



Squirrelpox Detection in the Central Lowlands 2017-2020

Summary

To set the context for squirrelpox detection work in the Central Lowlands, the history of squirrelpox virus in Scotland is summarised. Arriving first on the Scottish Border in 2005, squirrelpox spread steadily northward towards Edinburgh, as revealed by widespread squirrelpox testing of grey squirrels across south Scotland. The establishment of a systematic monitoring scheme in 2012 included the Central Lowlands, where the disease was expected to spread next. Sporadic positive serological test results occurred at isolated locales across Glasgow and northward to Loch Lomond and north-eastward to Plean, south east of Stirling. However, to date there has been no evidence of ongoing incremental spread across the intervening landscape, countering expectations of a more rapid spread north of Edinburgh-Glasgow than there had been to the south.

Squirrelpox testing results for the four years 2017-2020 are presented, showing the sporadic pattern of disease continuing and even possibly slowing down. The possibility of the impact of the well-documented continuing reestablishment of pine marten in the region is considered, with a call for further research into the dynamic between this native predator, the two squirrel species, the squirrelpox virus and the influence of urban habitats.

Background

Squirrelpox Virus (SQPV) is a viral disease endemic at sub-clinical¹ level in grey squirrel populations which is transmissible to red squirrels with invariably fatal results. Recognised in England and Wales for several decades, antibody testing of grey squirrel blood samples has shown it to have been present in Scotland only since 2005. First appearing at Dalton on the Cumbrian border, it initially spread along the wooded valleys of the Rivers Esk and Liddel. It continued to spread, despite concentrated efforts to contain it, up Annandale and Nithsdale to South and East Ayrshire and Renfrewshire. In 2011 it was detected in the east of the country on the Northumberland border at Cornhill-on-Tweed, and from here spread up the Blackadder, Whiteadder and Tweed Valleys and up the Gala and Lauder Waters, again in spite of intensive control work aimed at halting the spread.

The blood testing of culled grey squirrels to identify the prevalence of the disease in populations was carried out by the Moredun Research Institute from samples taken from across southern Scotland and particularly at the front of the disease spread in an effort to help the project to direct control efforts. Until 2011, the project's sampling area was restricted to landscapes little further north than the most northerly existing positive squirrelpox samples, although occasional sampling by other organisations or landowners further to the north occurred.

In 2012, following recommendations made by Gurnell (2012) a monitoring scheme was developed which expanded the spread of SQPV sampling. Rather than continue to try to predict the most likely routes of spread of the disease, the scheme sampled at approximately regular geographical intervals across the whole area of Scotland occupied by grey squirrels, in an attempt to locate early occurrences of the disease in new areas. The map of the area from the Scottish border to the Highland Line was overlain by a 20x20km grid, and one 10km square in each 20x20km square was selected for annual sampling.

From each sample square the project aimed to collect a minimum of 10 blood samples per annum. Although 20 samples are generally required to rule out the possibility of low seroprevalence of SQPV, the costs of collection and the capacity for testing were limiting, and discussions with the Moredun

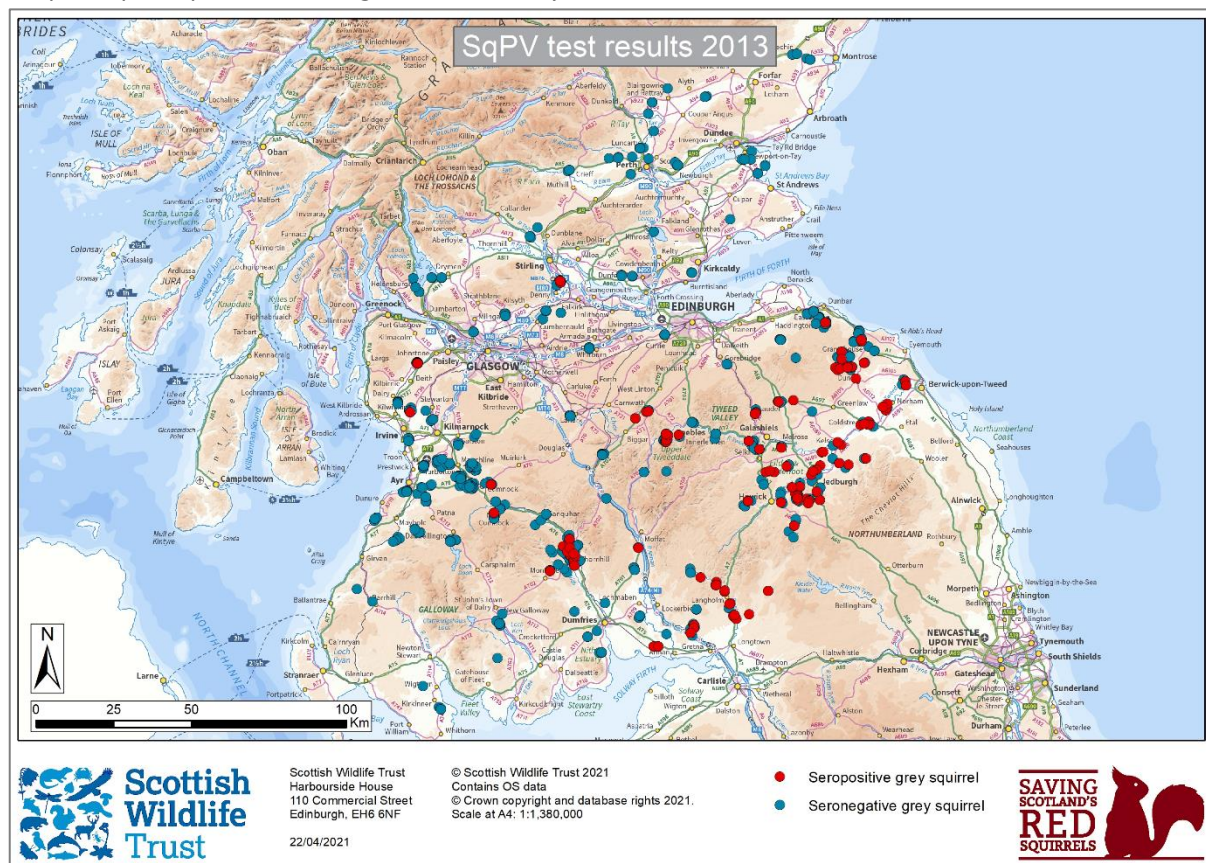
¹ Without disease [symptoms](#) that are detectable by [physical examination](#) .

