The Foulbroods; Know your enemy

Kirsty Stainton

NBU Technical manager

Animal & Plant Health Agency

European foulbrood

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European foulbrood:

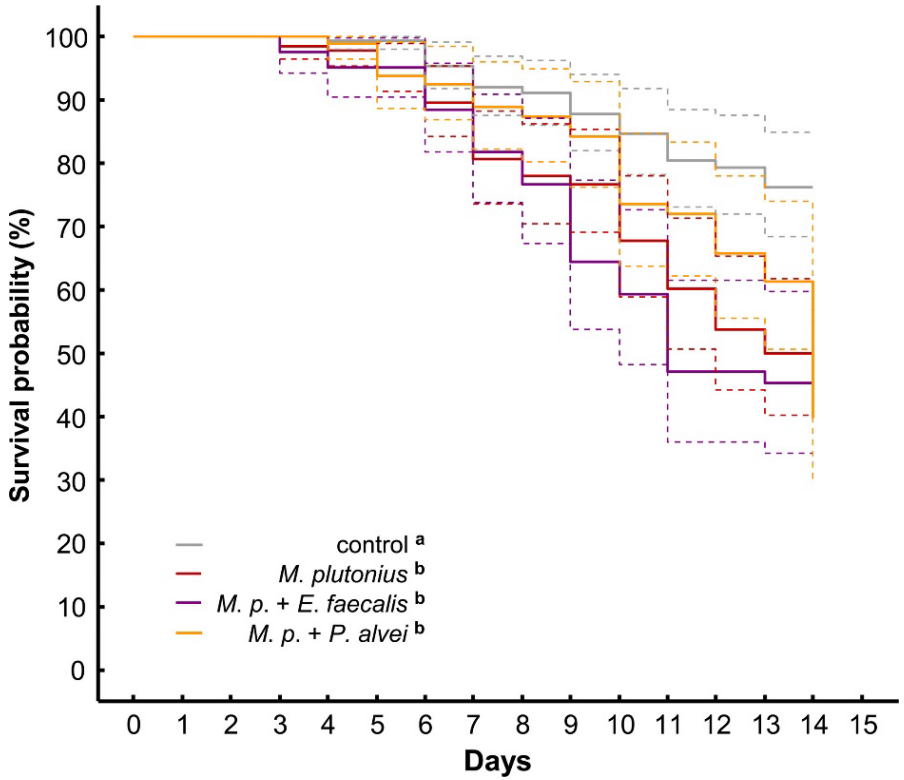
- EFB is caused by a bacterium called *Melissococcus plutonius*
- Intestinal infection of larvae caused by eating infected brood food; larvae of 4-5 days old are the most susceptible
- The bacteria then grow inside the gut and fill it up; the larvae die of starvation
- Larvae that survive to become adults that can discharge the bacterium in their faeces, re-infecting the colony
- Adults are asymptomatic

- A statutory notifiable disease in the UK

European foulbrood: secondary infections

- Secondary invaders include *Paenibacillus alvei*, *Enterococcus faecalis* and *Achromobacter Eurydice*
- They are harmless to healthy bees
- Study by Lewkowski *et al*. (2019) co-infected larvae with EFB and secondary bacteria

No significant difference in lifespan between EFB only and EFB with an additional infection



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Lewkowski, O., & Erler, S. (2019). Virulence of *Melissococcus plutonius* and secondary invaders associated with European foulbrood disease of the honey bee. *MicrobiologyOpen*, *8*(3), e00649.

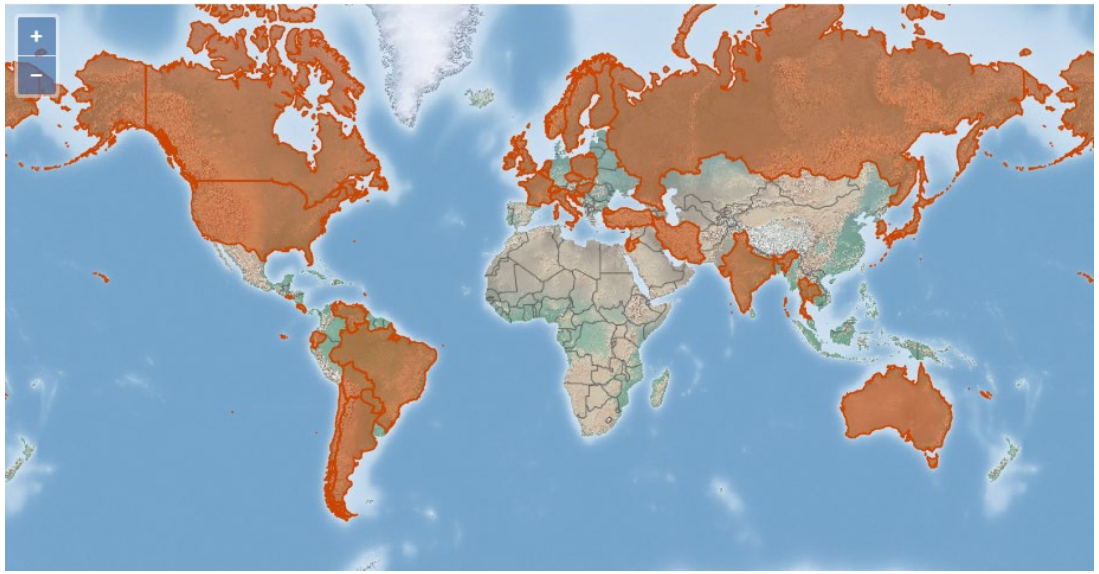
European foulbrood: distribution and prevalence

Distribution:

Widespread throughout Europe, North America, South America, Russia and some Asian countries. Absent from Africa, except South Africa; present in Australia but not Western Australia or its Northern Territory, nor New Zealand.

Prevalence:

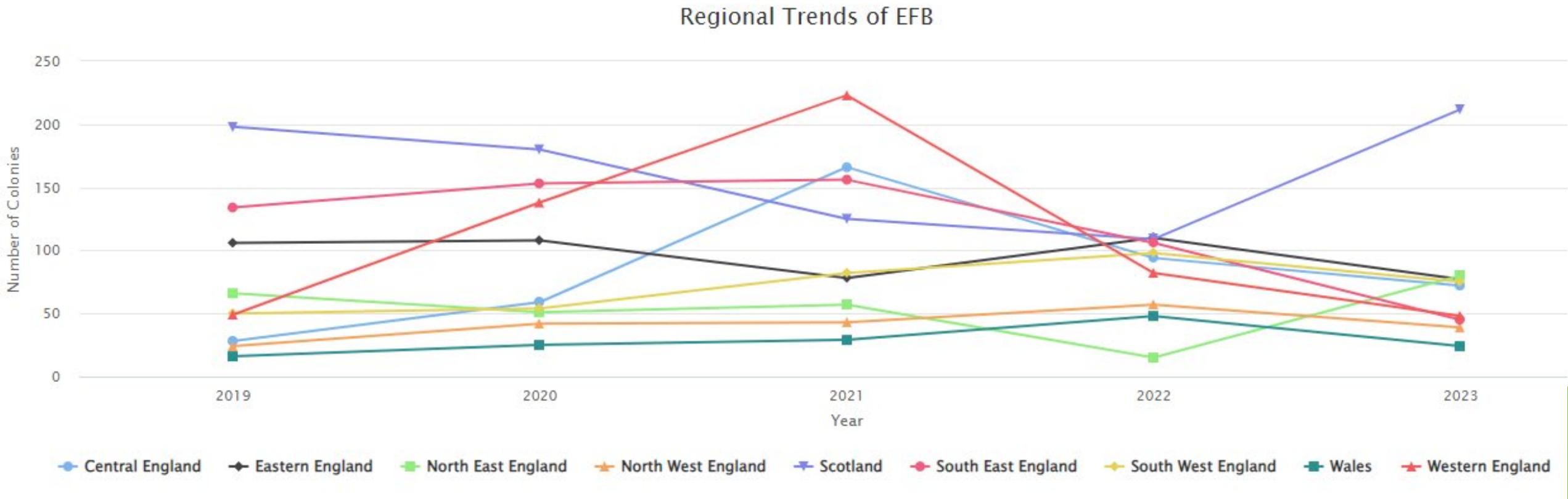
Varies by country and within country by year. In the UK, it fluctuates between 1 and 4%, while in North America, it is between 1 and 6.4%



