

Animal & Plant Health Agency

Annual Report (2020 to 2021) The National Reference Laboratory for *Trichinella* and *Echinococcus*

1 April 2020 to 31 March 2021



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APHA is an Executive Agency of the Department for Environment, Food and Rural Affairs and also works on behalf of the Scottish Government, Welsh Government and Food Standards Agency to safeguard animal and plant health for the benefit of people, the environment and the economy.

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1. Introduction

1.1 The National Reference Laboratory (NRL) for Trichinella and Echinococcus

The Food Standards Agency (FSA) is the Competent Authority (CA) for the purpose of Regulation (EU) 2017/625 on Official Feed and Food Controls in the UK and has a legal obligation to appoint National Reference Laboratories (NRLs).

The Animal and Plant Health Agency (APHA) is an executive agency of Defra (Department for Environment, Food and Rural Affairs) and as such provides impartial advice and testing services (to government and other agencies) that may be used to inform and assist with policy decisions. With a wide range of experience in Parasitology, the APHA laboratories at York continue to maintain expertise and diagnostic capability and are thus well placed to cover the UK for parasitic diseases particularly for Trichinella and Echinococcus.

Staff at the York NRL have been involved in Trichinella testing in wildlife (including wild boar) on behalf of the FSA for many years and have also been trained in the auditing of self-testing and government contracted laboratories. The NRL supplies trichinae required for the Proficiency Testing (PT) scheme that assesses the performance of Official Control Laboratories (OCLs) in the detection of *Trichinella* in meat. The York NRL was United Kingdom Accreditation Service (UKAS) accredited in March 2017 for the wild boar digest assay for the detection of *Trichinella* (SOP PARA 050) and for a Polymerase Chain Reaction (PCR) method for the detection of *Echinococcus multilocularis, Echinococcus granulosus* and other cestodes (PARA 026, PARA 027 and PARA 029). There are currently 3 staff members within the NRL verified for competence to ISO 17025 standard across these test methods. The NRL supports and participates in a Quality Assurance (QA) Scheme for *Trichinella* through collaboration with colleagues at APHA Sutton Bonington QA Unit (VetQas). Additionally, the York laboratory takes part in an annual External Quality Assurance EQA Scheme for *Trichinella* and *Echinococcus* run by the European Reference laboratory for Parasites (EURLP).

1.2 Core functions and duties of the NRL for *Trichinella* **and** *Echinococcus*

- (a) co-operate internationally in their area of competence, including collaborating and participating in training courses and inter-laboratory comparative tests organised by international laboratories (where appropriate)
- (b) co-ordinate, for their area of competence, the activities of OCLs responsible for the analysis of official controls samples to ensure the verification of compliance with feed and food law

- (c) where appropriate, organise comparative tests between the official national laboratories and ensure an appropriate follow-up of such comparative testing; ensure the dissemination of any information required by the CA
- (d) provide scientific and technical assistance to the CA, especially for the implementation of Multi Annual National Control Plans
- (e) participate in relevant national and international workshops and training courses and, where necessary, conduct training courses for the staff of OCLs
- (f) upon request by the appropriate authority, actively assist in relevant foodborne incident and outbreak situations, should be equipped with, or have access to, the necessary equipment to perform their tasks in emergency situations and in cases of non-compliance of consignments, by carrying out confirmatory analysis
- (g) carry out research, evaluation and development of new and existing methods for the analysis of UK regulated and officially monitored foods and feed and emerging new risks to UK food safety
- (h) provide advice and expertise on standardisation of methods at CEN and ISO
- (i) obtain and maintain accreditation for official reference and other relevant regulatory methods for food and feed within the NRL area of competence
- (j) be responsible for carrying out other specific duties as required by the CA, where appropriate and by prior agreement
- (k) assess the capability and suitability of *Trichinella* testing laboratories prior to their designation as official laboratories, and, through regular auditing to agreed schedules, to ensure required standards for *Trichinella* testing are in operation
- (I) be impartial, free from any conflict of interests, and in particular not be in a situation which may, directly or indirectly, affect the impartiality of their professional conduct as regards the exercise of their tasks as NRLs
- (m) have contractual access to, suitably qualified staff with adequate training in analytical, testing and diagnostic techniques in their area of competence, and support staff as appropriate
- (n) possess, or have access to, the infrastructure, equipment and products needed to carry out the tasks assigned to them
- (o) ensure that their staff and any contractually engaged staff have good knowledge of international standards and practices and that the latest developments in research at national and international level are taken into account in their work
- (p) where relevant, validate the reagents and lots of reagents, establish and maintain up-to-date lists of available reference substances and reagents and of

