

Animal & Plant Health Agency

Annual Report (2023 to 2024)

The National Reference Laboratory for *Trichinella* and *Echinococcus*

1 April 2023 – 31 March 2024



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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 retained Regulation (EU) 2017/625 on Official Feed and Food Controls in the UK and has a legal obligation to designate 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 consequently well placed to cover the UK for parasitic diseases particularly for *Trichinella* and *Echinococcus*.

The NRL supplies trichinae required for the Proficiency Testing (PT) scheme that assesses the performance of Official Laboratories (OLs) 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). 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*

1.2.1 Core Activity 1 - Secretariat Services

- a) All relevant information/advice was reviewed, assessed and either discussed verbally with the FSA for immediate action/dissemination or included in quarterly interim reports if more appropriate.
- b) Contact information for the UK OLs was held on a central database and this information was regularly reviewed and updated. Contacts are well established with regular electronic as well as face to face communication through dissemination of proficiency testing results, technical enquiries, sample management and contract management. All new OLs were required to participate in one-to-one training and familiarisation visits by NRL technical staff, initiating an ongoing transparent and supportive relationship with OL staff.
- c) Results of initial and ongoing audits of OLs were communicated to the FSA within 4 weeks of each visit. All NRL/OL activity were formally reported to the FSA at least quarterly and more regularly (monthly) if appropriate and were documented:
 - PT results for each laboratory as well as results for a rolling 12-month period.
 - OL communications.
 - EURL ring trial results.
 - Scientific developments.
- d) A dedicated website was maintained by the NRL on the GOV.UK site and this will be reviewed at least quarterly to ensure that information on methods of analyses, SOPs, latest developments and other background information is up to date and relevant. The website will be revised on receipt of any relevant changes to regulations or standards for testing or upon change of any relevant contact details.

1.2.2 Core Activity 2 - Advice and representation within UK and Internationally

e) Scientific and technical advances in testing methodology and analysis (for both Trichinella and Echinococcus) was subject to ongoing (and documented) review by the NRL lead/test consultant via literature searches, participation in relevant research and surveillance and via APHA CPD training for disease consultants. The NRL lead/test consultant maintains awareness of any emerging analytical issues or developments at a national or international level and recommended action to address them, advising the FSA, OLs and other relevant laboratories accordingly.

- f) The NRL lead was available to provide technical assistance and advice on best scientific practice to the FSA for the implementation of coordinated control plans and was available to the OLs to provide technical advice and training for the duration of the contract. The NRL lead/test consultant and their deputy are conversant with the Trichinella Contingency Plan for the UK and the testing regimes in place for confirmation of suspect/ non-negative cases. The NRL will be available to respond to and notify the FSA in such cases.
- g) The NRL lead/test consultant is expected to attend the annual Workshop of the National Reference Laboratories for Parasites, usually held in May and hosted by the EURL at the Instituto Superiore di Sanita. However, due to the ongoing issues around Brexit, the NRL was not included within the invitation list for the 18th Annual Workshop of the National Reference Laboratories for Parasites hosted by the EURLP in 2023 and did not attend.

1.2.3 Core Activity 3 - Compliance Assessment via Audits and Ring trials

- a) The NRL maintained a pool of competent staff at ISO:17025 level for diagnostic testing and NRL function. These staff were available to carry out initial and ongoing training of OL laboratory staff during the period of the contract. Auditing of all OLs will be carried out on a biannual basis.
 - b) Proficiency testing of all the OLs will be coordinated as follows:
 - The NRL liaised with the Quality Assurance Unit (QAU) for continuation of the existing PT scheme and assure participation of all registered OLs.
 - The NRL maintained a supply of Trichinae for PT sample spiking using reference material purchased from the EURL.
 - The NRL provided the QAU with spiked reference material for use in quarterly proficiency test distributions (March, June, September and December).
 - The NRL advised on, directed and co-ordinated these 4 distributions per year of the PT scheme samples.

- PT data and results were stored on a database.
- The NRL evaluated, analysed and commented on the results and reported these to the labs involved and the FSA following collation of the results.
- Underperforming laboratories were visited for audit and retraining in the first and second instance if appropriate.

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 designations and it is a requirement that all designated *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 in the PT scheme 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 to 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 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

First Instance

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

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

Result	Action
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.