Map courtesy of CABI.org

European foulbrood: UK distribution

All graphs available from NBU website



European foulbrood: UK distribution

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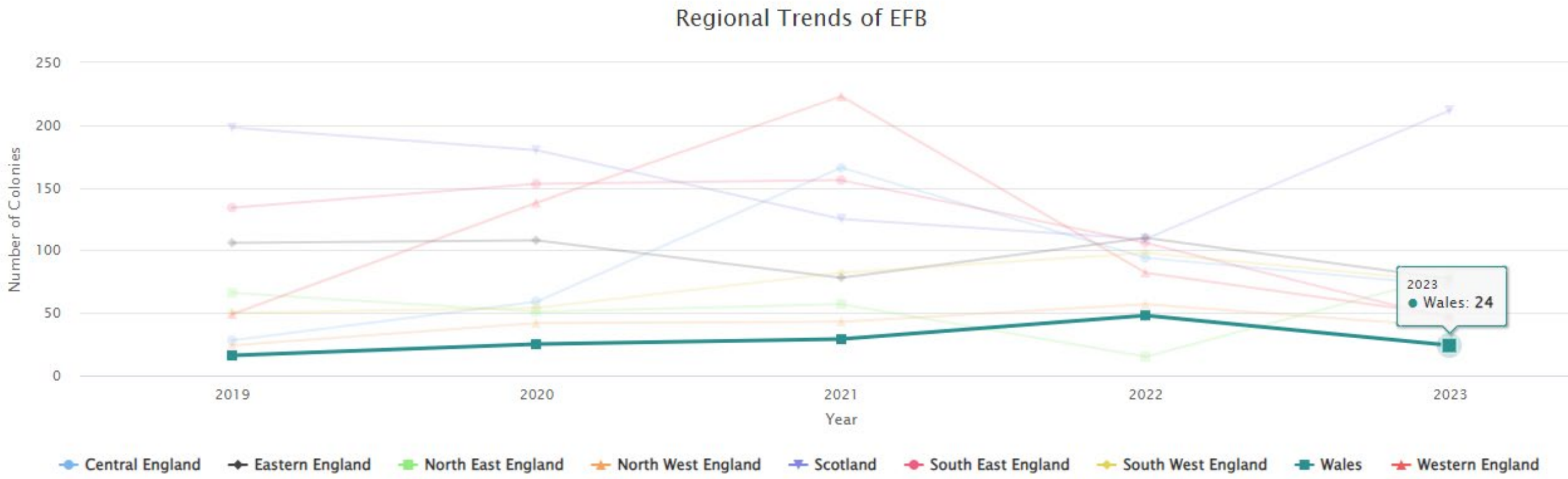
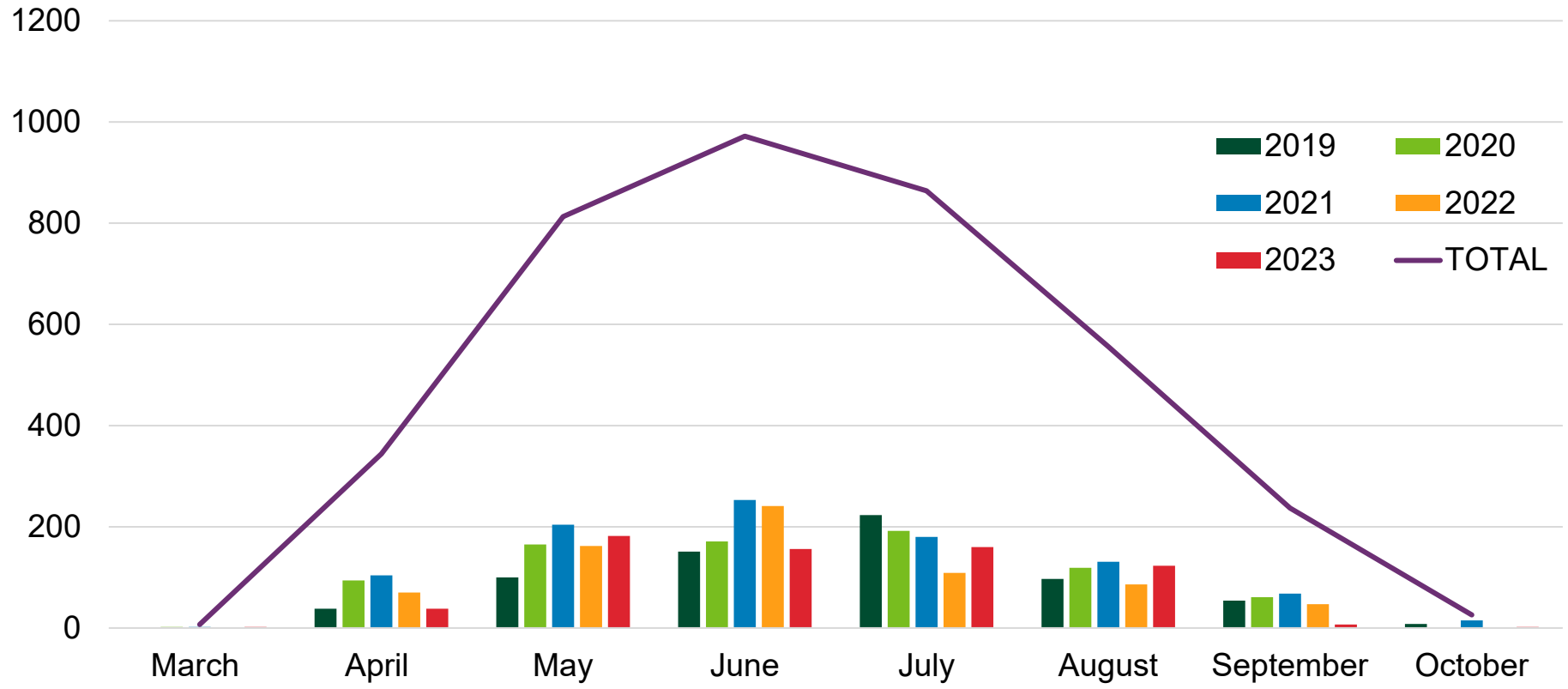


European foulbrood: UK seasonality*



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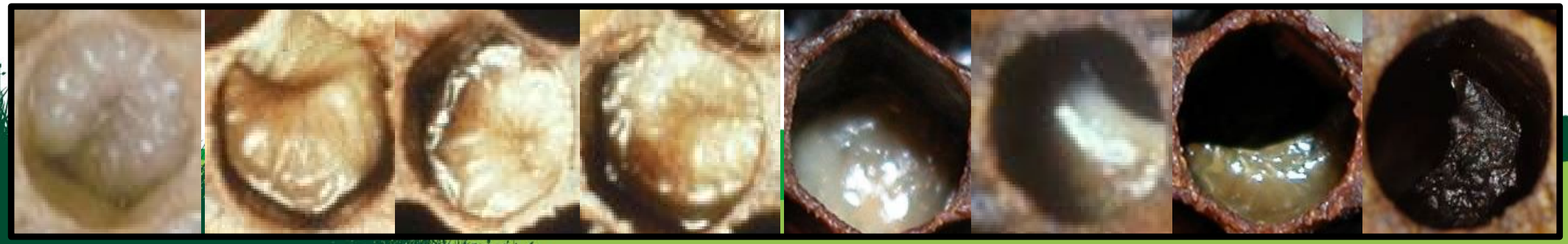
* England, Wales and Scotland

European foulbrood: signs of disease

- Younger larvae die and become transparent
- Older larvae twist and become flaccid (loss of segmentation) and turn yellow
- As decomposition progresses, the dead larval remains turn brown and eventually turn into a dark rubbery scale

Memory tip:
The ‘E’ in EFB is for ‘early’ – look for signs of disease *before capping*



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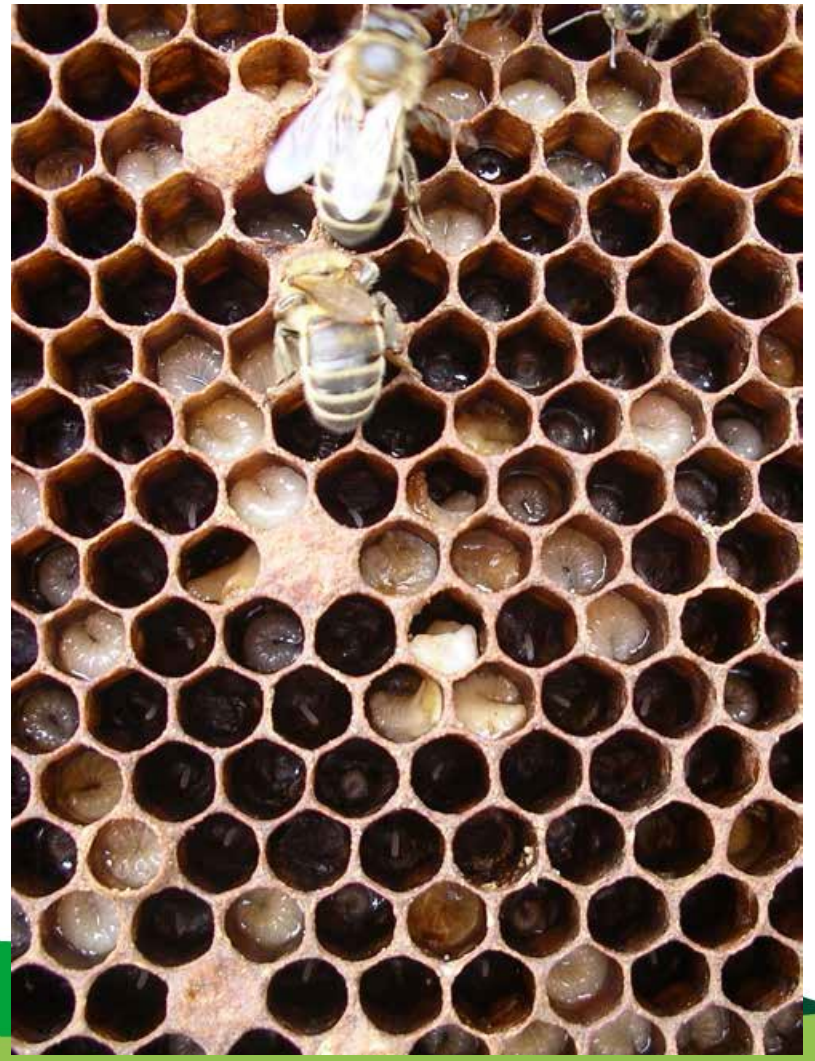
European foulbrood: signs of disease

Healthy brood is:

C shaped

Pearly white

Nicely segmented



EFB infected brood is:

Twisted and irregular

Yellow or brown

Melted

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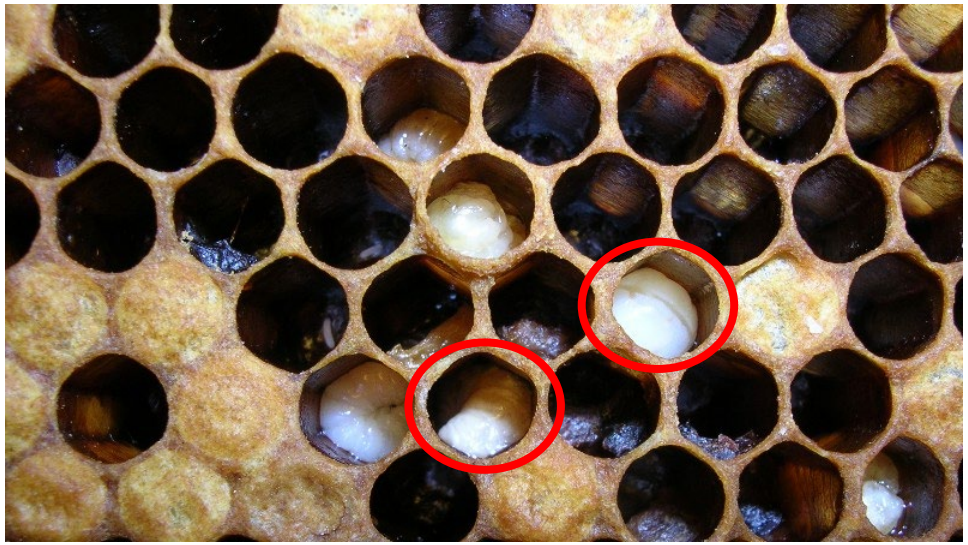
European foulbrood: signs of disease

Early stages of disease



- Larvae still white but start to twist and turn in their cells
- Segmentation pattern looks less plump

Late stages of disease

- Larvae are yellow or brown
- Brown and caramel coloured scales
- Brood pattern breaks down – ‘pepperpot’ brood pattern

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European foulbrood: Transmission