manufacturers and suppliers of such substances and reagents and secure access to any reference materials required in order to fulfil their responsibilities and support the relevant OCLs

- (q) be equipped with, or have access to, the necessary equipment to perform their tasks in emergency situations; and where appropriate, assist the CA in food incidents by carrying out diagnosis and/or testing of samples, when necessary
- (r) where relevant, be equipped to comply with relevant biosecurity standards
- (s) maintain a list of the accreditation for the relevant OCLs
- (t) liaise with other CA-appointed NRLs (as and when required)
- (u) have experience of, and be able to operate in accordance with, the relevant sampling and analysis legislation, including maintaining specific UK Accreditation Service (UKAS) accreditation (or equivalent) for the relevant analytes, and satisfactory performance in proficiency test schemes
- (v) be familiar with the enforcement system in operation in the UK

1.3 Proficiency Testing

A significant part of the NRL function is to train, audit and monitor approved *Trichinella* testing facilities in the UK. The NRL is responsible for reporting to the FSA who grant and maintain approvals and it is a requirement that all approved *Trichinella* testing laboratories to participate in a Proficiency Testing (PT) scheme, which is an External Quality Assurance EQA scheme to monitor performance. The PT scheme is run by APHA Quality Assurance Unit (QAU). This is an independent unit that prepares, distributes and collates the results from a wide range of PT schemes. The NRL provides the trichinae samples for the *Trichinella* PT (PT0111) scheme to order. Trichinae of *Trichinella spiralis* are harvested from an infected mouse.

Trained staff in designated laboratories are required to examine the samples and participation will test their ability to detect and discern the presence or absence of trichinae in the samples. Four distributions are sent to participating laboratories throughout the year in March, June, September and December when each laboratory is sent 4 test samples. Trichinae are spiked into each of the 4 formalised samples and the concentration of trichinae (sample target) is varied between distributions and samples (with the numbers determined by the VetQas QAU). Sample targets are the same for all laboratories at each distribution.

The sum of total number of trichinae spiked into each of the 4 samples is then used as the target result and the sum of the actual recovery for each sample is used to calculate the percentage recovery for each laboratory. The results of the distributions are made available to the NRL by Vetqas QAU for scrutiny, comment and feedback to laboratories each quarter. Specific advice is given if low recovery was achieved.

The pass criteria for acceptable recovery rates are taken from the publication by Rossi and Pozio (2008) and Rossi et al. (2015). For samples spiked with 4 or more larvae, the detection of at least 50% is considered acceptable whereas for samples spiked with 1-3 larvae, the detection of at least 1 larva is considered acceptable. The UK *Trichinella* PT scheme is based on the use of formalized trichinae and the FSA stipulates that a result of at least 75% recovery rate is regarded as satisfactory. If this standard is not achieved, the NRL investigates the reasons why and if necessary, undertakes a reality check visit and makes a recommendation to the FSA on the course of action.

To ensure consistent action and to ensure that all self-testing laboratories are clear about the course of action, should their recovery drop below the required standard, a decision tree is used (Table 1).

Table 1: Action Decision Tree for assessing results from the quarterly PT exercises

Result	Action			
Nil Return	Not operational – NRL recommendation to FSA to revoke designation			
Nil Return	Operational – NRL to investigate and make recommendation to FSA			
Below 50%	NRL to investigate and make recommendation to FSA			
Below 75%	NRL to investigate and make recommendation to FSA			
75% or above	Satisfactory result received – No action by NRL			

First Instance

Second Instance (following an unsatisfactory first instance outcome)

Result	Action
Nil Return	Not operational – NRL recommendation to FSA to revoke designation
Nil Return	Operational – NRL recommendation to FSA to revoke designation

Below 50%	NRL recommendation to FSA to revoke designation
Below 75%	NRL to investigate and make recommendation on reason and course of action to FSA

Third Instance (following an unsatisfactory second instance outcome)

Result	Action
Below 75%	NRL recommendation to FSA to revoke designation. FSA to subsequently make the decision on removal of designation and send letter.