Research Institute led to a compromise of 10 samples, which should pick up presence of SQPV in most cases.

Thus the first squirrelpox sampling in the Central Lowlands occurred in 2012. In that year no evidence of squirrelpox disease in the Central Lowlands was detected by the sampling. The furthest north that the disease was detected was just south of Eyemouth on the east coast and around Mauchline in the west (East Ayrshire).

In 2013 (Map 1) the first instance of squirrelpox antibodies in grey squirrels in the Central Lowlands was detected at Plean, near Bannockburn, south of Stirling (NS88 in Maps 2-5). Rapid spread south of Edinburgh-Glasgow was also evident, with positive results coming from near Innerwick in the eastern Lammermuirs, the Blyth Bridge area in South Lanarkshire, and at Lochwinnoch just southwest of Glasgow.

Map 1. Squirrelpox Monitoring Results January to December 2013



*The term “seropositive” denotes that a test of the squirrel’s serum for antibodies to squirrelpox virus produced a positive result, i.e. the squirrel had been exposed to the virus and created antibodies. “Seronegative” denotes that the test of the serum for squirrelpox antibodies was negative.

At around this time, mathematical modelling of the spread of squirrelpox in real landscapes (White et al 2016) predicted that if squirrelpox should reach the central belt (Edinburgh-Glasgow), the densities of grey squirrels would be high enough to support rapid spread of squirrelpox northwards through the Central Lowlands grey squirrel populations.

In accordance with standard protocols, when a seropositive squirrelpox result is first detected in a new area, a much larger sample (ideally 40 grey squirrels) is then collected for testing, to enable a better estimate of the prevalence of exposure to the virus in grey squirrels. Around Plean in 2013 we were only able to obtain 22 animals suitable for testing, of which 2 tested positive. This suggested presence at a very low level.

In 2014, sampling at Plean again detected squirrelpox seropositive grey squirrels – this time 4 positives in a sample of 43 – a similarly low level of incidence. In the same year we detected 2 seropositives in a sample of 47 at Edinburgh Science Park, just south of Edinburgh at Roslin. The

sampling in the geographical area between these two sites returned no other suggestion of squirrelpox presence.

In 2015, sampling at Plean again detected squirrelpox exposure in 1 of the 21 samples taken. There was also a new site, Bearsden in Glasgow (NS57), where 2 positives were detected in a sample of 22, for the first time since sampling began there in 2012.

In 2016, only 2 samples were obtainable from the Plean 10km-square (due to access difficulties). Both were negative. However, seropositives were picked up at Erskine Bridge (NS47) for the first time, in 2 of 21 samples. At Bearsden we were only able to collect 1 sample for testing, which was negative. A strange result also came in the form of a positive test from a squirrel caught at the south west shore of Loch Lomond (NS38). Despite prolonged trapping, we were only able to find 1 more squirrel in the area for testing: it was negative. This remains an anomalous result, being so far outside the known range, and in a site with almost no grey squirrels present.

Since the detection of squirrelpox antibodies and Plean and at Bearsden, we have sampled most of the neighbouring 10km squares in addition to the monitoring scheme's sample square, to try to find dispersal/ transmission routes in the landscape, but without finding any other positive results.