*Not operational can be defined as a change in circumstance resulting in no legal requirement for Trichinella testing. It can also be defined as non-participation or consistent failure within the VetQas Proficiency testing scheme.

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 Official Laboratories

In March 2023, there were 10 designated *Trichinella* self-testing facilities and 3 contracted government laboratories in operation, including the NRL. Table 2 shows the list of all designated operational laboratories during 2023 to 2024 and a full list of contact details is maintained by the NRL and is also available on the <u>FSA website</u>.

Table 2: List of approved government contracted and self-testing officiallaboratories (OL) carrying out *Trichinella* testing in 2022 to 2023

OL ID	OL 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
3638	self-tester

*Official laboratory surrendered designation in July 2023.

2.0 Results for the *Trichinella* EQA PT scheme distribution between April 2023 to March 2024

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).

2.1 Results of the *Trichinella* EQA PT scheme distribution in March 2023

A total of 12 *Trichinella* testing facilities participated in the March 2023 distribution (Table 3). Of the participating laboratories, 85% successfully passed the March 2023 *Trichinella* PT distribution scoring between 85 to 100% in overall *trichinae* recovery rate. Testing facilities 1620 and 1632 failed this distribution. Testing facility 1620 scored an overall recovery rate of 46% while Lab 1632 recorded a false negative in sample 23/4020. Testing facility 2993 did not participate in this distribution. On investigation, the NRL was informed that this was due to the VetQas contact list not being updated following the departure of the responsible staff member. Consequently, the site did not receive any of the reminder or confirmation of sample sent emails and as a result, samples were not read and results not submitted.

Table 3: Individual sample results (raw data and %) from the March 2023 *Trichinella* EQA PT scheme for government contracted and self-testing laboratories (Note: results marked (F) and (O) denote failure and overcounting respectively)

Sample ID	23/4017	23/4018	23/4019	23/4020	Overall score (%)
Intended	0 (100%)	6 (100%)	4 (100%)	3 (100%)	13 (100%)
1150	0 (100%)	6 (100%)	2 (50%)	3 (100%)	11 (85%)
1443	0 (100%)	6 (100%)	4 (100%)	3 (100%)	13 (100%)
1447	0 (100%)	6 (100%)	4 (100%)	3 (100%)	13 (100%)
1620	0 (100%)	3 (50%)	1 (25%)	2 (66%)	6 (46%) (F)
1632	0 (100%)	6 (100%)	4 (100%)	0 (0%) (F)	10 (77%) (F)
1706	0 (100%)	6 (100%)	4 (100%)	3 (100%)	13 (100%)
1787	0 (100%)	6 (100%)	4 (100%)	3 (100%)	13 (100%)
1824	0 (100%)	6 (100%)	4 (100%)	3 (100%)	13 (100%)
1921	0 (100%)	6 (100%)	4 (100%)	3 (100%)	13 (100%)
2475	0 (100%)	6 (100%)	4 (100%)	3 (100%)	13 (100%)
2993					Nil Return
3145	0 (100%)	5 (83%)	4 (100%)	3 (100%)	13 (100%)
3638	0 (100%)	6 (100%)	4 (100%)	3 (100%)	13 (100%)

2.2 Results of the *Trichinella* EQA PT scheme distribution in June 2023

A total of 12 *Trichinella* testing facilities participated in the June 2023 distribution (Table 4). Of the participating laboratories 92% successfully passed this *Trichinella* PT distribution scoring between 75 to 100% in overall *trichinae* recovery rate. Testing facility 1447 (67%) failed the distribution.

No false positive or false negative results were recorded by any of the participating facilities.

Testing facility 1620 did not participate in this distribution. On investigation by the NRL, the reason behind the nil return was due to the Official Laboratory ceasing Trichinella testing. Their designation was subsequently surrendered in July 2023.