Reference material such as formalised trichinae, photographs of apparatus and videos of live trichinae are kept at the NRL and are readily available to testing laboratories for refresher training of staff. The laboratories continue to have the opportunity to request further interim QA samples from the NRL to assist with internal training and may request help with training or further inspection from the NRL to help identify problem areas at any time.

1.4 Participating Laboratories

There are currently 12 designated *Trichinella* self-testing facilities and 3 contracted government laboratories in operation, including the NRL. Table 3 shows the list of all designated operational laboratories during 2020 to 2021 and a full list of contact details is maintained by the NRL. Of the 15 currently operational facilities, 2 labs (3638 and 2658) were recently designated and will commence participation in the Vetqas *Trichinella* EQA PT scheme March and June 2021 distributions respectively. Laboratory 3323 began participating in this PT scheme from June 2020.

Table 2: List of approved government contracted and self-testinglaboratories carrying out *Trichinella* testing 2020 to 2021

Vetqas Laboratory Reference Number	OCL Type
1706	government
1787	government
1921	government
1150	self-tester
1443	self-tester
1447	self-tester
1620	self-tester
1632	self-tester
1824	self-tester
2475	self-tester
2993	self-tester
3145	self-tester
3323	self-tester
3638	self-tester
2658	self-tester

Laboratory 3638 was audited by NRL and designated by FSA 18 December 2020.

Laboratory 2658 was audited by NRL and designated by FSA (NI) on 31 December 2020.

2. Results for the *Trichinella* EQA PT scheme March to December 2020

Coronavirus lockdown which started on 24 March 2020 and all other subsequent lockdowns had no impact on the preparation and dispatch of the *Trichinella* PT samples to Vetqas. The NRL implemented a contingency plan which allowed the PTs to be delivered on time and to the same standard. Results of the *Trichinella* EQA PT scheme for this reporting period will cover the performance of 13 testing laboratories (10 self-testers and 3 government contracted labs) except for the March 2020 distribution.

2.1 Overall trichinae recovery for government and selftesting laboratories for March 2020

A total of 12 *Trichinella* testing facilities participated in the March 2020 distribution (Table 3 and Figure 1). Of the participating laboratories 83% successfully passed the March 2020 *Trichinella* PT distribution scoring between 80 to 100% in overall trichinae recovery rate. One lab (1620) over scored in one sample (4016) and under scored in 2 samples (4013 and 4015) thus falsely recording a 100% overall recovery rate. Testing facility 1632 failed the March 2020 distribution scoring 53% overall recovery rate. No false positive or false negatives were recorded by any of the participating facilities.

Table 3: Individual sample results (raw data and %) from the March 2020*Trichinella* EQA PT scheme for government contracted and self-testinglaboratories.

Note: any numbers marked as red denote a failure and any numbers in blue denote overcounting.

OL ID	Sample 20/4013	Sample 20/4014	Sample 20/4015	Sample 20/4016	Overall score (%)
Intended result	6 (100%)	0 (100%)	4 (100%)	5 (100%)	15 (100%)
1150	5 (83%)	0 (100%)	4 (100%)	4 (80%)	13 (87%)
1443	6 (100%)	0 (100%)	4 (100%)	5 (100%)	15 (100%)
1447	5 (83%)	0 (100%)	3 (75%)	4 (80%)	12 (80%)
1620	5 (83%)	0 (100%)	3 (75%)	7 (140%)	15 (100%) overcounting
1632	3 (50%)	0 (100%)	2 (50%)	3 (75%)	8 (53%) failure
1706	6 (100%)	0 (100%)	4 (100%)	5 (100%)	15 (100%)
1787	6 (100%)	0 (100%)	4 (100%)	5 (100%)	15 (100%)
1824	6 (100%)	0 (100%)	4 (100%)	5 (100%)	15 (100%)
1921	6 (100%)	0 (100%)	4 (100%)	5 (100%)	15 (100%)
2475	6 (100%)	0 (100%)	4 (100%)	5 (100%)	15 (100%)
2993	6 (100%)	0 (100%)	4 (100%)	5 (100%)	15 (100%)
3145	6 (100%)	0 (100%)	4 (100%)	5 (100%)	15 (100%)