Squirrelpox Management during Saving Scotland's Red Squirrels – Developing Community Action (SSRS-DCA) 2017-2020

Saving Scotland's Red Squirrels (2009-2022) is a project aimed at protecting red squirrels in all parts of the Highlands, Argyll, North East Scotland, northern Tayside and Stirling where there are currently no grey squirrels, to enable red squirrels to recover their former range in North East Scotland, and to defend priority red squirrel populations in South Scotland from the threats of replacement by grey squirrels and from disease.

In the 5-year National Lottery Heritage funded "Developing Community Action" phase of the project (2017-2022), the aim is to secure the long-term future of red squirrels in Scotland by building capacity among communities of volunteers and land managers, empowering them to take on and take ownership of some of the essential protection measures required to save the nation's red squirrels.

The work in the Central Lowlands is essentially to contain grey squirrel encroachment northwards of the Highland Line – a geographical fault line that delineates a marked change of landscape between the two sides of the line. South of the line red and grey squirrels both occur in the landscape, and grey squirrel populations are constantly being replenished by dispersal from the "grey-only" central belt to the south of the Campsies, the Kilsyth Hills and the Sidlaw Hills in Tayside. The work of SSRS has restored red squirrels to much of the terrain from which it was vanishing within the 10km strip south of the Highland Line, thus helping to control the spread of grey squirrels from this zone into the "red-only" populations to the north of the line. Where grey squirrel densities are kept low, squirrelpox disease cannot circulate freely through the grey population because there are too few susceptible animals to keep the infection cycle active, so SSRS is at the same time as managing grey squirrel densities, also reducing the likelihood that squirrelpox will reach Scotland's "red-only" population.

The same principle is also in play in SSRS's work to protect red squirrels in South Scotland's Priority Areas for Red squirrel Conservation (PARCs). In this region the project has accepted that squirrelpox is now endemic in grey squirrel populations, and can be detected in up to 60% of grey squirrels south of Edinburgh and Glasgow.

An important finding from squirrelpox research in other parts of the UK, is that squirrelpox viral disease in red squirrel populations does not tend to spread rapidly. The onset of symptoms is so rapid that the virus seems to have only enough time to transmit to local red squirrels, and the outbreak burns out before it gets passed on more widely (White et al, 2016). Thus if grey squirrels can be prevented from moving in when a local red squirrel population crashes, the ground stays vacant for neighbouring reds to expand into.

Squirrelpox Testing during SSRS-DCA in 2017-2020

In 2015 we took the decision to cease testing grey squirrels for squirrelpox in South Scotland, since this information was not telling us anything new. However, red squirrels with disease symptoms are still sent for testing by post-mortem, electron microscopy for viral particles and sometimes PCR² testing for viral DNA in diseased red squirrel tissues. Serological testing of red squirrels rarely reveals the presence of antibodies, since most individuals have died before a sufficient immune response can be generated – hence the requirement for alternative means of diagnosis. Since 2016, all the sampling has been of grey squirrels from Edinburgh and Glasgow northwards, with a few samples coming in from other agencies or individuals from the south.

In the Central Lowlands, there are 34 10km-squares from which we endeavour to collect 10 samples each year to send for squirrelpox serological testing at the Moredun Research Institute. Because of the difficulty of finding enough volunteers to carry out sampling across the whole area, we have never managed to test in all 34 squares in any one year. We focus most of our resources towards testing the sites from which we have previously had positive results, and the 10km-squares and most likely dispersal routes around them. The aim is to detect any spread of the virus, so that plans can be drawn up by interested parties for any necessary containment measures as early as possible.

Some of the sample 10km-squares do not have sufficient grey squirrels present to enable 10 individuals to be caught and tested. Here we trap in the square with at least 10 traps for 10 days and then move on, even if we have not caught 10 squirrels. The assumption is that in these areas there are too few grey squirrels to maintain actively circulating disease, and therefore of low risk to red squirrels – effectively “negative” for presence of squirrelpox.

In 2017 (Map 2), only one squirrel of 10 tested in NS88, the Plean sample square, returned a positive result. In Bearsden (NS57) and Erskine (NS47) we detected no evidence of squirrelpox, and nor did we detect it the neighbouring square to the south (NS56).

In 2018 (Map 3), we again collected a seropositive sample from Erskine (NS47). Unfortunately, we were unable to sample in Bearsden in this particular year. Interestingly we were unable to detect any seropositive squirrels in NS88 (the Plean square) or surrounding squares, for the first time since 2012. The Moredun again tested a sample of grey squirrels from Pentland Science park (NT36), and came back with a seroprevalence of 80%, signifying actively circulating virus in local grey squirrels, and verifying the sensitivity of the testing procedure.

