

Can the Enferplex test help improve the sensitivity of bTB detection?

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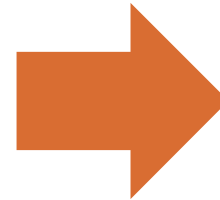
Synergy Farm Health Ltd and Surefarm Ltd



The problem

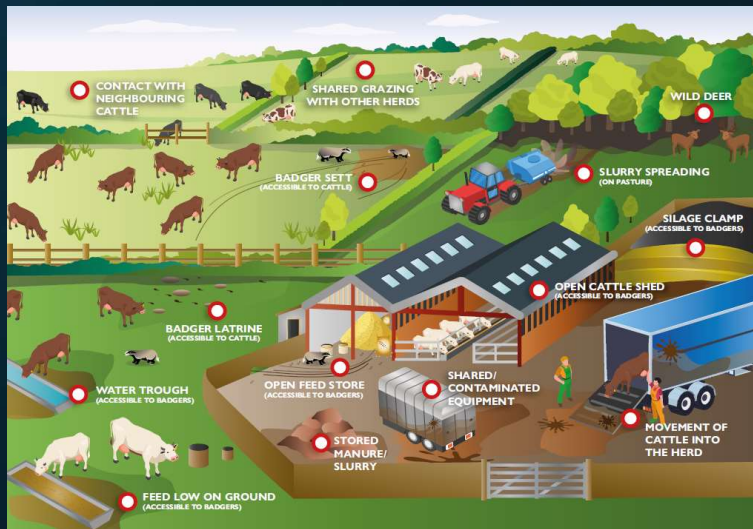
“We discussed in 2018 the growing evidence for substantial undiagnosed bovine TB infections in the national herd. Since then, the evidence has mounted, reinforcing the importance of testing and surveillance.”

- Extract from the 2025 Godfrey report



- Clear recognition of undisclosed infection in the national herd
- Explicit call for more sensitive diagnostics and discussion of the potential for milk-based testing

A failure to identify infection at the herd level has the potential recognised negative consequences for the control of Bovine Tuberculosis:



- Increased within herd prevalence of the disease as infected animals can infect naïve animals which will result in:
 - a. More cost and difficulty to the farm and the UK taxpayer in removing the disease when it is identified
 - b. The risk of spread of infection from the herd to local wildlife reservoir
 - c. Increased environmental contamination by M bovis
 - d. Increased risk of local transmission to neighbouring cattle
- Increased risk of spread to new areas via movement (e.g. Cumbria post Foot and Mouth, Republic of Ireland)



Can the Enferplex Bovine TB Antibody Test help fulfill this need?



World Organisation
for Animal Health
Founded as OIE

WOAH Procedure for Registration of Diagnostic Kits Validation Studies Abstract

Name of the diagnostic kit: Enferplex Bovine TB antibody test

Manufacturer: Enfer Scientific ULC

WOAH original approval number: 20190113

New procedure/approval number: 111824

Date of Registration: May 2023

Disease: Bovine tuberculosis

Pathogen Agent: *Mycobacterium bovis*

Type of Assay: Indirect chemiluminescent multiplex ELISA

Purpose of Assay:

Certified by the WOAH as fit for the detection of antibody to *Mycobacterium bovis* in bovine milk samples (May 2023) to be used as an ancillary test in conjunction with other methods for serological prevalence surveys, or diagnosis and management of *M. bovis* infection within herds, in particular for the following purposes:

1. To confirm, but not negate, diagnosis of suspect or clinical cases, including confirmation of positive screening tests in individual animals and in herds based on detection of antibodies in individual bovine milk samples excluding colostrum and first milk samples taken within 4 days of calving.
2. As a screening test to identify herds with *Mycobacterium bovis* infection based on detection of antibodies in bovine bulk tank milk samples excluding colostrum and first milk samples taken within 4 days of calving.