Figure 1: Percentage of trichinae recovered by government contracted and self-testing laboratories for *Trichinella* EQA PT scheme (March 2020)

Failure: laboratory 1632

Overcounting: laboratory 1620

2.2 Overall trichinae recovery for government and selftesting laboratories for June 2020

Results for the PT distribution for self-testing and government laboratories for June 2020 are shown in Table 4 and Figure 2. The June 2020 PT distribution had an unusual trichinae spike level (0, 0, 3, 0). The NRL approved the request as it was a good opportunity to test the ability of the operator in eliminating the detection of false positives.

A total of 13 *Trichinella* testing facilities participated in the June 2020 PT distribution. Of those, 8 laboratories (62%) successfully passed the PT scoring 100% across all 4 samples and overall recovery rate. None of the laboratories scored false positives or false negatives. Two labs (1921 and 3145) over scored by 1 trichinae in the single positive sample taking their overall score to 113%.

Three labs (1620, 1632 and 3323) detected 2 of the 3 trichinae in sample 4029 and although this level of detection is acceptable, they failed this PT based on the 75% required pass rate set by the FSA. According to Rossi and Pozio (2008) and Rossi et al. (2015) for *Trichinella* PT samples spiked with 4 or more larvae, the detection of at least 50% is considered acceptable whereas for samples spiked with 1 to 3 larvae, the detection of at least 1 larvae is considered acceptable.

Table 4: Individual sample results (raw data and %) from the June 2020 *Trichinella* EQA PT scheme for government contracted and self-testing laboratories

Note: any numbers marked as red denote a failure and any numbers in blue denote overcounting.

OL ID	Sample 20/4027	Sample 20/4028	Sample 20/4029	Sample 20/4030	Overall score (%)
Intended result	0 (100%)	0 (100%)	3 (100%)	0 (100%)	3 (100%)
1150	0 (100%)	0 (100%)	3 (100%)	0 (100%)	3 (100%)
1443	0 (100%)	0 (100%)	3 (100%)	0 (100%)	3 (100%)
1447	0 (100%)	0 (100%)	3 (100%)	0 (100%)	3 (100%)
1620	0 (100%)	0 (100%)	2 (67%)	0 (100%)	2 (67%) failure
1632	0 (100%)	0 (100%)	2 (67%)	0 (100%)	2 (67%) failure
1706	0 (100%)	0 (100%)	3 (100%)	0 (100%)	3 (100%)
1787	0 (100%)	0 (100%)	3 (100%)	0 (100%)	3 (100%)
1824	0 (100%)	0 (100%)	3 (100%)	0 (100%)	3 (100%)
1921	0 (100%)	0 (100%)	4 (113%)	0 (100%)	4 (113%) overcounting
2475	0 (100%)	0 (100%)	3 (100%)	0 (100%)	3 (100%)
2993	0 (100%)	0 (100%)	3 (100%)	0 (100%)	3 (100%)
3145	0 (100%)	0 (100%)	4 (113%)	0 (100%)	4 (113%) overcounting
3323*	0 (100%)	0 (100%)	2 (67%)	0 (100%)	2 (67%) failure

*First *Trichinella* PT participation since designation by the competent authority on 28th April 2020.





Failure: laboratory 1620, 1632, 3323

Overcounting: laboratory 1921, 3145

2.3 Overall trichinae recovery for government and selftesting laboratories September 2020

A total of 13 testing labs participated in the September 2020 *Trichinella spiralis* PT distribution (Table 5 and Figure 3). Eleven of the participating laboratories (85%) successfully passed this distribution scoring between 75 to 100% in trichinae recovery rate. Two self-testing laboratories, 1620 and 3323 failed the PT scoring 69 and 63% respectively.