Table 4: Individual sample results (raw data and %) from the June 2023 *Trichinella* EQA PT scheme for government contracted and self-testing laboratories. (Note: results marked (F) and (O) denote failure and overcounting respectively)

Sample ID	23/4024	23/4025	23/4026	23/4027	Overall score (%)
Intended	2 (100%)	4 (100%)	0 (100%)	6 (100%)	12 (100%)
1150	2 (100%)	4 (100%)	0 (100%)	5 (83%)	11 (92%)
1443	2 (100%)	4 (100%)	0 (100%)	6 (100%)	12 (100%)
1447	1 (50%)	3 (75%)	0 (100%)	4 (67%)	8 (67%) (F)
1620					Nil Return
1632	3 (150%) (O)	2 (50%)	0 (100%)	6 (100%)	11 (92%)
1706	2 (100%)	4 (100%)	0 (100%)	6 (100%)	12 (100%)
1787	1 (50%)	4 (100%)	0 (100%)	7 (117%) (O)	12 (100%)
1824	2 (100%)	3 (75%)	0 (100%)	6 (100%)	11 (92%)
1921	2 (100%)	4 (100%)	0 (100%)	6 (100%)	12 (100%)
2475	2 (100%)	2 (50%)	0 (100%)	6 (100%)	10 (83%)
2993	2 (100%)	2 (50%)	0 (100%)	5 (83%)	9 (75%)
3145	2 (100%)	4 (100%)	0 (100%)	5 (83%)	11 (92%)
3638	2 (100%)	4 (100%)	0 (100%)	6 (100%)	12 (100%)

2.3 Results for the *Trichinella* EQA PT scheme distribution in September 2023

A total of 12 *Trichinella* testing facilities participated in the September 2023 distribution (Table 5). All participating laboratories successfully passed this *Trichinella* PT distribution scoring between 90 to100% in overall *trichinae* recovery rate.

No false positive or false negative results were recorded by any of the participating facilities.

Testing facility 1620 did not participate in this distribution following the surrender of their designation in July 2023.

Table 5: Individual sample results (raw data and %) from the September 2023 *Trichinella* EQA PT scheme for government contracted and self-testing laboratories.

Sample ID	23/4037	23/4038	23/4039	23/4040	Overall score (%)
Intended	5 (100%)	0 (100%)	3 (100%)	2 (100%)	10 (100%)
1150	5 (100%)	0 (100%)	2 (100%)	2 (100%)	9 (90%)
1443	5 (100%)	0 (100%)	3 (100%)	2 (100%)	10 (100%)
1447	5 (100%)	0 (100%)	3 (100%)	2 (100%)	10 (100%)
1620					Nil Return
1632	5 (100%)	0 (100%)	2 (100%)	2 (100%)	9 (90%)
1706	5 (100%)	0 (100%)	3 (100%)	2 (100%)	10 (100%)
1787	5 (100%)	0 (100%)	3 (100%)	2 (100%)	10 (100%)
1824	5 (100%)	0 (100%)	3 (100%)	2 (100%)	10 (100%)
1921	5 (100%)	0 (100%)	3 (100%)	2 (100%)	10 (100%)
2475	5 (100%)	0 (100%)	3 (100%)	2 (100%)	10 (100%)
2993	5 (100%)	0 (100%)	3 (100%)	2 (100%)	10 (100%)
3145	4 (80%)	0 (100%)	3 (100%)	2 (100%)	9 (90%)
3638	5 (100%)	0 (100%)	3 (100%)	2 (100%)	10 (100%)

2.4 Results for the *Trichinella* EQA PT scheme distribution in December 2023

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A total of 12 *Trichinella* testing facilities participated in the December 2023 distribution (Table 7). Of the participating laboratories 92% successfully passed this *Trichinella* PT distribution scoring between 90 to 100% in overall *trichinae* recovery rate.

Testing facility 1632 failed the distribution by recording a false negative in sample 23/4058.

Testing facility 1620 did not participate in this distribution following the surrender of their designation in July 2023.

Table 6: Individual sample results (raw data and %) from the December 2023 *Trichinella* EQA PT scheme for government contracted and self-testing laboratories. (Note: results marked (F) and (O) denote failure and overcounting respectively)