- Robbing or drifting bees from infected colonie, swarms or sale of bees
- Bacteria can be present in comb, honey and wax, or be present on the surface of boxes, tools or other equipment
- Study by Forsgren *et al.* (2005) founds bacteria present in honey and larvae of symptomatic colonies
- Also detected the bacteria in asymptomatic colonies (using PCR)

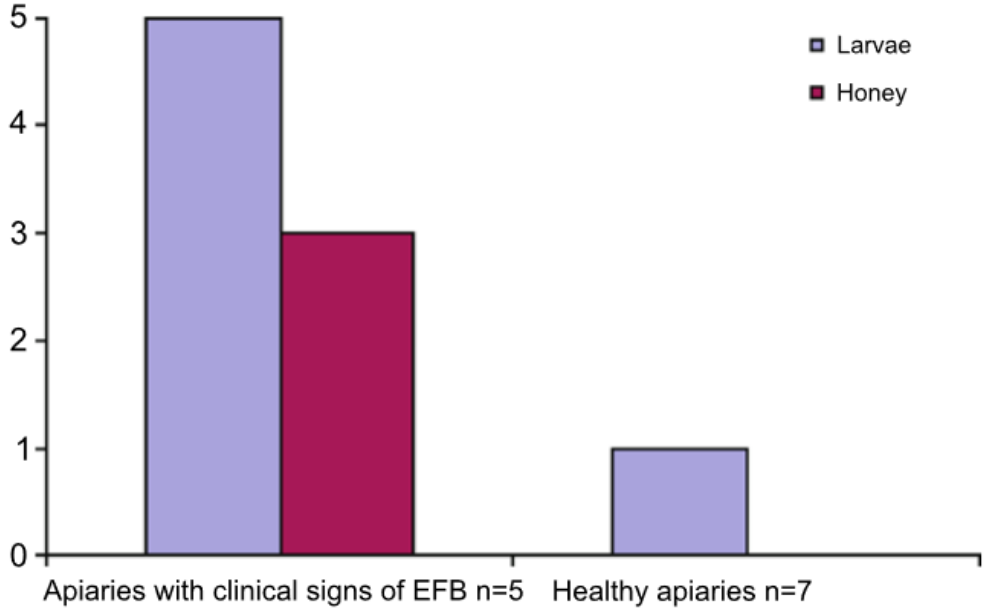


Figure 2. Number of apiaries where *M. plutonius* was detected by PCR in honey and pooled samples of larvae.

Forsgren E, Lundhagen AC, Imdorf A, Fries I. Distribution of Melissococcus plutonius in honeybee colonies with and without symptoms of European foulbrood. Microbial ecology. 2005 Oct;50:369-74.

European foulbrood: Transmission

- Infection with EFB strongly correlated with proximity to other infected hives
- In non symptomatic colonies within 500m to 1km to an outbreak, almost 40% of colonies contained the bacteria
- In non symptomatic colonies over 1km from an outbreak, around 10% of colonies contained the bacteria

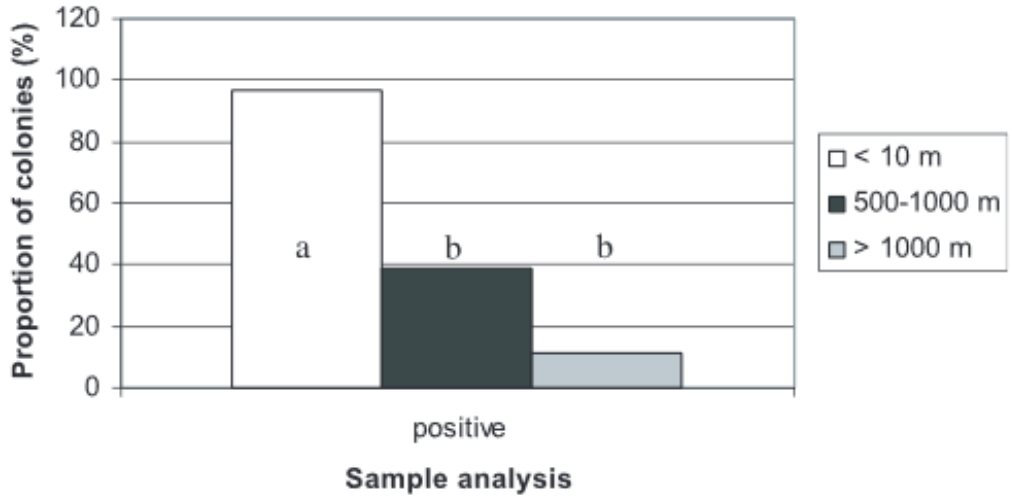
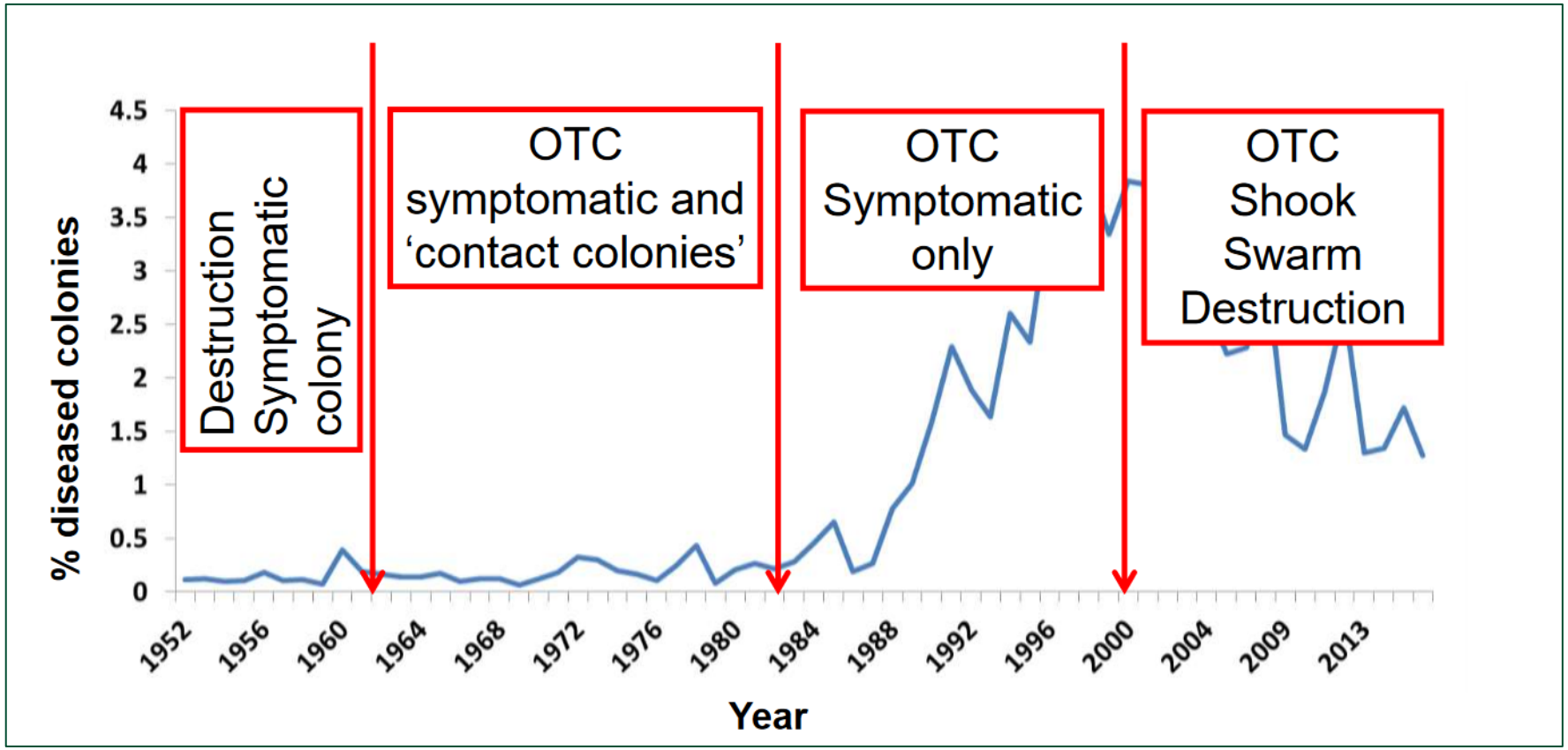


Figure 1. Proportion of samples positive for *Melissococcus plutonius* in colonies within apiaries with clinical EFB present (< 10 m, 6 apiaries, 32 colonies) and in non-symptomatic apiaries at two different distance categories (500–1000 m, 3 apiaries, 23 colonies; >1000 m, 2 apiaries, 9 colonies).

Belloy, L., Imdorf, A., Fries, I., Forsgren, E., Berthoud, H., Kuhn, R. and Charrière, J.D., 2007. Spatial distribution of Melissococcus plutonius in adult honey bees collected from apiaries and colonies with and without symptoms of European foulbrood. *Apidologie*, *38*(2), pp.136-140.

European foulbrood: Treatment (UK)

Historically



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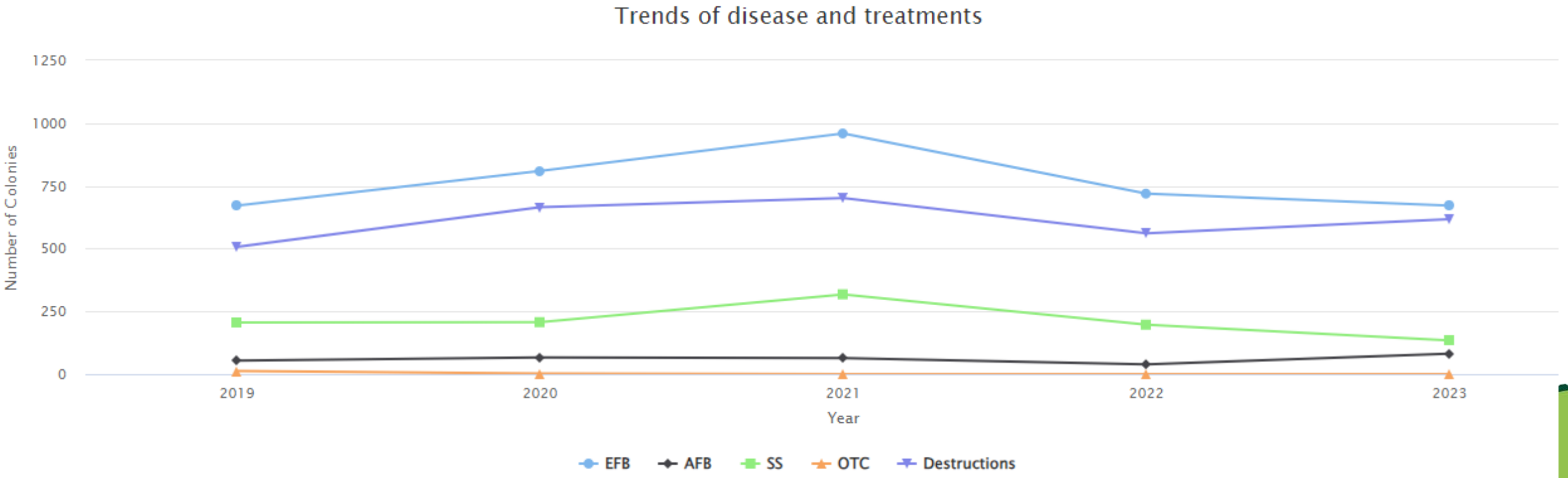
European foulbrood: Treatment