Species and Specimens

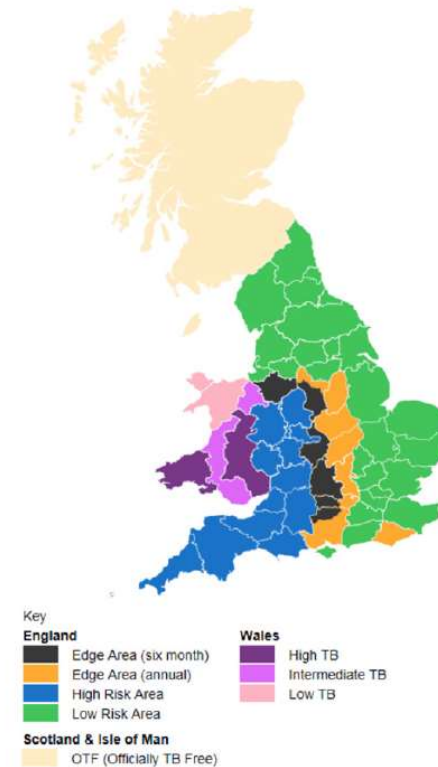
The test has been validated and approved for testing individual and bulk tank milk samples from cattle.

The GB Bovine Tuberculosis Bulk Milk Study

Independent longitudinal GB wide study (~5000 dairy farms) funded by Innovate UK on the outcomes of testing bulk milk with the WOAAH validated Enferplex bTB Antibody test.



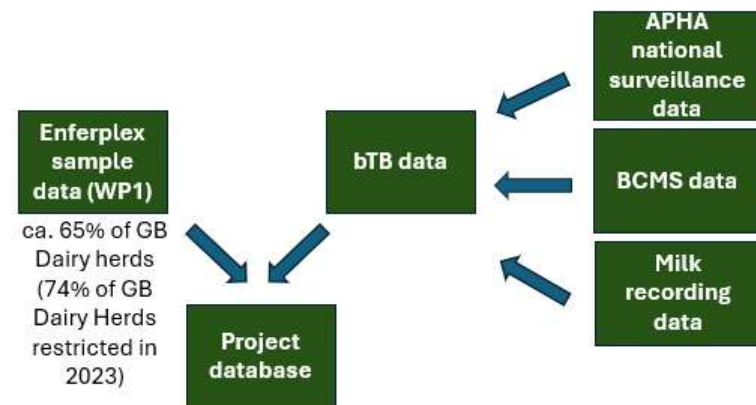
Figure 1.1: Risk map for Great Britain (Edge Area boundaries pre-2018)



Project Outline

Sample collection and testing - NMR

- Three sample collection rounds Feb, May & Aug 2023 (4830 herds) plus monthly sampling from a small cohort of herds enrolled directly via their vets.
- Testing at NML Hillington; equipment and training provided by Enfer.
- Results associated with the herd's CPH and the sample date sent to SRUC for independent analysis.