Table 5: Individual sample results (raw data and %) from the September2020 Trichinella EQA PT scheme for government contracted and self-testing laboratories

OL ID	Sample 20/4047	Sample 20/4048	Sample 20/4049	Sample 20/4050	Overall score (%)
Intended result	4 (100%)	7 (100%)	0 (100%)	5 (100%)	16 (100%)
1150	4 (100%)	7 (100%)	0 (100%)	5 (100%)	16 (100%)
1443	4 (100%)	7 (100%)	0 (100%) 5 (100%)		16 (100%)
1447	4 (100%)	5 (71%)	0 (100%)	3 (60%)	12 (75%)
1620 3 (75%) 4 (57%) 0 (100%)		0 (100%)	4 (80%) 11 (69%) failure		
1632	5 (12%)	5 (71%)	0 (100%)	5 (100%)	15 (94%)
1706	4 (100%)	7 (100%)	0 (100%)	5 (100%)	16 (100%)
1787	4 (100%)	7 (100%)	0 (100%)	5 (100%)	16 (100%)
1824	4 (100%)	7 (100%)	0 (100%)	5 (100%)	16 (100%)
1921	4 (100%)	7 (100%)	0 (100%)	5 (100%)	16 (100%)
2475	4 (100%)	7 (100%)	0 (100%)	5 (100%)	16 (100%)
2993	4 (100%)	7 (100%)	0 (100%)	5 (100%)	16 (100%)
3145	4 (100%)	7 (100%)	0 (100%)	4 (80%)	15 (94%)
3323	4 (100%)	5 (71%)	0 (100%)	1 (20%)	10 (63%) failure

Note: any numbers marked as red denote a failure.

Figure 3: Percentage of trichinae recovered by government and self-testing laboratories for *Trichinella* EQ PT scheme (September 2020)



Failure: laboratory 1620, 3323

2.4 Overall trichinae recovery for government and selftesting laboratories December 2020

A total of 12 (92%) of the 13 designated *Trichinella* testing labs participated in the December 2020 PT distribution (Table 6 and Figure 4). Ten of the participating laboratories (83%) successfully passed this distribution scoring between 88 and 100% in trichinae recovery rate. Two self-testing laboratories, 1620 and 3323 failed the PT both scoring 63%. One testing facility, 1447 failed to participate in the December 2020 PT.

Table 6: Individual sample results (raw data and %) from the December2020 Trichinella EQA PT scheme for government contracted and self-testing laboratories

OL ID	Sample 20/4059	Sample 20/4060	Sample 20/4061	Sample 20/4062	Overall score (%)
Intended result	0 (100%)	3 (100%)	0 (100%)	5 (100%)	8 (100%)
1150	0 (100%)	2 (67%)	0 (100%)	5 (100%)	7 (88%)
1443	0 (100%)	3 (100%)	0 (100%)	5 (100%)	8 (100%)
1447					Nil Return
1620	0 (100%)	2 (67%)	0 (100%)	3 (60%)	5 (63%) failure
1632	0 (100%)	3 (100%)	0 (100%)	5 (100%)	8 (100%)
1706	0 (100%)	3 (100%)	0 (100%)	5 (100%)	8 (100%)
1787	0 (100%)	3 (100%)	0 (100%)	5 (100%)	8 (100%)
1824	0 (100%)	3 (100%)	0 (100%)	5 (100%)	8 (100%)
1921	0 (100%)	3 (100%)	0 (100%)	5 (100%)	8 (100%)
2475	0 (100%)	3 (100%)	0 (100%)	5 (100%)	8 (100%)
2993	0 (100%)	2 (67%)	0 (100%)	5 (100%)	7 (88%)
3145	0 (100%)	3 (100%)	0 (100%)	4 (80%)	7 (88%)
3323	0 (100%)	2 (67%)	0 (100%)	3 (60%)	5 (63%) failure

Figure 4: Percentage of trichinae recovered by government and self-testing laboratories for *Trichinella* EQ PT scheme (December 2020)