Sample ID	23/4055	23/4056	23/4057	23/4058	Overall score (%)
Intended	6 (100%)	0 (100%)	4 (100%)	1 (100%)	11 (100%)
1150	6 (100%)	0 (100%)	4 (100%)	1 (100%)	11 (100%)
1443	6 (100%)	0 (100%)	4 (100%)	1 (100%)	11 (100%)
1447	6 (100%)	0 (100%)	4 (100%)	1 (100%)	11 (100%)
1620					Nil Return
1632	5 (82%)	0 (100%)	4 (100%)	0 (0%) (F)	9 (82%) (F)
1706	6 (100%)	0 (100%)	4 (100%)	1 (100%)	11 (100%)
1787	6 (100%)	0 (100%)	4 (100%)	1 (100%)	11 (100%)
1824	6 (100%)	0 (100%)	4 (100%)	1 (100%)	11 (100%)
1921	6 (100%)	0 (100%)	4 (100%)	1 (100%)	11 (100%)
2475	6 (100%)	0 (100%)	4 (100%)	1 (100%)	11 (100%)
2993	6 (100%)	0 (100%)	4 (100%)	1 (100%)	11 (100%)
3145	6 (100%)	0 (100%)	3 (75%)	1 (100%)	10 (91%)
3638	6 (100%)	0 (100%)	4 (100%)	1 (100%)	11 (100%)

2.5 Results for the *Trichinella* EQA PT scheme for a 12month rolling period

To summarise the performance of the contracted government (1706, 1787 and 1921) and self-testing laboratories in the *Trichinella* EQA exercises, the average trichinae recovery rates have been calculated for each EQA distribution over the last 12 months (Table 7). All false positives, nil returns and over scoring were removed during this calculation.

On average, the contracted government laboratories performed better than the self-testing laboratories, averaging a 98% recovery over the last 4 PT distributions. This represents a slight increase from the 95% recovery rate achieved during the 4 distributions in 2022-2023.

Over the last 12 months, self-testing laboratories have also generally performed well and achieved an average recovery rate of 89% for this period. This also represents a slight increase from the 88% recovery rate recorded in 2022-2023.

Table 7: Overall trichinae recovery rate (%) recovered by government contracted and self-testing laboratories over a 12-month rolling period. (Note: results marked (F) and (O) denote failure and overcounting respectively). 12-month average only includes samples participated in and without overcounting, false positives and false negatives.

OL ID	March 2023	June 2023	September 2023	December 2023	Over 12 months
1150	85	92	90	100	92
1443	100	100	100	100	100
1447	100	67 (F)	100	100	88
1620	46 (F)	Designation Surrendered	N/A	N/A	46
1632	77 (False Negative)	83 (O)	90	82 (False Negative)	86
1706	100	100	100	100	100
1787	100	92 (O)	100	100	98
1824	100	92	100	100	98
1921	100	100	100	100	100
2475	100	83	100	100	90
2993	N/A	75	100	100	92
3145	100	92	90	100	96
3638	100	100	100	100	100

Overall, the NRL is pleased with the results achieved over the last 12-month rolling period of *Trichinella* PT's. However, lab 1632 has under-performed during *Trichinella* PT's over the last 12 months, recording false negatives in both March and December distributions.

Following their poor performance in the March distribution, lab 1632 was placed on an improvement action plan consisting of NRL training, practice PT panels, and an audit of their facility.

3.0 NRL EQAs for 2023

3.1 Trichinella EQA March 2023

The NRL participated in the EURLP led International Proficiency Testing in March 2024 (week 11 to 16 March 2024). Meatballs inoculated with live *Trichinella* larvae, fish fillets spiked with *Anisakis* sp. larvae and *Echinococcus* (worms for sedimentation and DNA for molecular diagnosis and speciation) were received at the NRL on 12 March 2024 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 13th March 2024 and reported onto the EURLP website using the provided logon details. The result of the EURLP led International Trichinella Proficiency Testing Scheme (PT-01) from March 2024 is shown in Table 8.

Table 8: EURLP PT-01 distribution from March 2024 showing trichinae recovery rate (raw data and %), and the difference between observed and reported result (Λ).

Meat	Weight (g)	Code	Spiked Larvae	Recovered Larvae (Raw data and %)	Λ (Delta)
Pork	100	3039	0	0 (100%)	0
Pork	100	7486	4	2 (50%)	2
Pork	100	2297	4	4 (100%)	0

3.2 Anisakis EQA March 2024

The Anisakis digests were conducted and reported on the 12th of March 2024. The NRL received a qualitative evaluation here the Λ (Delta) value equates to the number of missed larvae. The result of the EURLP led International Trichinella Proficiency Testing Scheme (PT-04) is shown in Table 9.