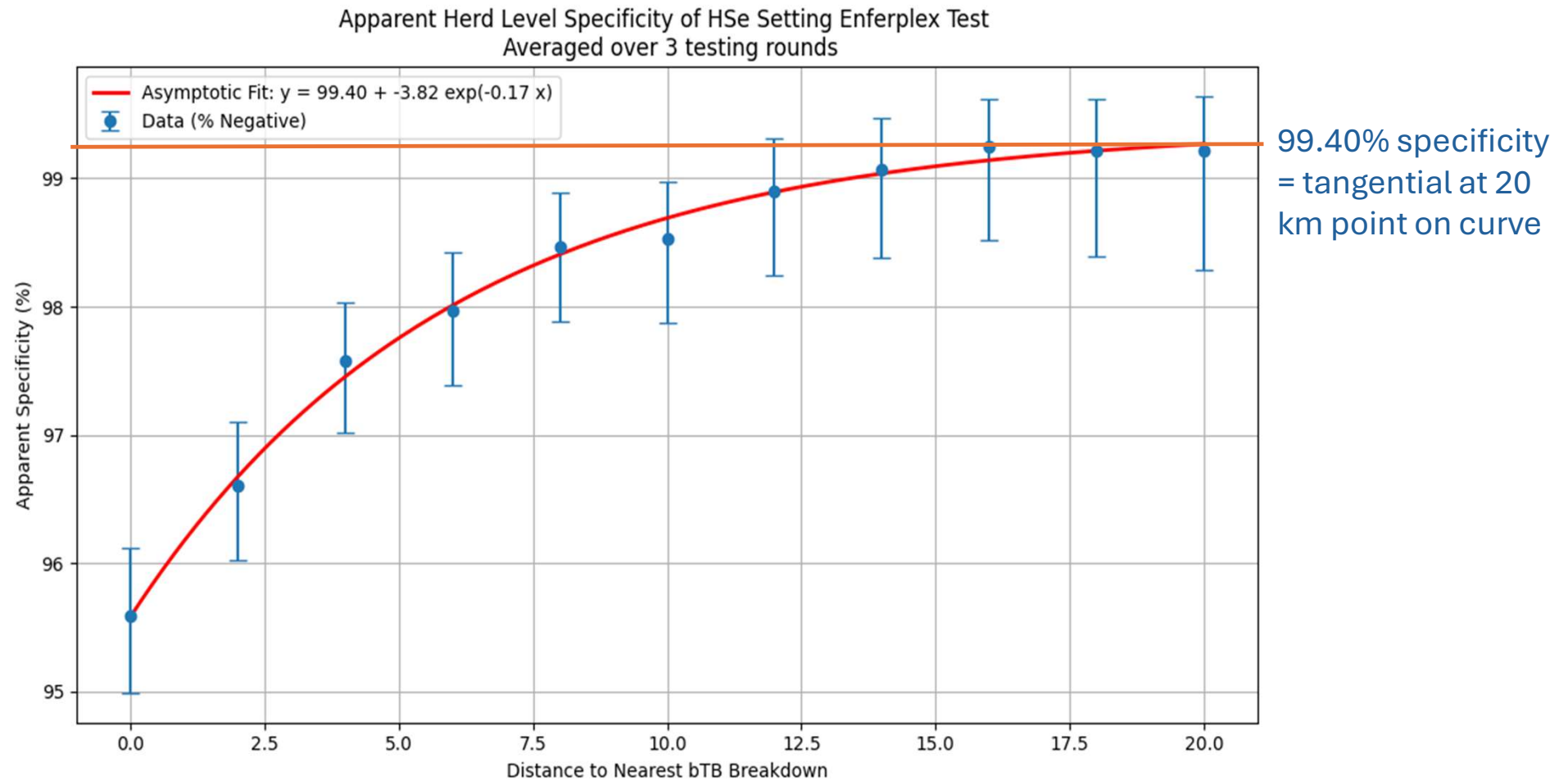
Enferplex Bulk Milk (EP) results and bTB restriction at trial

Definitions:

- EP+ herd: at least one Enferplex positive sample
- EP- herd: no Enferplex positive samples
- bTB+ herd: in bTB restriction at some point during the trial
- bTB- herd: bTB free (OTF) during the entire trial

WOAH Assessment of Specificity

- The test is already WOAHA validated (3431 international bulk milk samples) (Hayton et al, 2025)
- Criteria used for “True Negatives” was no bTB for 8 years and more than 10km from the nearest breakdown in the last 12 months
- Overall specificity was 99.8% at the high sensitivity (HSe) setting.
- Around 250 of these samples were from GB; specificity from these was slightly lower at 99.3%.



Apparent specificity plots of the Enferplex Bovine TB antibody test at the herd level for the Hse settings with asymptotic regression fit of data, bars indicate 95% CI

Conclusion

The Enferplex test used has high specificity
– i.e. there is a very low risk of false positives

Diagnostic specificity of the EP test at herd level from bTB free GB dairy cattle herds at the HSe setting (16 km criteria)¹

UK TB areas	Statistical variable ²	EP (Hse) ³			
		Round 1	Round 2	Round 3	All rounds
LRA	N	349	363	345	1,057
	n	5	1	2	8
	%	1.43	0.27	0.58	0.76
	95% CI	0.61-3.31	0.05-1.54	0.15-2.09	0.39 – 1.45
	Dsp (%) ⁴	98.57	99.73	99.42	99.24

Dairy herds with no history of bTB for at least 8 years and no bTB breakdown herds within 16 km in the last 12 months.