Failure: laboratory 1620, 3323

3. Results for the *Trichinella* EQA Proficiency Testing Scheme for a 12-month rolling period

3.1 Government contracted laboratories (March to December 2020)

The performance of the contracted government laboratories in the *Trichinella* EQA exercises carried out between March 2020 and December 2020 and calculated as the percentage of the target recovery for each of the 4 samples is shown in Table 7 and Figure 5 and can be summarized as follows:

Two government contracted laboratories (1706 and 1787) performed extremely well in this 12-month rolling period from March to December 2020 achieving 100% in all 4 quarterly *Trichinella* PT distributions. A similar result was seen for laboratory 1921 although they did over score in the June 2020 distribution by 1 trichinae which took their overall score to 133%.

Table 7: Number of trichinae (shown as a percentage) recovered bycontracted government laboratories for March 2020 - December 2020Trichinella EQA PT scheme

OL ID	March	June	September	December
1706	100	100	100	100
1787	100	100	100	100
1921	100	133 (overcounting)	100	100

Note: any numbers marked in blue denote overcounting.

3.2 Self-Testing Laboratories (March to December 2020)

Results for each of the PT distributions for the self-testing laboratories are shown in Table 8 and plotted as a graph in Figure 5. Results are presented as the percentage of the target recovery for each of the 4 samples. Five labs (1150, 1443, 1824, 2475 and 2993) performed very well passing each of the 4 quarterly distributions for March to December 2020 scoring between 87 to 100% in trichinae recovery. Lab 1447 had a good result for March to September 2020 scoring 75 to 100%. However, they failed to participate in the December 2020 PT distribution. Lab 3145 over scored during the June 2020 distribution but recovered well scoring 94% and 88% in trichinae recovery for the subsequent PT distributions. Lab 1632 failed the first 2 consecutive distributions for March to June 2020 but passed September and December 2020 scoring 94% and 100% respectively. Lab 1620 passed the March 2020 PT distribution but unfortunately failed the 3 consecutive distributions from June to December 2020. Lab 3323 failed all the *Trichinella* PTs following their designation in April 2020.

Table 8: Number of trichinae (shown as a percentage) recovered by self-testing laboratories for March to December 2020 Trichinella EQA PTscheme

Note: any numbers marked as red denote a failure and any numbers in blue denote overcounting.

OL ID	March	June	September	December
1150	87	100	100	88
1443	100	100	100	100
1447	80	100	75	NIL RETURN
1620	100 (overcounting)	67 (failure)	69 (failure)	63 (failure)
1632	53 (failure)	67 (failure)	94	100
1824	100	100	100	100
2475	100	100	100	100
2993	100	100	100	88
3145	100	113 (overcounting)	94	88
3323	Designated April 2020	67 (failure)	63 (failure)	63 (failure)





4. EQA Trends across the Year (March to December 2020)

Average recovery per quarter for the government contracted and self-testing laboratories were calculated using only results for laboratories that returned results and with all false positives/over scoring removed from the calculation. Results are shown in Table 9. On average, the contracted government laboratories performed better than the self-testing laboratories with 100% average recovery for each of the 2020 PT distributions. Average results were consistently lower for the self-testing laboratories (≥88%).

Table 9: Average percentage recovery of trichinae by governmentcontracted and self-testing laboratories for March to December 2020Trichinella EQA PT scheme

Laboratory	March	June	September	December
Government contracted	100	100	100	100
Self-Testing	90	90	90	88

5. Conclusion

The NRL is pleased with the overall results achieved for the March to December 2020 *Trichinella* PTs which is a reflection of the continuous *Trichinella* training provided to OCL staff. However, 2 labs have consistently failed to score the required \geq 75% trichinae recovery rate and these have been contacted and offered further training as soon as feasibly possible once lockdown restrictions are lifted.

5.1 Trichinella EQA PT scheme March 2021

The NRL completed participation in the March 2021 *Trichinella* EQA distribution on 26 March 2021. Results on the performance of the NRL as well as that of all the *Trichinella* OCL facilities will be received from Vetqas in May 2021.

