this project indicate that SBV infection will recur cyclically in the Irish national dairy herd therefore a prospective, national monitoring programme is recommended. This project presents the opportunity for a national approach to prospective monitoring, using the tools evaluated here, to be established.

6. Dissemination:

Main publications:

1. Collins, Á.B., Doherty, M.L., Barrett, D., and Mee, J.F., 2019. Schmallenberg virus: a systemic international literature review (2011-2019) from an Irish perspective. *Irish Veterinary Journal*, 72:9
2. Collins, Á.B., Mee, J.F., Doherty, M.L., Barrett, D., and England, M. 2018. Culicoides species composition and abundance on Irish cattle farms: implications for arboviral disease transmission. *Parasites & Vectors*, 11: 472.
3. Collins, Á. B., Mee, J. F. and Kirkland, P. (2018). Pathogenicity and teratogenicity of Schmallenberg virus and Akabane virus in experimentally infected chicken embryos. *Veterinary Microbiology*,

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Popular publications:

1. Collins, A., Kenneally, J., Heffernan, J. and Mee, J.F. (2019). Schmallenberg virus- lessons learned from the emergence of a novel virus. Moorepark 2017 Open Day Book, p.175-176.
2. Collins, A., Kenneally, J., Heffernan, J. and Mee, J.F. (2017). Schmallenberg virus- significant re-emergence and re-circulation in Ireland in 2016. Irish Dairying - Resilient Technologies Moorepark 2017 Open Day p.166-167.
3. Collins, A., Mee, J.F., Doherty, M., Barrett, D. and England, M.E. (2015). *Culicoides* biting midges as arbovirus vectors. *Veterinary Ireland Journal* 5 (10) p.476-478.
4. Collins, A., Kenneally, J., Heffernan, J. and Mee, J.F. (2015). Schmallenberg - it is history or could it re-emerge? Irish Dairying - Sustainable Expansion Moorepark15 Open Day p.119-120,
5. Collins, A., Mee, J.F., Doherty, M. and Barrett, D. (2014). Schmallenberg virus: three years of research progress. *Veterinary Ireland Journal* 4 (10) p.518-520.
6. Collins, A., Kenneally, J. and Mee, J.F. (2014). Schmallenberg-a new disease of cattle and sheep. *TResearch* 9 (3) Autumn, p.10-11.
7. Collins, Á. B., Mee, J. F. and Kirkland, P. (2018) Schmallenberg virus: pathogenicity and teratogenicity in the embryonated chicken egg (ECE) model. In: Proceedings of British Socitey of Animal Science, Dublin, April 9th -11th, 2018
8. Collins, Á. B., Mee, J. F., Doherty, M. L., Barrett, D. and England, M. E. (2018) *Culicoides* species composition and abundance, and new geographical species identification, on Irish cattle farms. In: Proceedings of British Socitey of Animal Science, Dublin, April 9th -11th, 2018
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10. Collins, Á. B., Barrett, D., Doherty, M. L., McDonnell, M. and Mee, J. F. (2017). Evidence of significant re-emergence and re-circulation of Schmallenberg virus in previously exposed dairy herds in Ireland in 2016. In: Proceedings of the 5th European Buiatrics Forum 4th-6th Oct 2017. p.163.
11. Collins, Á. B., Barrett, D., Doherty, M. L., McDonnell, M. and Mee, J. F. (2017). Cattle Association of Veterinary Ireland – Proceedings of the Annual Conference 2017 13th-15th October 2017 - Douglas Co. Cork. Schmallenberg returned once – will it return again in the future? In: Proceedings of the Cattle Association of Veterinary Ireland. p.129 – 138.
12. Collins, Á. B., Barrett, D., Doherty, M. L., McDonnell, M. and Mee, J. F. (2017). Schmallenberg virus: is there evidence the virus continues to circulate? In: Proceedings of Society of Veterinary Epidemiology and Preventive Veterinary Medicine, Inverness, Scotland, 29th – 31st March, 2017. http://www.svepm.org.uk/posters.html. p.1
13. Collins, Á. B., Barrett, D., Doherty, M. L., McDonnell, M. and Mee, J. F. (2017). *Culicoides* biting midges in cattle herds. ? In: Proceedings of Society of Veterinary Epidemiology and Preventive Veterinary Medicine, Inverness, Scotland, 29th – 31st March, 2017. http://www.svepm.org.uk/posters.html. p.2
14. Collins, Á. B., Mee, J. F. and Kirkland, P. (2017) Teratogenicity of Schmallenberg virus (SBV) and Akabane virus (AKAV) in chicken embryos – a novel experimental model to study SBV in ruminants. In: Proceedings of the 5th European Buiatrics Forum 4th-6th Oct 2017. p.161
15. Collins, Á. B., Mee, J. F., Doherty, M. L., Barrett, D. and England, M. E. (2017). Cattle Association of Veterinary Ireland – Proceedings of the annual conference 2017 13th-15th October 2017 - Douglas Co. Cork. What do we know about midges spread disease on Irish cattle farms? In: Proceedings of the Cattle Association of Veterinary Ireland. p.120-138
16. Collins, Á. B., Mee, J. F., Doherty, M. L., Barrett, D. and England, M. E. (2017). *Culicoides* biting midges on Irish cattle farms and their role in the transmission of Schmallenberg virus and Bluetongue virus. In: Proceedings of the 5th European Buiatrics Forum 4th-6th October 2017, Bilbao. p.162
17. Collins, Á. B., Mee, J. F. and Kirkland, P. (2017). Cattle Association of Veterinary Ireland – proceedings of the annual conference 2017 13th-15th October 2017 - Douglas Co. Cork. Can we use chick embryos instead of cattle to study Schmallenberg virus? In: Proceedings of the Cattle Association of Veterinary Ireland. p.129-137
18. Collins, A.B., Barrett, D., Doherty, M.L. and Mee, J.F. (2016). Predicting within-herd prevalence of infection with Schmallenberg virus in dairy herds using bulk-tank milk antibody levels. In Proceedings of British Cattle Veterinary Association, Leicestershire, UK , 20th – 22nd October, 2016. p.5.
19. Collins, A.B., Barrett, D., Doherty, M.L., Mee, J.F. and England, M.E. (2016). *Culicoides* arbovirus vectors: species composition, abundance and new geographical species identification on Irish dairy