N – number of samples; n – number of herds positive, % - percent positive; n - 95% CI – 95% confidence interval

Hse – High sensitivity setting ⁴Dsp – Diagnostic specificity

Percentage of herds under bTB restriction at some point during the study – overall incidence as measured by the two tests was very similar

24% of herds were under bTB restriction during the study period

25% of herds were Enferplex positive (EP+ve) at least once during the study period

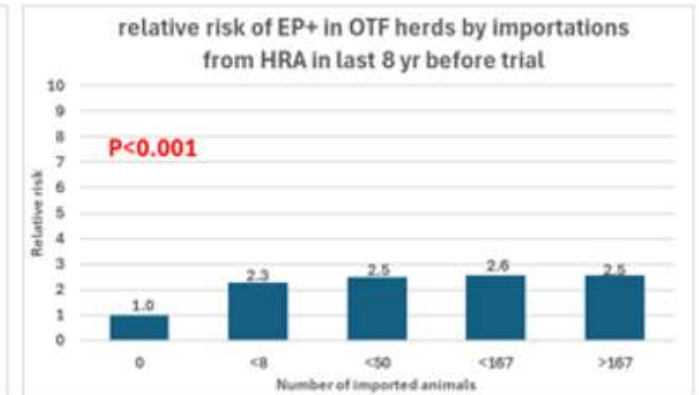
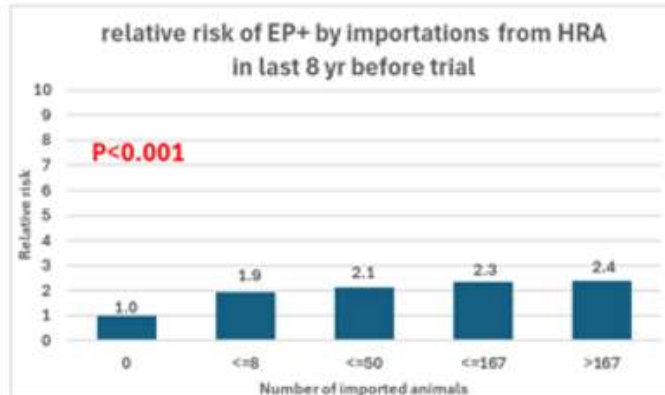
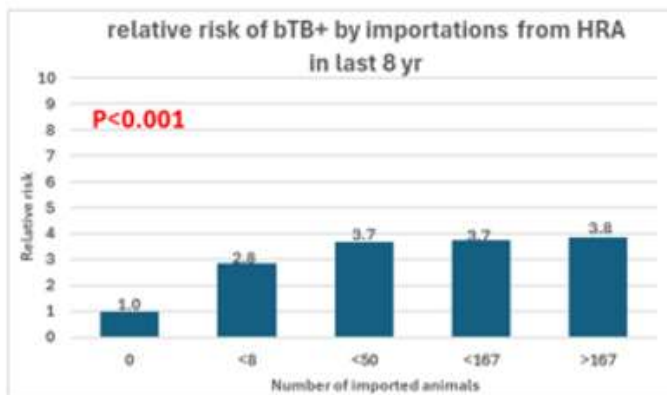
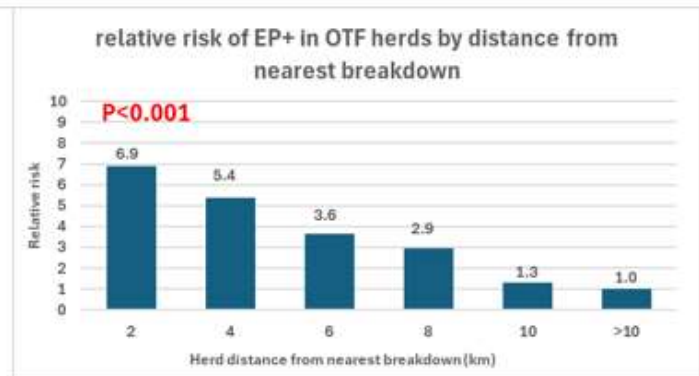
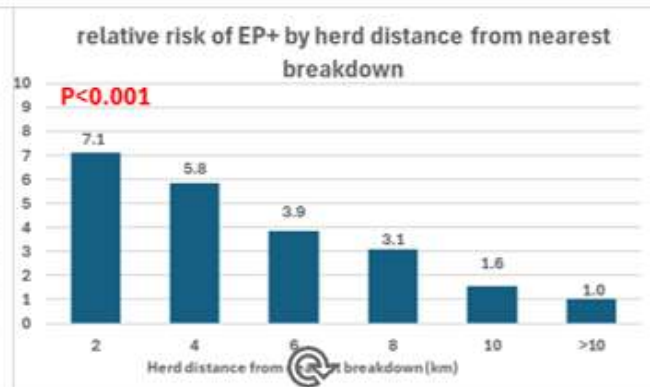
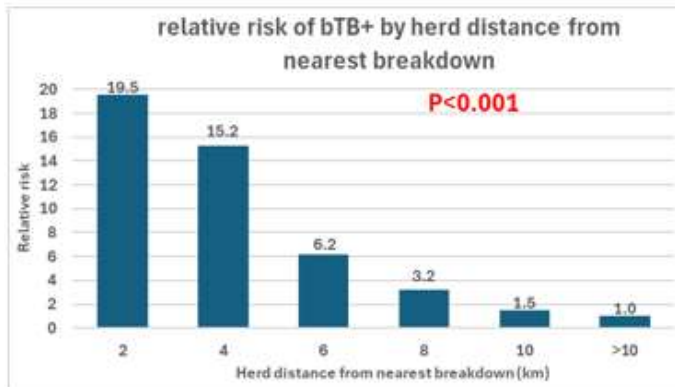
Conclusion