Today:

- Shookswarm
- Destruction
- OTC not used



European foulbrood: Avoiding antibiotics

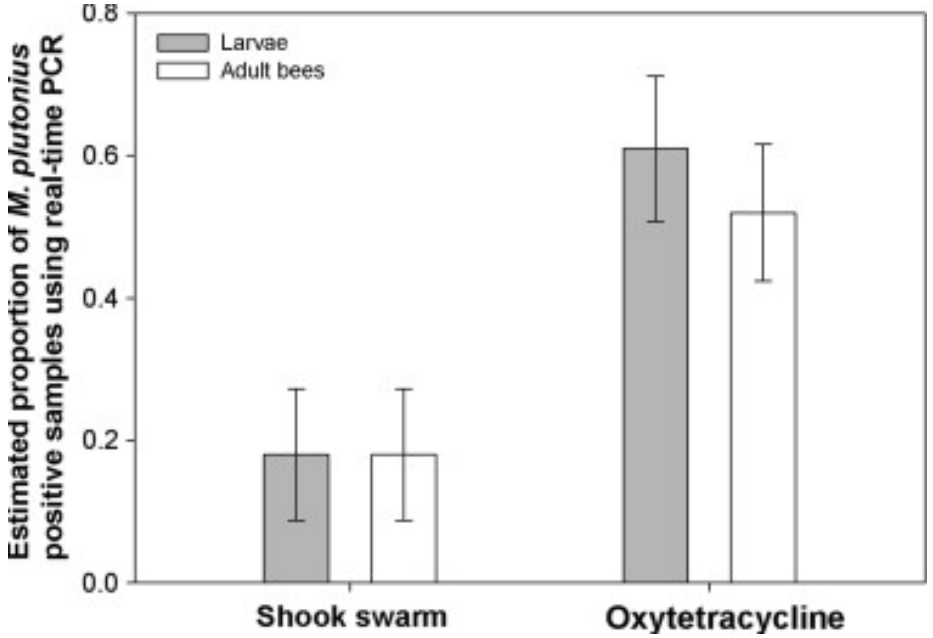
- Some strains of EFB are completely resistant to OTC
- A number of strains identified by Nicola Burns in specific regions of the UK (such as ST5 in East Anglia)
  Burns, N (2021) PhD thesis
- OTC thought to ‘mask’ the symptoms of EFB
- Some strains can re-occur after shookswarm so it’s not a panacea

European foulbrood: Avoiding antibiotics

- Shook swarm can be more effective than OTC

- Following treatment, OTC treated colonies are:
  - three times more likely to still have EFB in spring
  - five times more likely in summer

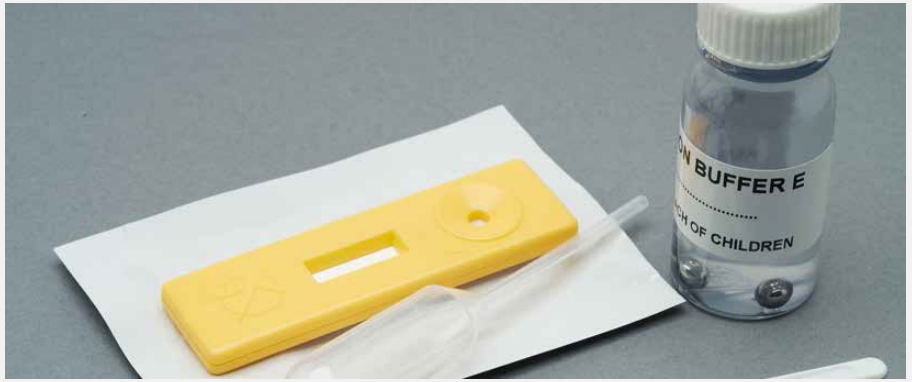
(compared to SS)

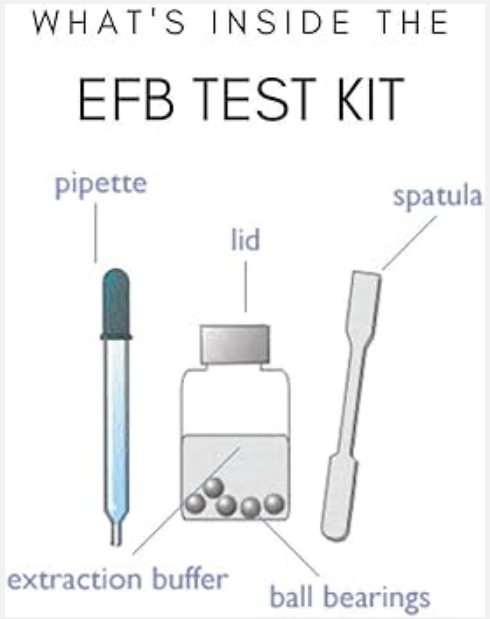


Budge, G.E., Barrett, B., Jones, B., Pietravalle, S., Marris, G., Chantawannakul, P., Thwaites, R., Hall, J., Cuthbertson, A.G. and Brown, M.A., 2010. The occurrence of Melissococcus plutonius in healthy colonies of Apis mellifera and the efficacy of European foulbrood control measures. *Journal of invertebrate pathology*, *105*(2), pp.164-170.

European foulbrood: Tracking strains in the UK

- All (registered) outbreaks in the UK collected by NBU Bee inspectors
- Buffer bottle from EFB kits sent back to Fera Science Ltd
- All samples ‘sequence typed’ to determine bacterial strain present
- Every EFB sample since 2014





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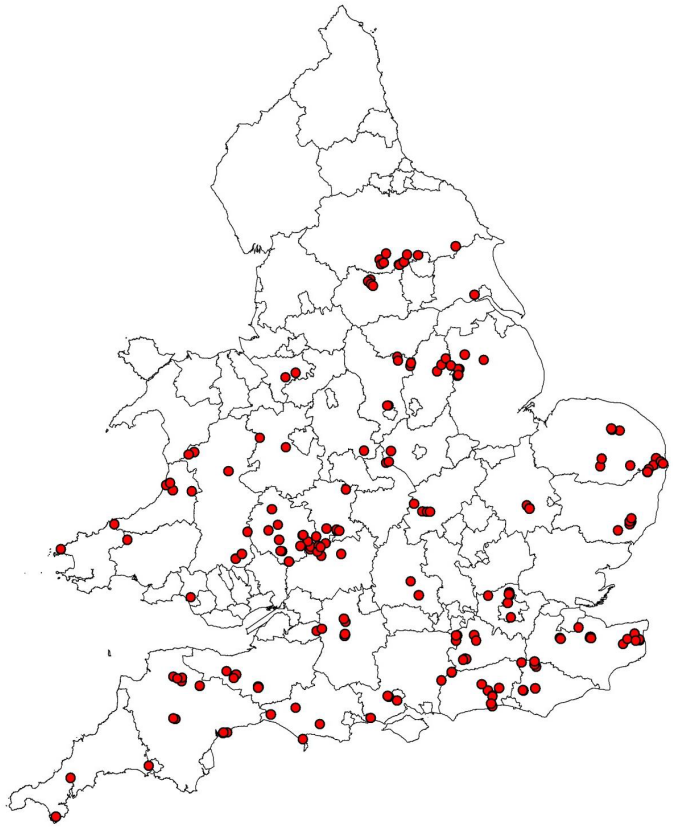
European foulbrood: Tracking strains in the UK

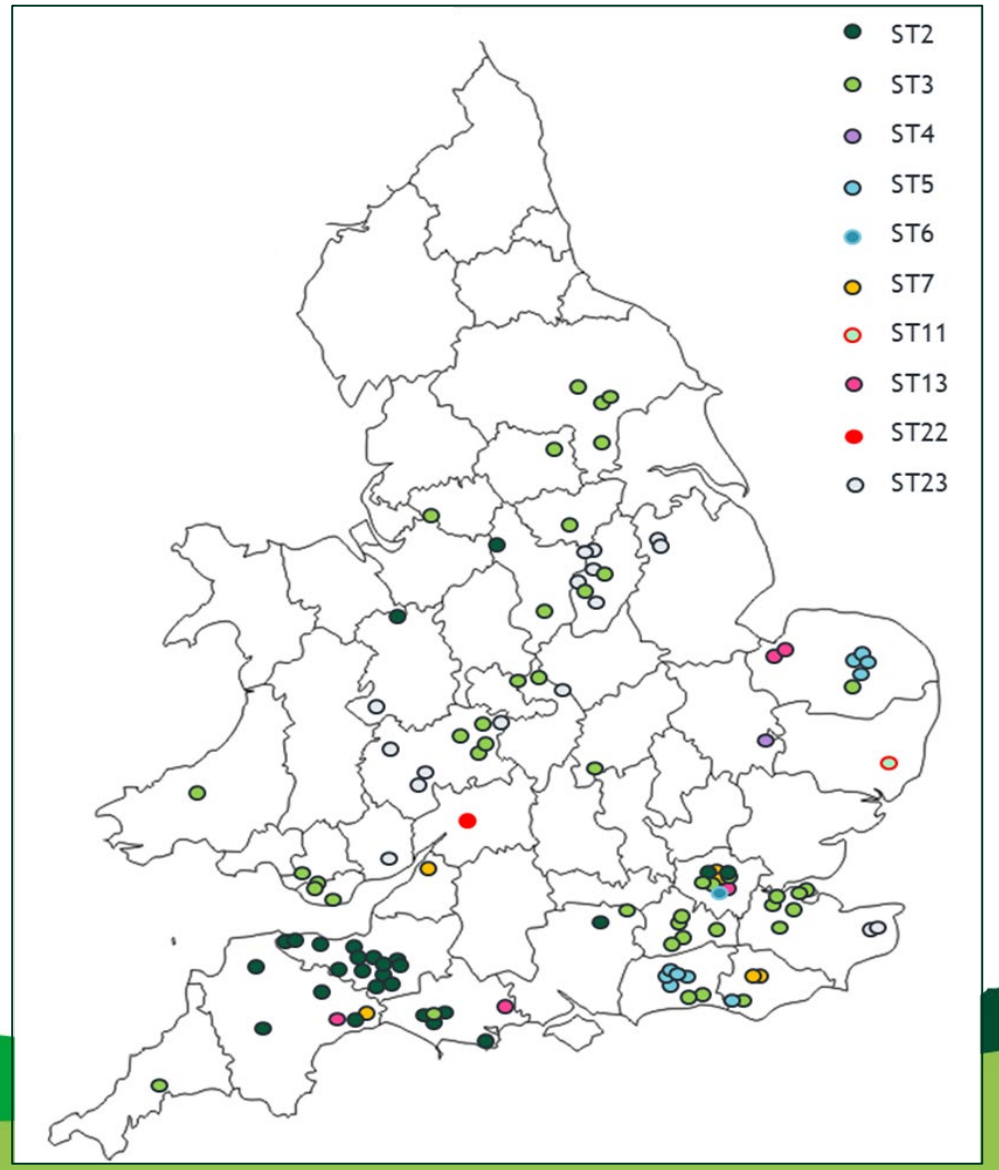
- Multi-locus sequence typing (MLST) compares differences between 4 genes
- Each samples is then allocated a ‘sequence type’
- 39 different sequence types in the world; up to 28 in the UK
- Strains vary in virulence, resistance, persistence



