Wally Thrale of Bedfordshire BKA, the coordinator for the East Anglian Beekeepers' Research Student scheme, writes:

Emma Bradford is our second Phd student (EARS 2). She is working at Aberdeen University on Deformed Wing Virus and Varroa with Dr Alan Bowman, looking to see how Varroa makes the virus more pathogenic than it was prior to the arrival of Varroa. Emma has recently started her 3rd year of research and a report of her work so far follows.

Most of the Associations in the East of England contribute to the support this work so I think it is important that all beekeepers have the opportunity to read about the work that we help to fund.

EARS2 PhD Project Progress Update

Any scientific papers which I write during the course of my PhD that are published in peer-reviewed journals will be sent to EARS to be distributed to all Eastern Region associations.

Project Background

The European honey bee is a globally economically important pollinator, however in recent years the managed honey bee population has been under intense stress due to the impact of parasites and pathogens. The main parasite of the honey bee is *Varroa destructor*. This parasitic mite negatively affects the health of the bees, mainly by its ability to vector honeybee viruses. The main virus affecting honey bees in the UK is Deformed Wing Virus (DWV). DWV was first identified in in honey bees in the 1970s (decades before *Varroa* arrived). *Varroa*'s arrival has been found to change the DWV viral landscape - its strain diversity. In the absence of *Varroa,* bees will have many different strains of DWV but at low levels. However, after *Varroa* has arrived bees have much higher viral loads, of fewer DWV strains. The two main DWV strains are DWVA and DWVB. DWVB is considered more pathogenic to the bees and most associated with *Varroa*. My project intends to help fill some of knowledge gaps that exist about the complex relationship between *Varroa*, DWV and honey bees.

My Project

Investigating DWV replication in Tick cell lines

Tick cell lines have been used in investigations regarding Arboviruses (Arthropod borne viruses) for many years, allowing us look at viral replication just within cells. There are currently no honey bee or *Varroa* cell lines; due to this fact there is very little known about how DWV interacts with cells. I was supplied with pure DWV from a collaborator in Germany, which I then used to infect cell lines. I screened 24 tick cell lines, and found that the DWVB strain (which is most associated with *Varroa*) replicated at low levels in all of the cell lines, whereas the DWVA strain (which is the more classical strain associated with honey bees) only replicated in 3 cell lines at very low levels. Given that *Varroa* and ticks are reasonably closely related, it was not that surprising that DWVB replicated better in the tick cells compared to DWVA. I performed this work was at The Pirbright Institute in Surrey for a few months in 2015 in collaboration with a large EU project.

Designing a new method for DWV quantification

Currently, most methods for DWV quantification are very strain specific, resulting in an underestimation of viral load. They also do not consider the impact of strain pathogenicity, with certain strains being more damaging to honey bee health than others. These are important aspects of infection to consider, so I have designed a new assay for looking at DWV viral loads. This new assay allows both strain specific, and total DWV levels to be analysed and will ensure that all aspects of DWV infection can be looked at, giving a more completed view of viral infection. This new assay is a positive reference sample for use in qPCR (quantitative Polymerase Chain Reaction). This positive reference sample is a plasmid containing three distinct sections, with each section being specific to a certain area of the DWV genome. Two of the sections are strain specific: DWVA and DWVB, while the third is a universal section which detects both strains, giving total DWV levels. These three sections have been combined into a single construct which can be used in a dilution series of known amounts to allow quantification of DWV levels within samples.

This new assay was tested using honey bee samples from multiple locations in the UK, including an area which is still *Varroa* free, areas which regularly treat for *Varroa* and an area which does not treat as regularly for *Varroa*. The results showed that bees from areas with no *Varroa* had extremely low viral levels; whereas bees from an area which does treat

regularly and therefore has high *Varroa* loads have extremely high viral levels. During this testing period four sets of samples from the EARS area were also tested, one each from Essex, Bedfordshire, Mid-Suffolk, and East-Suffolk. I am currently writing this work up as a manuscript for publication with me as the first author and EARS being acknowledged.

Investigating the potential use of antivirals to reduce viral levels

Antivirals are used in the treatment of viral infection; they work by inhibiting aspects of viral replication. At the moment there are no completely clean bees without DWV and, once a bee has DWV, there is no way to treat them. The ability to treat for DWV infection would be important as it could allow lab based work to be done looking at re-infection, and how the viral diversity changes. While this is extremely important for research, it is not a practical treatment for DWV nor is it designed to help find a cure for DWV. So far I have been looking at 2 antiviral compounds and 3 delivery methods – injecting honey bee pupae, feeding adult bees and soaking *Varroa*. I have been using my new assay for quantifying the viral load in these preliminary investigations. The preliminary results are encouraging indicating that there is an effect. This exciting area of work will be continued.

DWV strain transmission from *Varroa*

At the University of Aberdeen, we have an artificial feeding system for *Varroa*, which allows us to keep *Varroa* alive in the lab following removal from the hive. I have been using this system to investigate levels of DWV transmission during *Varroa* feeding, using my new assay for viral quantification. This allows us to see how much virus the mites could be injecting into the bees during feeding. The system can also be used to look at potential *Varroa* treatments, antiviral testing, and other investigations into the *Varroa* life. The work on the *Varroa* feeding system is currently being written up; I am a co-author, with an acknowledgment to EARS.

Investigating DWV in other pollinators

So far DWV has been found on bumble bees near honey bee colonies. I am planning on taking this research a step further, and looking to see if DWV is replicating within other pollinators. This is important as, if DWV is found to be replicating in other species, then it would imply that the virus is spilling over into different host species. I am planning on looking at a range of other pollinators including bumblebees, wasps and several species of solitary bees. I will not only be looking for replicating DWV, but also at which strain of DWV they are infected with. This will give us a valuable insight into potential viral dynamics of DWV in other species.

Conference attendance

There are two main ways to spread your research to the scientific community: publish research papers and conference attendance. I have presented written posters at three conferences including a Microbiology Society focus meeting on “Arboviruses and their Vectors”, Glasgow, for which I successfully applied for a £500 travel grant from the society to help towards the costs of my attendance. I also presented talks at two conferences including the International Congress of Entomology (ICE2016) held in Orlando, Florida, USA. I successfully applied for a £550 travel grant from the University to help towards my attendance of this conference. This was an excellent opportunity for me to present my research and meet and hear talks from research within the entomological and bee communities.

EARS Talks

During November I came down to the EARS regions, and gave a series of talks to 4 of the EARS associations. I spoke at the Lincolnshire BKA, Bedfordshire BKA, Ipswich and East Suffolk BKA, and West Suffolk BKA. I really enjoyed the opportunity to meet members of EARS and to be able to tell those who were able to attend about the work I have been doing, and what I plan to continue to do during the rest of my PhD.

Emma Bradford December 2016

We are fortunate that Emma’s supervisor is Professor Alan Bowman, the pre-eminent Varroa & DWV researcher in Europe and perhaps the world. It is his view that we may soon decide to switch the weight of research from Varroa to DWV since, even if we conquer Varroa, we shall still have DWV as it is transmitted in honey bee brood food, sperm and eggs.