The test detects a significant number of herds missed by the current surveillance systems

	EP status	
bTB restriction status during trial	negative	positive
negative	81%	19%
positive	60%	40%

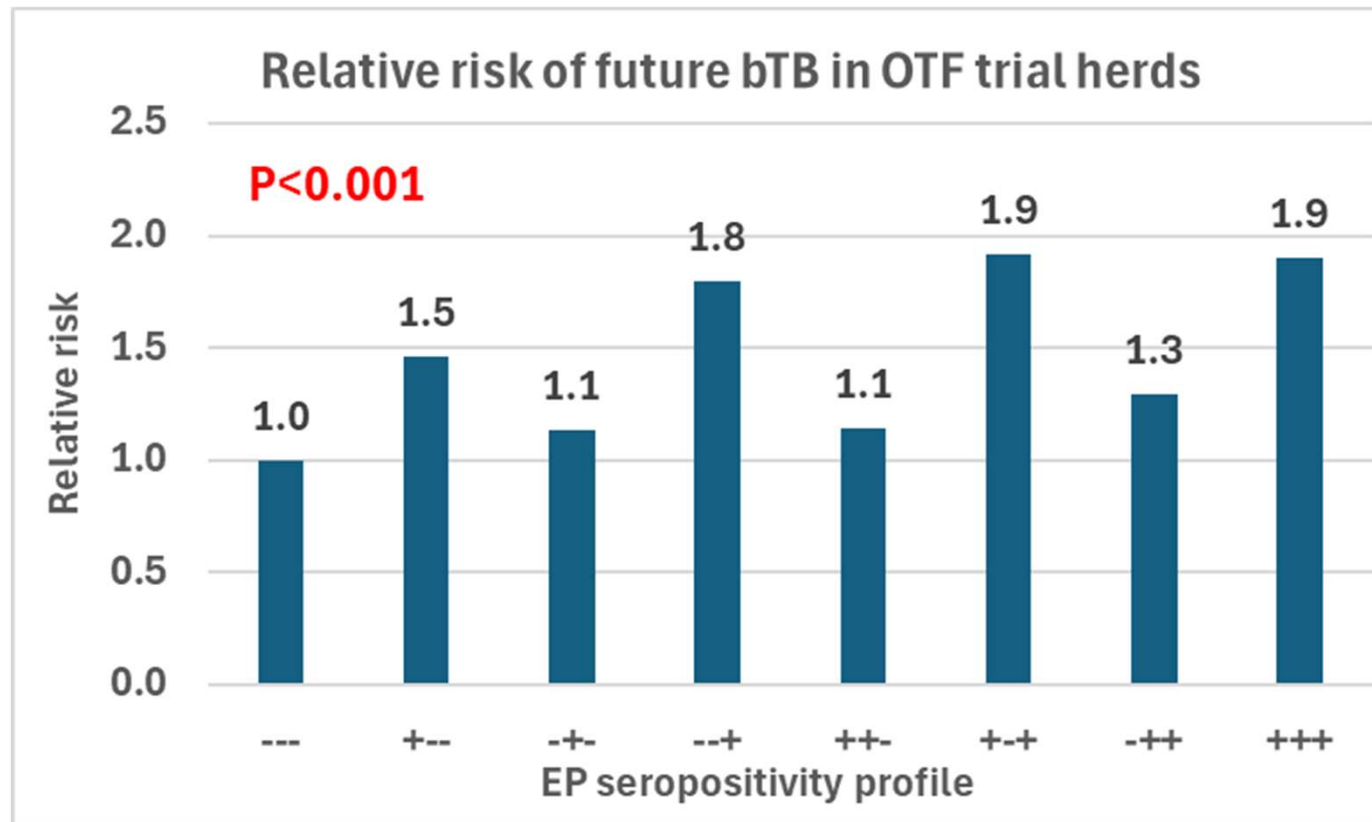
Conclusion

The patterns for risk of Enferplex positivity are entirely consistent with accepted epidemiology for risk of infection.