European foulbrood: Tracking strains in the UK

- Cases logged in the UK:
  - 2018: 248 cases
  - 2019: 671 cases
  - 2020: 810 cases
  - 2021: 959 cases
  - 2022: 719 cases
  - 2023: 672 cases

- Geographical resolution on STs



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European foulbrood: Outbreak identification case studies

- Case study 1:

BK1 sold honey to BK2 located 54 km away. BK2 lived in an area where there was no EFB in past 10 years. MLST typing linked the new outbreak to the honey from BK1 (rare ST9)

- Case study 2:

BK1 sold diseased bees to BK2 located 84 km away. Bacteria from both BKs was identitical; the ST was found nowhere else in the UK (rare ST11)

Haynes, E., Helgason, T., Young, J.P.W., Thwaites, R. and Budge, G.E., 2013. *Environmental Microbiology Reports*, *5*(4), pp.525-529.

https://www.nationalbeeunit.com/bee-health-improvement/scientific-journal-publications/

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European foulbrood: Tracking strains in the UK

- Can identify new incursions of unique STs
- Can identify if strains have been eradicated
- Can examine how different STs respond to treatment
- Can perform experiments to examine the biological differences between STs

European foulbrood: Prevention

- Sign up to BeeBase
- Never buy colonies of bees unless you can verify that they are disease free
- Keep swarms in an isolation apiary until confirmed disease free
- Perform regular brood inspections (increase in frequency if there is an alert in your email)

National Bee Unit - disease alert

nbu@beebaseadmin.fera.co.uk Mon, Sep 4, 7:07 PM
to me

BeeBase

THIS IS AN AUTOMATED EMAIL SENT TO YOU BY THE NATIONAL BEE UNIT (NBU)

Dear Ms Kirsty Stainton.

This email has been sent out as part of a 'disease alert' service to beekeepers, implemented by the National Bee Unit (NBU). At the end of each day, beekeepers will be notified if they have an apiary that is within 3KM of a new disease outbreak.

Please be advised that today, the following disease has been confirmed within 3KM of one or more of your apiaries

Disease Diagnosed: European Foul Brood

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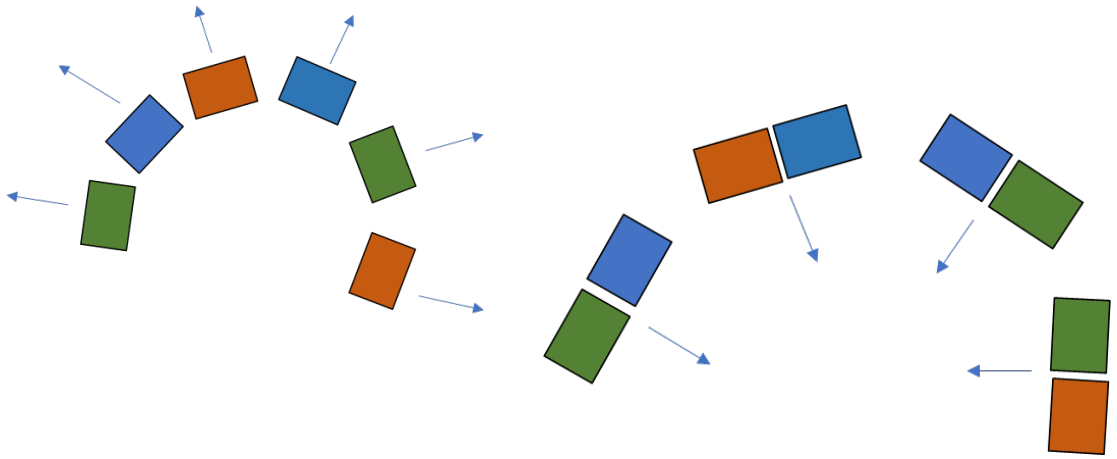
European foulbrood: Prevention

- Using optical trackers, *Bordier et al.* (2017) found that 10 -16 % of bees drifted into neighbouring colonies
- Some viruses can increase this; i.e. IAPV
- More likely to drift from the centre hives to the end hives than the other way around
- Can recognise height & the colour below the entrance better than above it



Bordier, C., Pioz, M., Crauser, D., Le Conte, Y. and Alaux, C., 2017. Should I stay or should I go: honeybee drifting behaviour as a function of parasitism. Apidologie, 48(3), pp.286-297.

European foulbrood: Management

- In the UK, bee inspectors co-ordinate treatment with the beekeeper
- Shookswarm only performed if appropriate

Examples of suitability for colony shook swarm:

Weak colony ✗	Heavily infected ✗	Autumn/ Winter ✗
Strong colony ✓	Lightly infected ✓	Spring/ Summer ✓

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Figure A: Flow Chart of European Foulbrood Control - Destruction

Apiary inspection

EFB suspected
Standstill Notice placed on apiary

EFB confirmed in the field by means of an LFD

No

EFB confirmed at the NBU laboratory

No

No evidence of EFB

Yes

Yes

Heavily infected, small colony or beekeepers preferred action or previous treatment failed

Infected colony killed by Beekeeper/ Bee Inspector

Bees, frames, combs and honey destroyed by fire

After six Weeks or at the commencement of the following season, re-inspection of apiary

Signs of reportable disease

No signs of reportable disease

Standstill Notice withdrawn

European foulbrood: Decontamination

<u>0.5% bleach in cold water</u> and scrubbing (in a well ventilated space)

- '*Strong*' bleach isn't superior and can be toxic
  (*1hr of 0.5% bleach is just as effective as 4% at killing anthrax spores)*
- Heat decomposes sodium hypochlorite making it ineffective [wash off with hot water]
- Non-porous objects (i.e. hive tool) > 10 minutes
- Porous objects (i.e. boxes) > 30 minutes

NBU factsheet

<u>4% soda crystals in hot water (must be >80°C)</u> and scrubbing

Please use PPE with bleach or boiling soda crystals (mask, gloves, specs & apron)

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European foulbrood: Decontamination

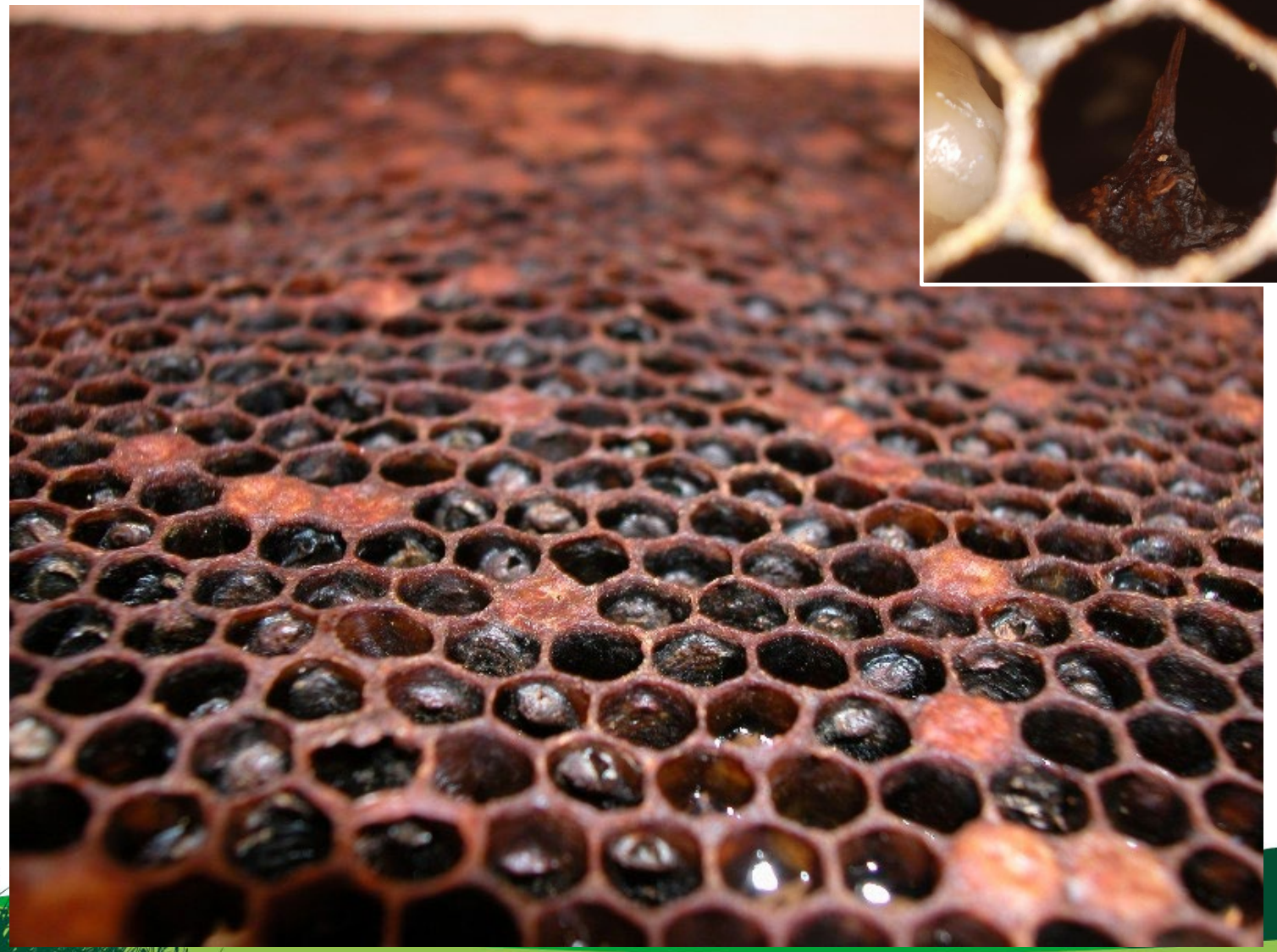
- Question from audience member:
  Would freezing, or cycles of freezing/thawing, destroy EFB?