6. Inspection and designation of new *Trichinella* laboratories

Two facilities contacted the NRL requesting support in setting up their facilities for *Trichinella* testing capability and designation in order to be able to export to the EU after the end of the EU transition period. The NRL supported both establishments through the provision of all the requirements for setting up a testing facility.

One facility (3638) was inspected on 1 December 2020 and designated by the FSA on 18 December 2020. Four members of staff from this establishment attended training in the use of the magnetic stirrer method at the NRL (on 22 and 23 October 2020; 9 and 15 December 2020). This laboratory will participate in the *Trichinella* PT Scheme organised by Vetqas in March 2021.

The second facility (2658) was late in requesting auditing/training due to the uncertainty of the EU transition negotiations and clarity on whether 100% of pigs from controlled environments would still require testing for *Trichinella* post-transition. The NRL was in contact with the laboratory manager throughout providing all necessary advice. The laboratory was inspected by NRL staff on 22 December 2020 and designated by FSA, Northern Ireland (NI) on 31 December 2020. Minor actions were followed up by the NRL and confirmation of their designation was communicated to FSA (NI) on 26 February 2021.

Two members of staff from this establishment had attended training in the use of the magnetic stirrer method at the NRL on 22 November 2019. Due to COVID restrictions staff members from this facility were not able to attend the refresher training offered to them by the NRL in December 2020. The NRL then arranged for a member of staff from a government contracted laboratory (1921) who had been trained at the NRL in August 2019 to provide refresher training. This laboratory will participate in the *Trichinella* PT Scheme organised by Vetqas in June 2021.

7. *Trichinella* testing in wild boar (WB) (contract OG0236)

Summaries detailing wild boar sample ID, collection date, sample origin, kill date, test date, report date, digest number and results are provided to FSA operations on a monthly basis. Numbers of wild boar samples submitted per month from April 2020 to March 2021 are shown in Table 10. No *Trichinella* species were isolated from any of the submitted samples.

Table 10: Monthly figures for *Trichinella* Testing of Wild Boar from April2020 to March 2021

Month	No Samples	Samples - Running Total
April	3	3
Мау	20	23
June	44	67
July	73	140
August	65	205
September	62	267
October	61	328
November	64	329
December	45	427
January	36	473
February	64	537
March (up to 26 March 2021)	34	571

9. European Union Reference Laboratory (EURLP) EQA

9.1 Criteria for the evaluation

The PT result evaluation is expressed as "correct" if one or more *Echinococcus* spp. adult worms is detected in a spiked sample or no worms are detected in a non-spiked sample. The evaluation is considered "incorrect" if any false positive or false negative results are submitted, irrespective of the number of worms in the sample/s. The final evaluation is only based on qualitative evaluation and is expressed as "positive" if the results of all samples are correct or "negative" if at least one result is incorrect.

9.2 NRL EQAs for March 2021

The NRL participated in the EURLP led International Proficiency Testing in March 2021 (week 15to 19 March 2021). Meatballs inoculated with live *Trichinella* larvae and *Echinococcus* (worms for sedimentation and DNA for molecular diagnosis and speciation) were received at the NRL on 16 March 2021 and processed by the NRL technical staff at York. Three samples were received for each test and results were reported within the deadline submission date. The live *Trichinella* digests were conducted on the 16 March 2021 and reported onto the EURLP website using the provided logon details. The *Echinococcus* Sedimentation and Counting Technique (SCT) for the detection of adult worms in the mucosa was conducted and reported on 17 March 2021. The results for the 3 DNA Taeniid speciation PT were completed and reported on 19 March 2021. The NRL is awaiting confirmation of the results from the EURLP.

10. *Echinococcus multilocularis* surveillance in UK foxes

Echinococcosis caused by *Echinococcus multilocularis* is often expensive and complicated to treat, and may require extensive surgery and/or prolonged drug therapy. Humans are infected through ingestion of parasite eggs in contaminated food, water or soil, or through direct contact with animal hosts. Regulation (EU) No 1152/2011 lays down legislation for the treatment of dogs travelling into member States (MSs) under the PET travel scheme.