Conclusion

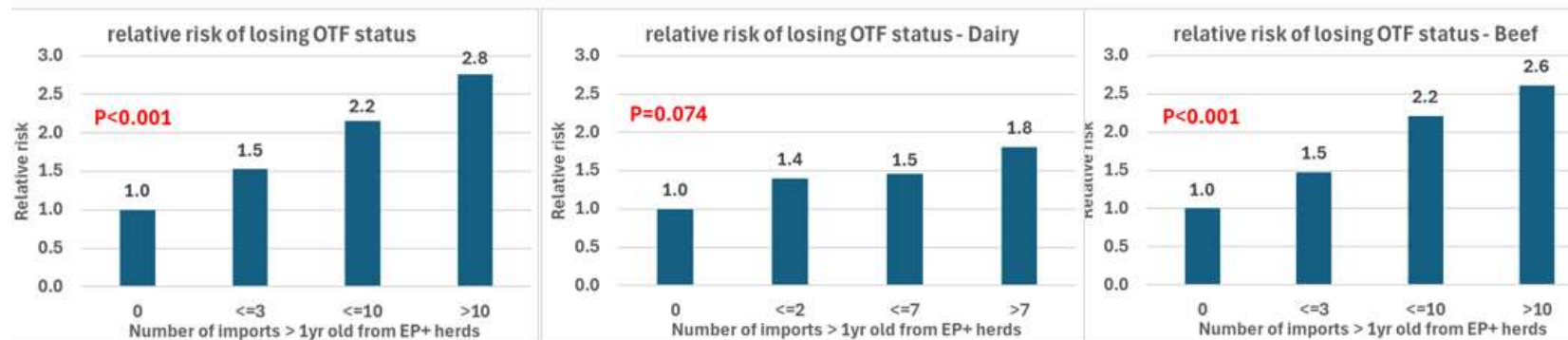
The risk of loss of OTF status is increased if herds are Enferplex test positive.



Conclusion

Importation of animals over a year of age from Enferplex positive herds is associated with increased frequency of OTF loss.