  [a video suggests leaving equipment (i.e. frames) in freezer for a couple of weeks would be sufficient to re-use]

NBU factsheet

- Answer:
  - Many bacteria can easily survive freezing
  - Some bacteria survive freeze/thawing
  - Survival dependent on a number of factors:
    Strain of bacteria (gram + less susceptible), pH of matrix, temperature and cycles of freeze/thawing

American foulbrood



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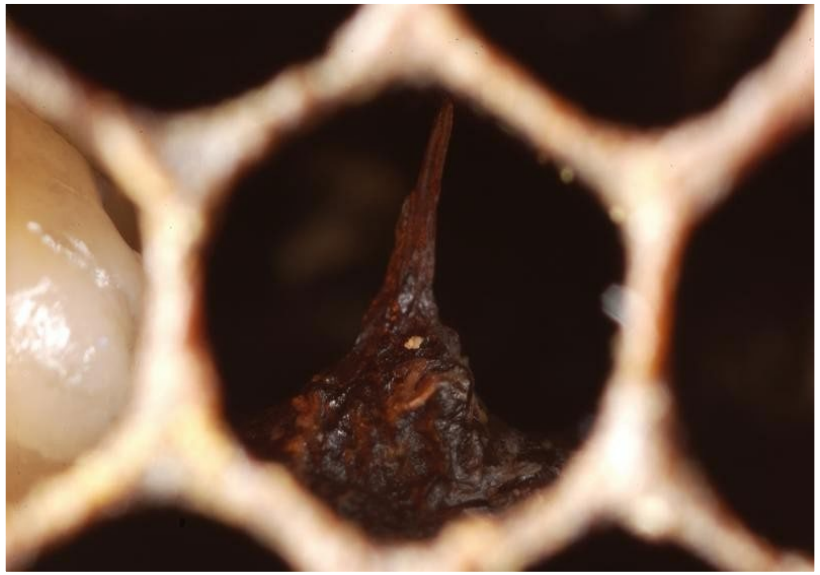
American foulbrood

- AFB is caused by a spore-forming bacterium called *Paenibacillus larvae*
- Intestinal infection of larvae caused by eating infected brood food; larvae of 12 to 36 hrs old are the most susceptible
- Only 10 spores needed to cause infection
- Spore germinates in larval gut; bacteria secrete toxins which damage the gut and spread to rest of the body, causing rapid death



American foulbrood: distribution and prevalence

Secondary infections: *N*one. AFB secretes toxins to inhibit other bacteria

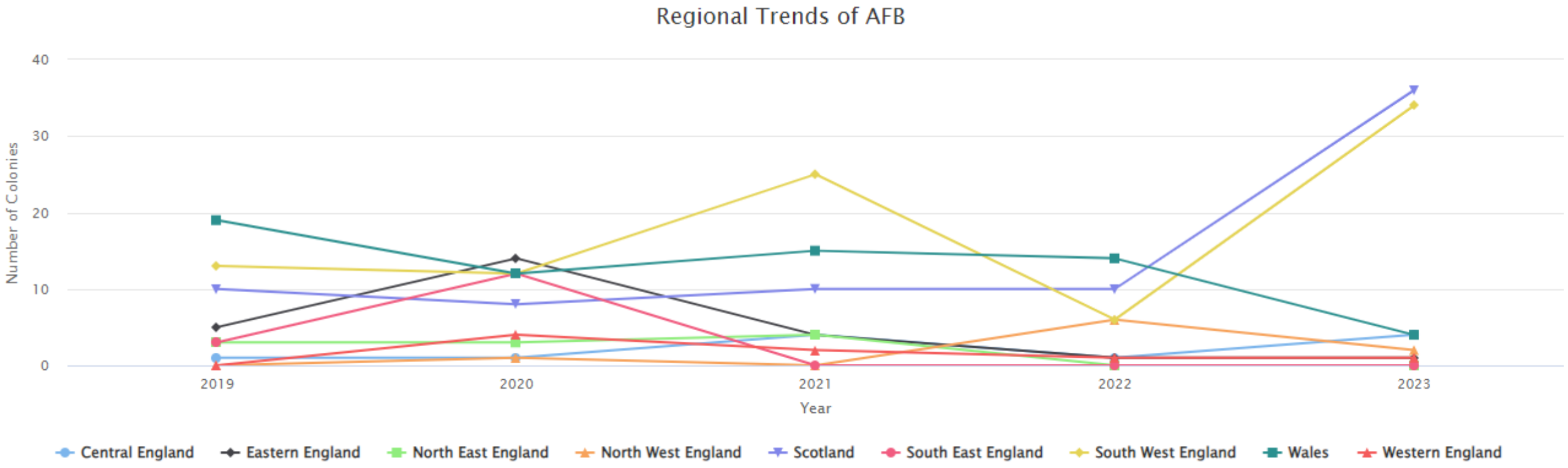
***Distribution*:** Widespread in Asia, Europe, North America, Australia, found in some South American countries. Not found in Russia or Africa, except South Africa and Guinea-Bissau

Prevalence: In the UK, less than 1% of colonies are affected per year: fewer than 100 colonies per year out of approximately 260,000 registered colonies

	AFB cases	EFB cases
2019	54	671
2020	67	810
2021	64	959
2022	39	719
2023	82	672

American foulbrood: UK distribution

All graphs available from NBU website



American foulbrood: UK distribution

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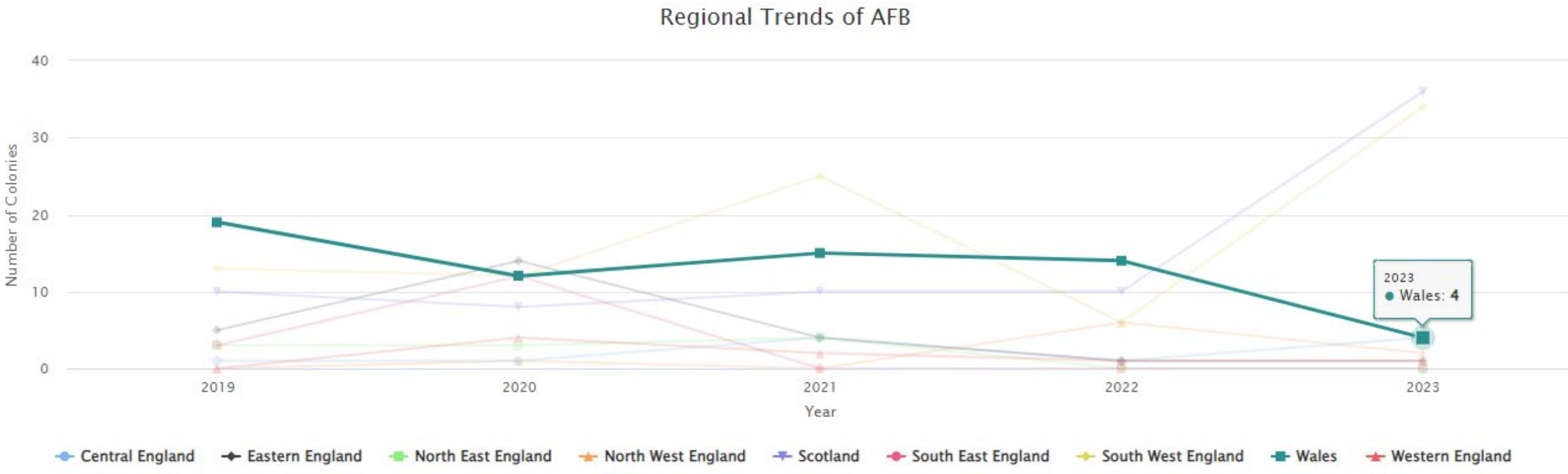