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20. Collins, A.B., Doherty, M., Grant, J., Hallinan, A., Barrett, D. and Mee, J.F. (2016). Schmallenberg virus lacto/sero-surveillance: A novel exploration of milk and blood sample relationships. In: Proceedings of XIII Congreso Internacional de Medicina Bovina. p.246-247.
21. Collins, A.B., Doherty, M., Grant, J., Hallinan, A., Barrett, D. and Mee, J.F. (2016). Schmallenberg virus sero-surveillance: relationships between herd bulk milk and individual cow blood antibody values in exposed dairy herds. In: Proceedings of World Buiatrics Congress. p.360.
22. Collins, A.B., Hallinan, A., Barrett, D., Doherty, M.L., Larska, M. and Mee, J.F. (2016). Three years after the Irish Schmallenberg epidemic: does the virus continue to circulate?. *Veterinary Record*. p.8
23. Collins, A.B., Larska, M., Doherty, M., Hallinan, A., Barrett, D. and Mee, J.F. (2016). Post-epidemic Schmallenberg surveillance: prospective bovine serological and *Culicoides* virological studies. In: Proceedings of World Buiatrics Congress. p.361.
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25. Collins, A. & Mee, J. F. (2016) Monitoring for re-emergence of Schmallenberg virus. *Veterinary Record*, 179, 549-550
26. Collins, A., Grant, J., Hallinan, A., Doherty, M., Barrett, D. and Mee, J.F. (2015). Schmallenberg: is there evidence that the virus continues to circulate? In: Proceedings of the Congress on Controversies & Consensus in Bovine Health, Industry & Economics, Berlin, Germany, 27-Aug-2015.
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