Summary

A potential hotspot area, defined by APHA, is an area in England or Wales of enhanced surveillance where TB breakdowns with confirmed disease of uncertain origin are emerging in a region of historically low TB incidence.

This paper provides an update on HS21, an area in east Cumbria where linked cattle and badger infection was first identified in 2017. Linked cattle-badger infection has also recently been identified in HS23, located across the Lincolnshire-Leicestershire border. A summary of cattle breakdowns and found-dead wildlife surveillance is provided.

In order to understand the extent of infection in the wildlife in these parts of the Low Risk Area (LRA), APHA is continuing to test carcasses that are reported through the Defra Rural Service Helpline (03000 200 301)

Hotspot 21 – Cumbria

A potential ‘hotspot’ area was declared in east Cumbria in the LRA of England during 2016 (HS21). This was due to the emergence of a cluster of breakdowns associated with *Mycobacterium bovis* genotype 17:z. This genotype had not previously been identified in Great Britain, and investigations concluded that this was most likely introduced by cattle imported from Northern Ireland.

Whilst cattle contact was identified as the transmission route for some of the breakdowns, others were unclear. Infected badger carcasses with the same genotype as the cattle breakdowns were found also in the area. The novel genotype in this area provides clear evidence that local spread of TB is occurring due to the close genetic relationship of the *M. bovis* strain in badgers and cattle. Following the declared potential hotspot protocol, enhanced cattle and wildlife surveillance was initiated in September 2016.

From the index case in November 2014 to 29 July 2020, there has been a total of 39 breakdowns across 33 premises associated with genotype 17:z.

From the initiation of found-dead wildlife surveillance in September 2016 to 29 July 2020, only three *M. bovis* positive badgers have been identified out of the 61 Post Mortem
Examinations (PME) conducted\(^1\), with no new confirmations since 2018. Eight deer carcases have also been collected, two were unsuitable for sampling, six were negative. APHA is continuing to test found-dead badger and deer carcases in HS21 reported via the Defra Rural Service Helpline (03000 200 301).

As published in March 2020\(^2\), 317 badgers were removed from the Area 32-Cumbria during the 2019 cull operations. Of these, 116 were controlled shot and 201 cage-trapped. A publication in March 2020\(^3\) described the surveillance carried out by APHA on the cage-trapped badgers from the area. It was reported that a small number of culture results were still pending, all of which have now being resolved.

As an update, three out of 313 tested badgers (1.0%) were positive, all with the genotype 17:z, 309 were negative\(^4\). The prevalence in the central minimal infected area (MIA)\(^5\) was 14.3% (3/21). No badgers tested positive for *M. bovis* genotype 17:z in the outer cull area\(^6\) (0/233). The remaining 59 carcases from parcels that span both the MIA and outer cull area were negative.

As part of the surveillance, Whole Genome Sequencing (WGS) was carried out on all *M. bovis* isolates from cattle and badgers. As of 29 July 2020, there were 26 unique genetic sequences (or clades) found in Area 32 (Fig 1) of which:

- Three clades were found in both species, including the original clade which is the most likely ancestor of the epidemic and which all the other clades are descended from,
- 17 clades were found in badgers only,
- 6 clades were found in cattle only.

WGS could not be completed on five samples due to poor sequence quality.

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\(^1\) A total of badgers 86 badger carcases were collected, with 25 deemed unsuitable for PME

\(^2\) Natural England (2020) Summary of 2019 badger control operations

\(^3\) Defra (2020) TB surveillance in badgers during year 2 badger control operations in eastern Cumbria, Low Risk Area

\(^4\) A further *M. bovis* positive badger was reported in March 2020. This was found to be most likely genotype 9:c, which has not previously been identified in HS21, but can be found in other areas of Great Britain. As this infected badger is not linked to the outbreak of 17:z in HS21, and no linked cattle breakdowns have been disclosed, this is classed as negative for the 17:z badger prevalence estimation.

\(^5\) Minimal Infected Area; the location of the infected badgers, associated farms and contiguous breakdown areas, plus a radius of the estimated average social group territory based on main sett distribution

\(^6\) Outer cull area; based on estimated average badger social group territory size, surrounding the minimum infected area, to take into account the possibility that infection may have already spread in the badger population. The boundary was adjusted to adhere to natural barriers to badger movement as far as practical to minimise the risk of any possible perturbation effects
There are fewer sequences available from cattle, possibly due to the increased frequency of cattle surveillance (to six-monthly whole-herd testing) which should be removing infected animals earlier and thus before they have developed visible lesions and can be easily cultured.

The presence of shared sequences across the two species provides more evidence that possible cattle-badger and/or badger-cattle transmission has occurred in the area. However, direction of transmission cannot currently be inferred from this data.

Figure 1. WGS tree for all *M. bovis* isolates from HS21, where the orange clade far left represents the original imported strain and each column represents a one SNP difference.
Hotspot 23 – Lincolnshire

A potential ‘hotspot’ area\(^1\) was established following the disclosure of \(M.\) \(bovis\) in a cattle herd in south west Lincolnshire in the LRA of England, near the border with north east Leicestershire, in December 2017. Due to its proximity to, and shared genotype with, a cluster of Officially TB Free status Withdrawn\(^7\) (OTFW) breakdowns in north east Leicestershire, the hotspot is situated partially in the LRA and partially in the Edge Area.

Additional surveillance measures have been implemented in cattle and wildlife across the whole hotspot, including the collection of ‘found dead’ badger and wild deer carcases\(^8\).

From the disclosure of the index case in December 2017 to 13 August 2020, a total of 24 new breakdowns across 23 herds have been confirmed in the area: 19 OTFW and five OTFS\(^9\). Fifteen of the OTFW breakdowns share genotype 25:a and Whole Genome Sequencing (WGS) indicates that they are part of a distinct local cluster\(^10\).

To date, a total of 34 badger carcases were reported to the APHA, with 23 of those carcases considered to be suitable for PME. Three badger carcases have been confirmed as culture positive for \(M.\) \(bovis\), and 16 badger carcases have tested negative. Results from four further badger carcases are pending. One of the positive badgers was located in the Edge Area portion of the hotspot, and the two others were located in the LRA portion. The current sample size is too small to accurately estimate the prevalence of infection in the badger population in the area. Two wild deer carcases have been submitted for PME: one is negative and the other is pending.

The three positive badgers identified within HS23 were all found to have the genotype 25:a, the same as that found in the majority of cattle breakdowns within the hotspot. This genotype is thought to have been originally introduced to the Edge Area portion of the hotspot via cattle movements from the traditional homerange of genotype 25:a (Staffordshire, Shropshire, Cheshire or Derbyshire).

APHA is continuing to test found-dead badger and deer carcases in HS23 reported via the Defra Rural Service Helpline (03000 200 301).

\(^{\text{7}}\) A breakdown with infection confirmed by the presence of visible lesions and/or culture positive for \(M.\) \(bovis\)

\(^{\text{8}}\) Badger and wild deer carcases from HS23 are submitted for PME and tissues are sampled in an attempt to culture \(M.\) \(bovis\). If a positive culture is identified, this then undergoes WGS to establish if there are links to known cattle infection

\(^{\text{9}}\) Nine in the LRA portion (six OTFW and three OTFS) and 15 in the Edge Area portion (13 OTFW and two OTFS)

\(^{\text{10}}\) The other four OTFW breakdowns are; three genotype 25:b and one culture negative