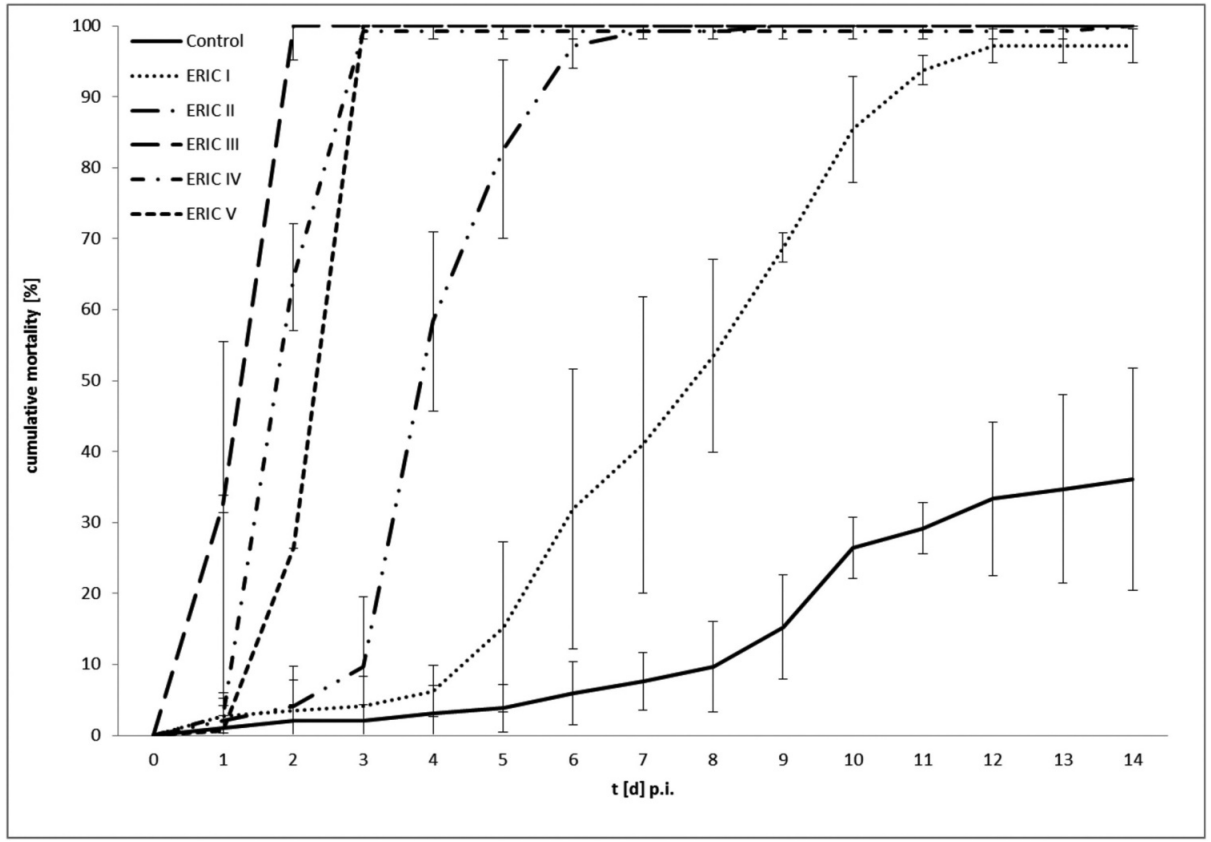
American foulbrood: virulence

- AFB is extremely virulent
- Mortality of *larvae* after infection with different *P. larvae* genotypes
- Larvae of <30 hr old were infected with **500 spores** of *P. larvae*

  Death occurs:

  ERIC I – up to 12 days

  ERIC II-IV – 6 to 7 days



Beims, H. et al. 2020. *International Journal of Medical Microbiology*, *310* (2), p.151394.

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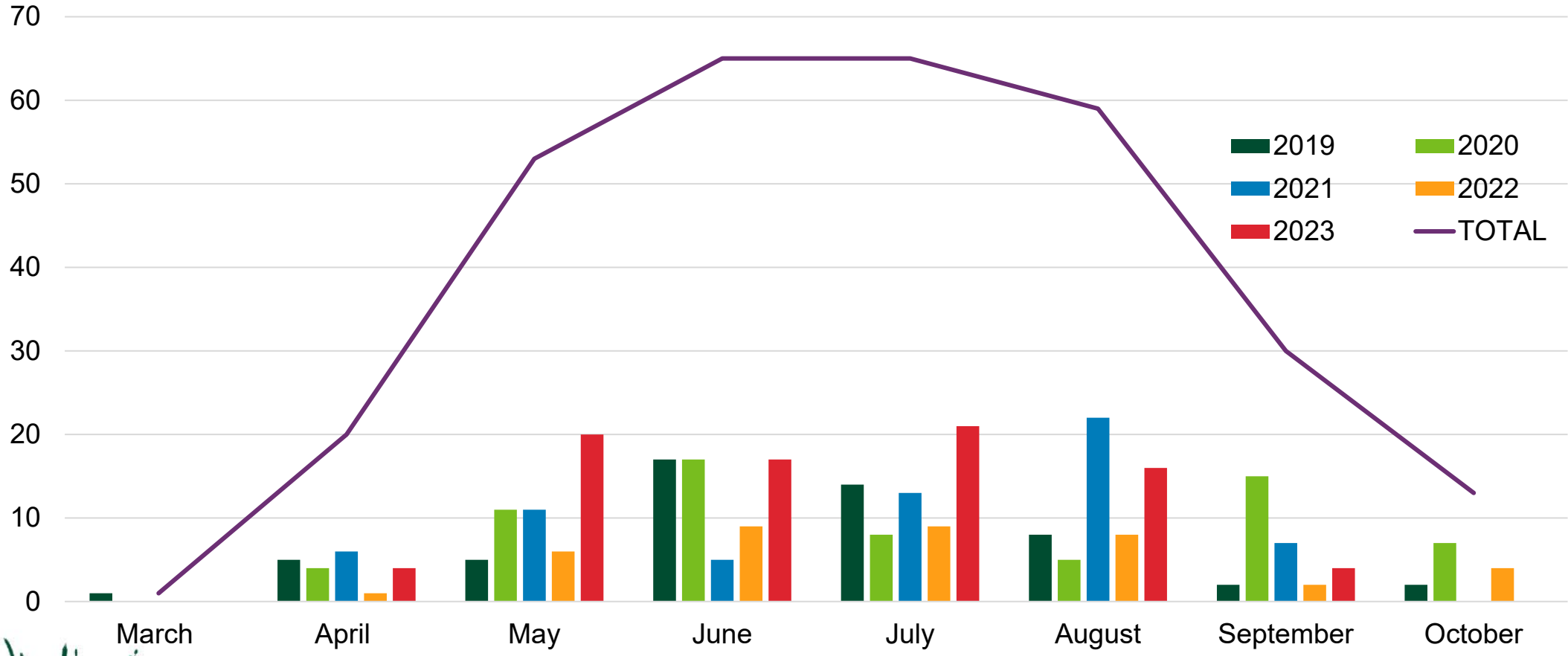
American foulbrood: UK seasonality*



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* England, Wales and Scotland

American foulbrood: signs of disease

- Uncapped larvae are not symptomatic; symptoms appear after capping
- Infected larvae die in their cells; cells turn dark and greasy and may look sunken
- The dead remains turn brown/black and eventually turn into a dark rubbery scale
- Causes colony collapse

Memory tip:
The ‘A’ in AFB is for ‘after’ – look for signs of disease *after capping*

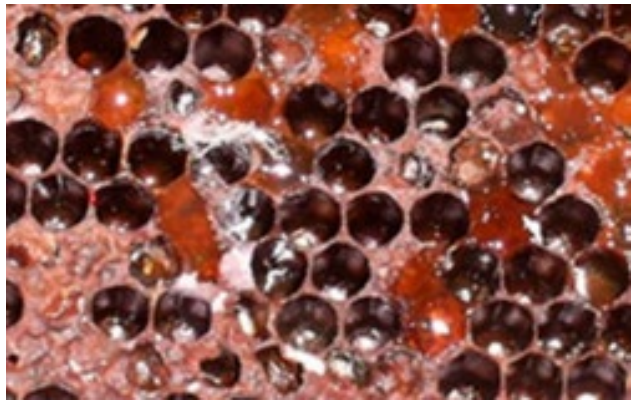

Image reproduced under the Creative Commons License 4.0:
Matovic et al. (2023)
Vet. Sci. 10 (3)

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American foulbrood: signs of disease

Healthy cappings:

Biscuit coloured

Consistent pattern

AFB cappings:

Sunken

Greasy

Dark

Pepperpot

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American foulbrood: signs of disease

AFB roping

- Bacteria secrete sticky proteins which aggregate
- Long roping

AFB scale

- After death and decomposition, dark scales form
- Triangular shaped
- Bottom of cells

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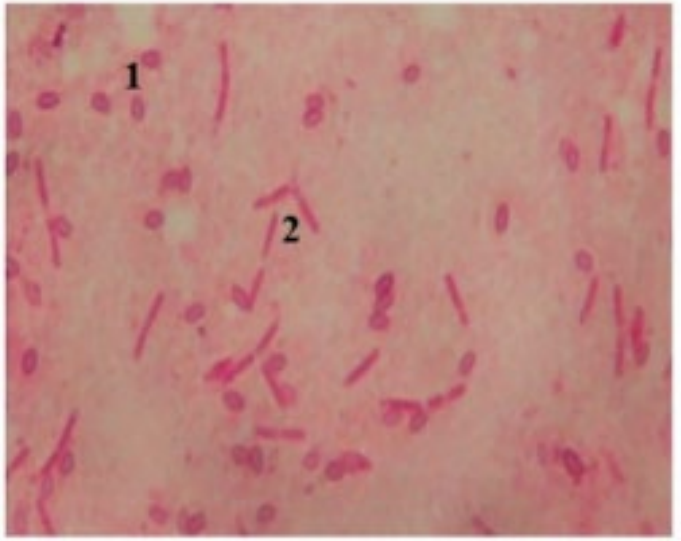
American foulbrood: Transmission

- Robbing or drifting bees from infected colonies, swarms, or sale of bees

- Bacteria can be present in comb, honey and wax, or be present on the surface of boxes, tools or other equipment

- **Spores can persist for decades**
- Symptoms appear only late in disease progression
- A single infected larva may contain 2500 million spores; scales even more highly infectious
- 10 spores to cause infection: **colonies highly infectious once symptoms appear**



P. larvae spores (A) and cells (B) under a microscope. Figure reproduced under the Creative Commons License 4.0: Matovic et al. (2023) Vet. Sci. 10 (3)

American foulbrood: Treatment (UK)

- **Destruction**

Petrol tipped into crown board to kill the bees

Combs and bees destroyed by burning

Everything else is sterilised with a blow torch

- **AFB is a death sentence for the colony**

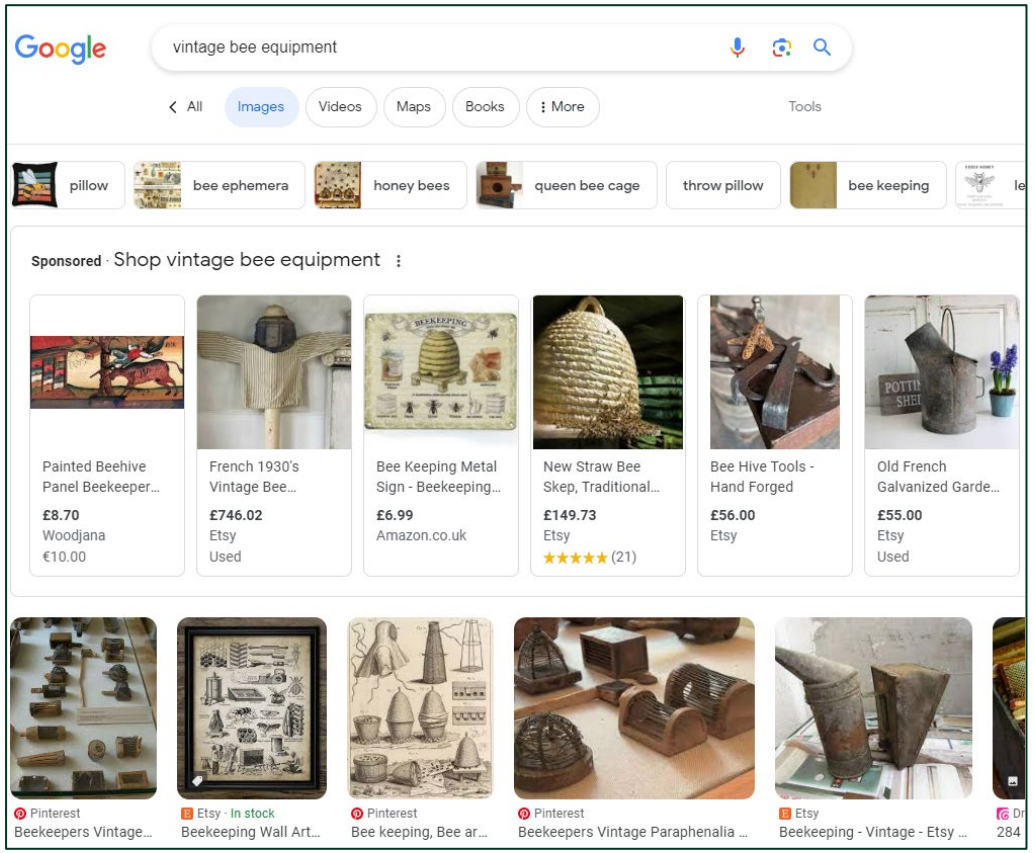
This approach helps prevent further transmission