Table 9: EURLP PT-04 distribution from March 2024 showing *Anisakis* sp. recovery rate (raw data and %), and the difference between observed and reported result (Λ).

Meat	Weight (g)	Code	Spiked Larvae	Recovered Larvae (Raw data and %)	Λ (Delta)
Fish	100	A1218	1	1	0
Fish	100	A1219	1	1	0
Fish	100	A1220	1	1	0

3.3 Echinococcus EQA March 2024

The *Echinococcus* Sedimentation and Counting Technique (SCT) for the detection of adult worms in the mucosa (PT-05) was conducted and reported on the 14th March 2024. The NRL received a final evaluation of "positive", as one or more *Echinococcus* spp. adult worms were recovered in spiked samples and no worm was recovered in the samples which were not spiked (Table 10).

Table 10: Results of the EURLP PT-05 showing the recovery of *Echinococcus* spp. adult worms from each sample.

Sample	Number of Spiked Worms	Number of Recovered Worms	Evaluation
EG4	25	9	Correct
EG5	0	0	Correct
EG6	0	0	Correct

The results for the molecular identification of *Echinococcus* at the species level (PT-08) were completed and reported on 19th of March 2024. The NRL received a final evaluation of "positive" as all species were correctly identified (Table 11).

Table 11: Results from the EURLP PT-08 showing the recovery of*Echinococcus* spp. adult worms from each sample.

Item Code	Result Observed	Result Expected	Evaluation
EGM1	E. granulosus s.l	E. granulosus s.l	Correct
EGM2	Negative	Negative	Correct
EGM3	E. multilocularis	E. multilocularis	Correct

4. Other NRL Activities

4.1 NRL recruitment

The NRL successfully recruited one Assistant Parasitologist who joined the team in November 2023.

5. References

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Date: 08/05/2024

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Appendix 1 - Additional *Trichinella* and *Echinococcus* Surveillance Contracts

1. *Trichinella* testing of wild boar (WB) in England and Wales (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 to August have been consistent throughout the year to date (Table 11). Numbers of wild boar samples submitted per month from April 2023 to March 2024 are shown in Table 12. No *Trichinella* species were isolated from any of the submitted samples.

2. *Trichinella* testing of wild boar in Scotland (Project OG0248)

The Service Level Agreement (SLA) for monitoring *Trichinella* in Feral Wild Boar in Scotland signed between APHA and Food Standards Scotland (FSS) remains ongoing. Summaries detailing wild boar sample ID, collection date, sample origin, kill date, test date, report date, digest number and results are provided to FSS operations on a monthly basis. Between April 2023 and March 2024, the NRL received a total of 31 samples. No *Trichinella* species were isolated from any of the submitted samples.

Table 12: Monthly figures for *Trichinella* Testing of Wild Boar from April2023 to March 2024

Month	Sample Number	Cumulative Samples
April 2023	28	28
May 2023	22	50
June 2023	30	80
July 2023	55	135
August 2023	57	192
September 2023	61	253
October 2023	39	292
November 2023	50	342
December 2023	22	364
January 2024	26	390
February 2024	44	434
March 2024	39	473

3. *Echinococcus multilocularis* surveillance in UK foxes 2022 to 2023

For the 2022 to 2023 surveillance, the NRL collected a total of 868 foxes provided by a network of pest controllers between March 2022 to February 2023. Foxes underwent post-mortem and NRL technical staff retrieved faecal samples which were frozen for subsequent analysis. A total of 348 faecal samples retrieved from foxes across the UK were spatially selected as unique and were included in the 2022 to 2023 surveillance. An additional 174 duplicate samples were also tested for *E. multilocularis* using the methodology described by

Learmount et al. (2012). The final report was sent on 19 June 2023 with the conclusion that no positive result for *E. multilocularis* was observed.

4. *Echinococcus multilocularis* surveillance in UK foxes 2023 to 2024

For the 2023 to 2024 surveillance, the NRL had collected a total of 858 foxes provided by a network of pest controllers between March 2023 to February 2024. Foxes underwent post-mortem and NRL technical staff retrieved faecal samples which were frozen for subsequent analysis. A total of 362 faecal samples retrieved from foxes across the UK were spatially selected to be included in the 2023 to 2024 surveillance and tested for *E. multilocularis* using the methodology described by Learmount et al. (2012).