Effects on bTB status after trial (to Dec 2024) in OTF herds that imported > 1 yr olds from EP+ herds



Increasing number of imports increases the relative risk across all holding types and particularly in beef herds

Conclusion

- The Enferplex test used has high specificity.
- The test detects a significant number of herds missed by the current surveillance systems
- The test detects a significant proportion of restricted herds seropositive indicating residual infection after removal of skin test reactors.
- The patterns for risk of Enferplex positivity are entirely consistent with accepted epidemiology for risk of infection.
- The risk of loss of OTF status is increased if herds are Enferplex test positive.
- Importation of animals from Enferplex positive herds is associated with increased frequency of OTF loss.
- Monthly testing is showing a progressive increase in Enferplex seropositivity over quarterly testing.

Individual Animal Testing

Enferplex positivity using serum from SICCT negative or IFN γ negative animals with lesions

bTB infection status NEGATIVE by:	Statistical Variable*	Enferplex test setting	
		Hse	Hsp
SICCT test bTB lesion positive Boosted	N RSn CI	127 88.2% 81.4 – 92.7	127 81.9% 74.3 – 87.6
IFN γ test bTB lesion positive Boosted	N RSn CI	55 83.6% 71.7 – 91.2	55 74.6% 61.7 – 84.2

*N = number; DSn – diagnostic sensitivity; Relative sensitivity; CI – 95% Confidence Interval

Individual Animal Testing

Serum and Milk – decisions behind how ancillary testing will be key to success

The main areas of action

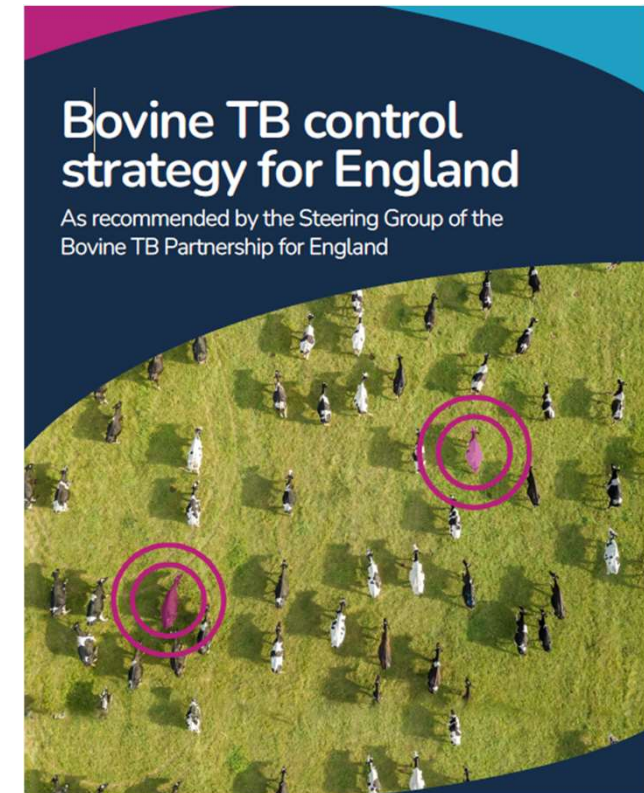
The recommended strategy focuses effort in three areas:

Stronger cattle-focused control

- More effective use of testing and earlier detection of infection.
- Clear and accessible herd risk information to support safer trading decisions.
- Stronger management of cattle with a higher likelihood of infection.
- Improved biosecurity and on farm decision making.
- Introduction of cattle vaccination, supported by a DIVA test.

Action beyond cattle

- Proportionate, science-informed management of TB in other farmed species, and wildlife.
- Expansion of badger vaccination in priority areas.
- Strengthening surveillance to better understand TB risk to cattle and inform action.



Fits with the needs of improving bTB control

- Need for more sensitive testing / surveillance - Godfray 2025
- Need for improved biosecurity

APHA study published 2025:

“There was consistent evidence that reducing contact with neighbouring herds and preventing cattle at higher bovine tuberculosis risk from entering the herd reduced a herd’s bTB risk”

Can biosecurity on farms reduce bovine tuberculosis risks in cattle in England? A review of observational and literature-based evidence.

Voller *et al* Veterinary Record (2025)