Spores are extremely resilient to physical and chemical interventions

- **MLST strain typing also performed but results less relevant due to destruction only policy**

American foulbrood: Prevention

- AFB spores are very resilient, surviving on infected boxes for decades
- UK outbreaks commonly caused by use of old equipment that has not been thoroughly decontaminated before use
- Brood inspections if outbreak alert – the highest levels of transmission of AFB occurs within 1 km of clinically diseased colonies
- BeeBase email alerts for AFB as well



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American foulbrood: Decontamination

Please use PPE with bleach or boiling soda crystals (mask, gloves, specs & apron)

Clean any second-hand equipment:

0.5% bleach in cold water and scrubbing (in a well ventilated space)

- '*Strong*' bleach isn't superior and can be toxic
  (*1hr of 0.5% bleach is just as effective as 4% at killing anthrax spores)*
- Heat decomposes sodium hypochlorite making it ineffective [wash off with hot water]
- Non-porous objects (i.e. hive tool) > 10 minutes
- Porous objects (i.e. boxes) > 30 minutes

4% soda crystals in hot water (must be >80°C) and scrubbing

Scorching only kills spores on the surface!

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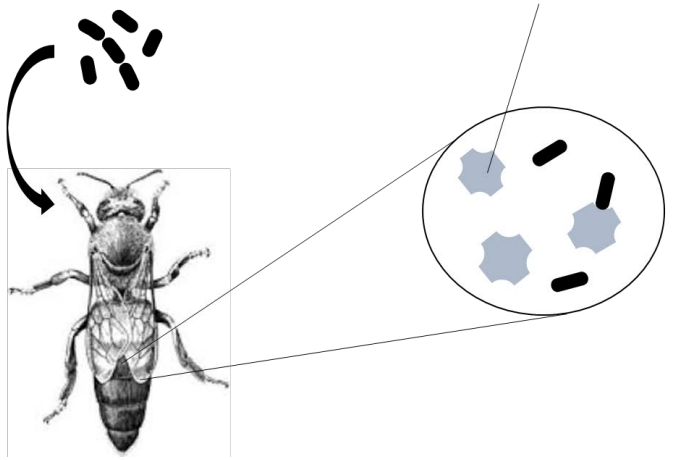
Future therapies for foulbrood?

- Vaccinations

Oral delivery of killed pathogen

Vitellogenin: a yolk protein, capable of transporting/transferring immune modulators, i.e. pathogen fragments



Protection to pathogen in next generation of larvae

frontiers | Frontiers in Veterinary Science

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The oral vaccination with *Paenibacillus larvae* bacterin can decrease susceptibility to American Foulbrood infection in honey bees—A safety and efficacy study

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Animal & Plant Health Agency

Future therapies for foulbrood?

- ~30% effective
- Not clear how it affects spore production
- **Available in the US under USDA 2 year conditional licence**

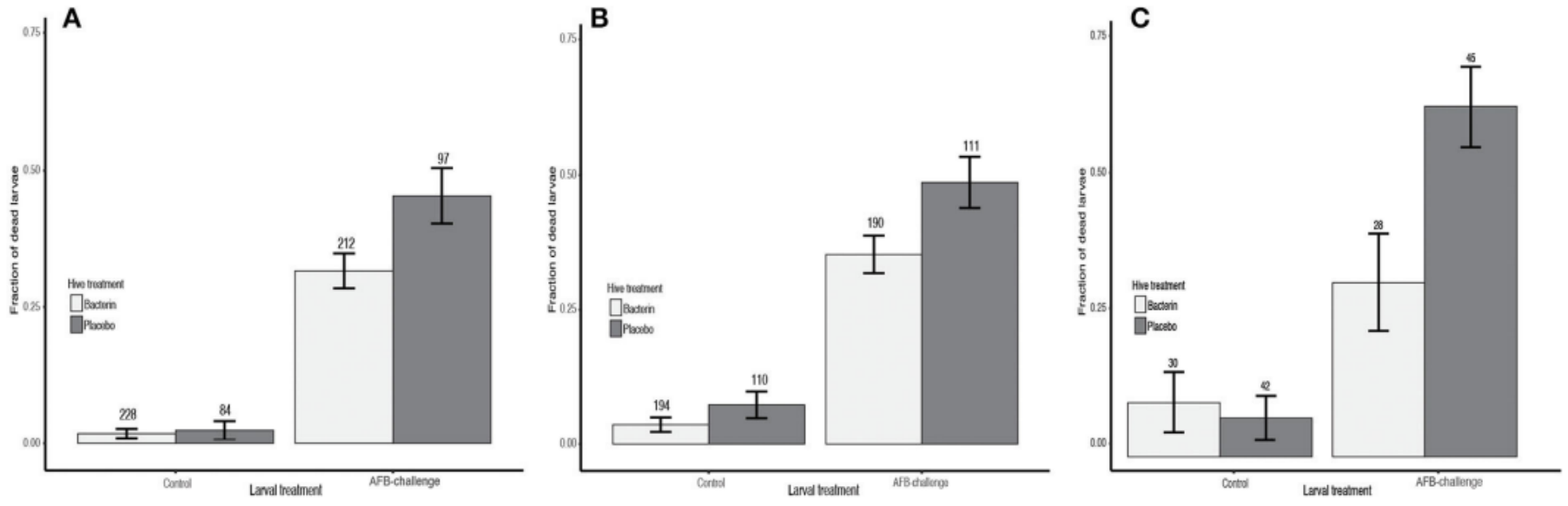

FIGURE 1
The efficacy of the vaccination to prevent the infection with AFB. Prevented Fraction showing the vaccination efficacy of *P. larvae* bacterin (light grey bars) vs. Placebo (dark grey bars) in a heterologous challenge, as mortality of larvae after 8 days. **(A)** Study Site A—first challenge, **(B)** Study Site A—second challenge, and **(C)** Study Site B—second challenge.

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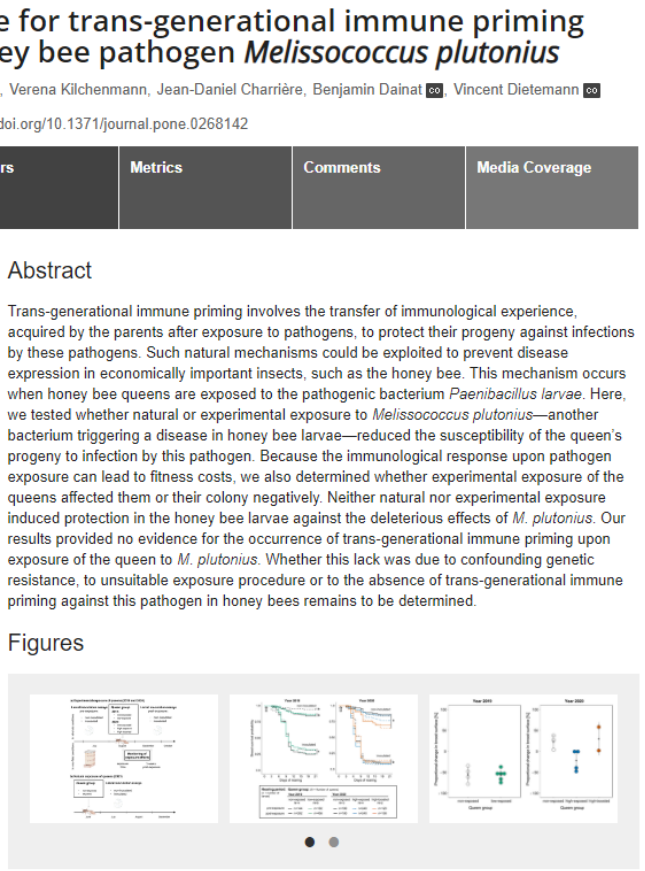
Future therapies for foulbrood?

- No evidence for efficacy against EFB



Lack of evidence for trans-generational immune priming against the honey bee pathogen *Melissococcus plutonius*

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Abstract

Trans-generational immune priming involves the transfer of immunological experience, acquired by the parents after exposure to pathogens, to protect their progeny against infections by these pathogens. Such natural mechanisms could be exploited to prevent disease expression in economically important insects, such as the honey bee. This mechanism occurs when honey bee queens are exposed to the pathogenic bacterium *Paenibacillus larvae*. Here, we tested whether natural or experimental exposure to *Melissococcus plutonius*—another bacterium triggering a disease in honey bee larvae—reduced the susceptibility of the queen's progeny to infection by this pathogen. Because the immunological response upon pathogen exposure can lead to fitness costs, we also determined whether experimental exposure of the queens affected them or their colony negatively. Neither natural nor experimental exposure induced protection in the honey bee larvae against the deleterious effects of *M. plutonius*. Our results provided no evidence for the occurrence of trans-generational immune priming upon exposure of the queen to *M. plutonius*. Whether this lack was due to confounding genetic resistance, to unsuitable exposure procedure or to the absence of trans-generational immune priming against this pathogen in honey bees remains to be determined.

Figures

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Summary

- EFB and AFB are statutory notifiable diseases in the UK
- If you suspect either condition, please contact the National Bee Unit

General Enquiries

National Bee Unit

The Animal and Plant Health Agency (APHA), Room 11G03 York Biotech Campus, Sand Hutton, York, YO41 1LZ.

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Thank you for listening...

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