During this reporting period the NRL collected a total of 843 foxes provided by a network of pest controllers from March 2020 to February 2021. Due to COVID-19 restrictions no foxes were collected during April to May 2020 of the first national lockdown (Figure 7). Foxes underwent postmortem by NRL technical staff and retrieved faecal samples were frozen for subsequent analysis. A total of 500 faecal samples retrieved from foxes across the UK were spatially selected to be included in the 2020 to 2021 surveillance and tested for *E*.

multilocularis using the methodology described by Learmount et al., (2012). At the time of writing this report approximately 270 faecal samples were processed for egg isolation and DNA extraction by 3 members of competent staff. Results of this surveillance will be submitted to DEFRA as supportive evidence to allow the UK to retain its disease-free listing latest by the beginning of May 2021.



Figure 7: Number of foxes processed for the Echinococcus multilocularis surveillance 2020 to 2021

11. Other NRL activities

11.1 Project OG0248

A Service Level Agreement (SLA) for monitoring *Trichinella* in Feral Wild Boar in Scotland was signed between APHA and Food Standards Scotland (FSS). The agreement is from 1 April 2020 to 31 March 2023.

11.2 Audits

The NRL was remotely audited on 5 August 2020 by UKAS. UKAS assessment visit looked at management and technical system requirements in accordance with ISO/IEC 17025 and accreditation was maintained.

On 1 February 2021 the NRL had the first ever assurance audit by APHA SHAW of our SAPO2 *Trichinella* activities/facility. The auditor looked through training/competence records and monitoring, servicing reports for equipment used in the laboratory, MSC

certification and airflow monitoring checks, validation documentation for disinfectants, inventory checks, emergency drill contingency exercises, validation certificates and maintenance reports, autoclave records and records of received and dispatched material containing biological agents. A handful of actions were raised which have been addressed. The NRL scored 90.2% in this SAPO assurance audit.

11.3 Guidance for *Trichinella* Testing in Feral Wild Boar

The Guidance for *Trichinella* Testing in Feral Wild Boar aimed at hunters was significantly updated and approved by FSA *Trichinella* policy. This guidance has been shared with FSA and the FSS and is now live on APHA surveillance website: <u>http://apha.defra.gov.uk/vet-gateway/surveillance/forms.htm</u>

11.4 NRL recruitment

The NRL has successfully recruited an Assistant Parasitologist (Administrative Officer) who joined the team on June 29 2020 and a laboratory manager (HEO) who assumed her duties on the 14 December 2020.

11.5 Publications

The NRL Lead collaborated and published several publications during this reporting period on nomenclature in Echinococcosis, *Taenia* species diagnosis, *Taenia hydatigena* in wild boar and *Taenia saginata* in UK cattle as follows:

- 1) Vuitton, Dominique A., et al. "International consensus on terminology to be used in the field of echinococcoses." Parasite 27 (2020): 41.
- 2) *Taenia hydatigena* cysticercosis in wild boar (Sus scrofa) from southern Italy: an epidemiological and molecular survey. Parasitology. 2020 Aug 24;1-7. DOI: <u>10.1017/S0031182020001559</u>
- Bovine cysticercosis outbreak in an indoor beef finisher farm in the North of England. Macrelli M, et al. Vet Rec Case Rep 2020; 8: e001178. doi:10.1136/vetreccr-2020-001178
- 4) CYSTINET: The European Network on Taeniosis/Cysticercosis. Collaborative studies for the detection of *Taenia* spp. infections in humans. Submitted to *Microorganisms*, special issue dedicated to "Cysticercosis, a Neglected Disease.

12. References

Learmount, J., Zimmer, I. A., Conyers, C., Boughtflower, V.D., Morgan, C. P., Smith, G. C. (2012). A diagnostic study of *Echinococcus multilocularis* in red foxes (*Vulpes vulpes*) from Great Britain. Vet. Parasitol. 190: 447-453.

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Rossi, P. and Pozio, E. (2008). Guidelines for the detection of *Trichinella* larvae at the slaughterhouse in a quality assurance system. Ann Ist Super Sanità, 44: 195-199.

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