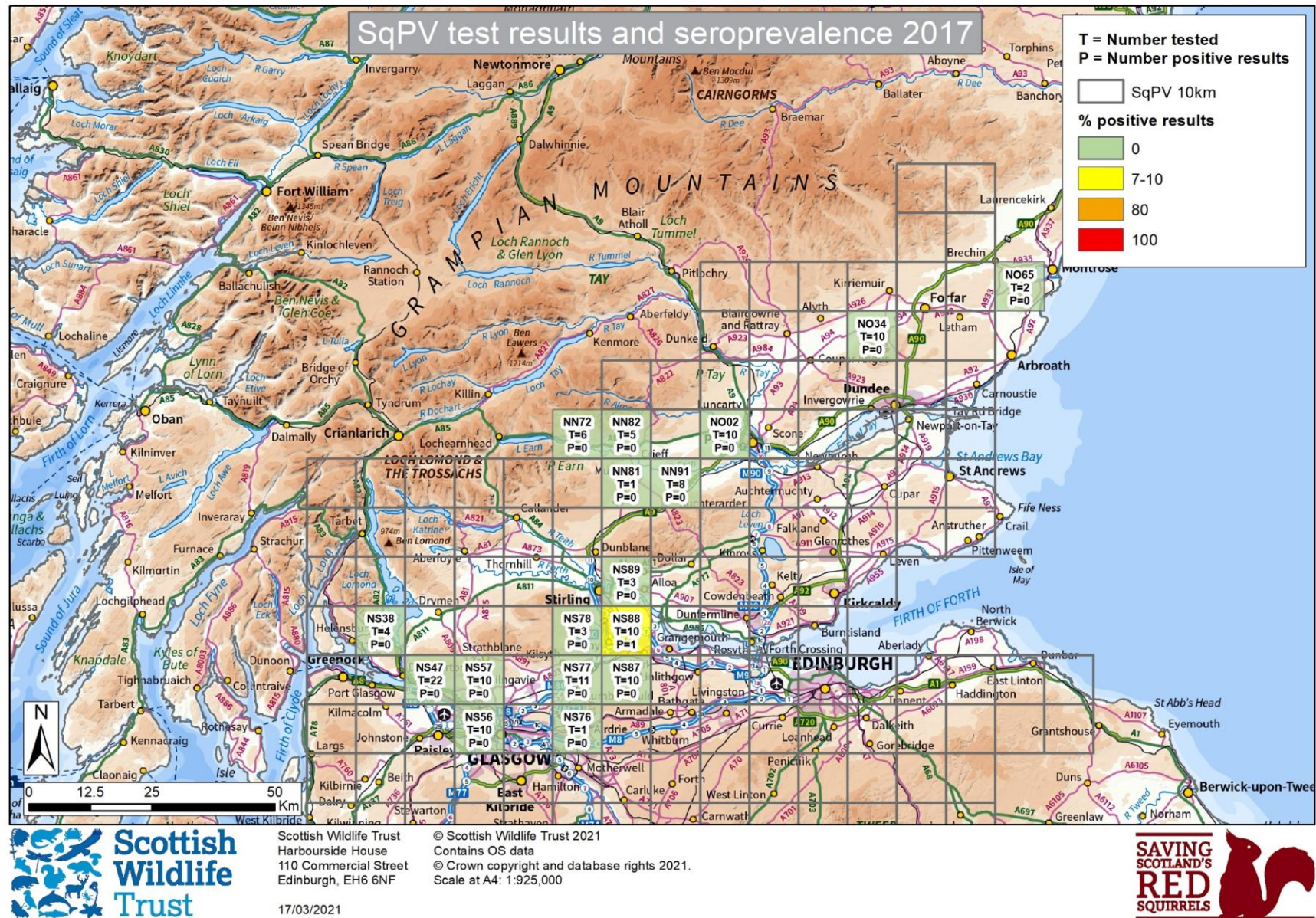
2019 (Map 4) was a second year with no positively testing animals coming from NS88 (Plean), suggesting that possibly the virus has died out there. We were again unable to test in Glasgow, but the Erskine square (NS47) came back as negative despite quite a large sample of 32. A questionable result that was only slightly over the cut-off level to be regarded as positive came from Strathallan (NN91), in one of ten squirrels tested. This kind of faintly positive result in one animal coming after earlier negative testing since 2017 (5 squirrels in 2018, and 8 in 2017) is hard to interpret. As neighbouring squares tested negative, this may be a false positive, which does occur occasionally.

The following year, 2020 (Map5), the first year of the Coronavirus Covid-19 pandemic, the project was unable to undertake testing in Central Belt areas, although some testing was possible on private land in the wider lowlands where workers on the land could work alone. Therefore, we have no data from Plean, Bearsden or Erskine. Six blood samples tested from Strathallan (NN91) were all negative.

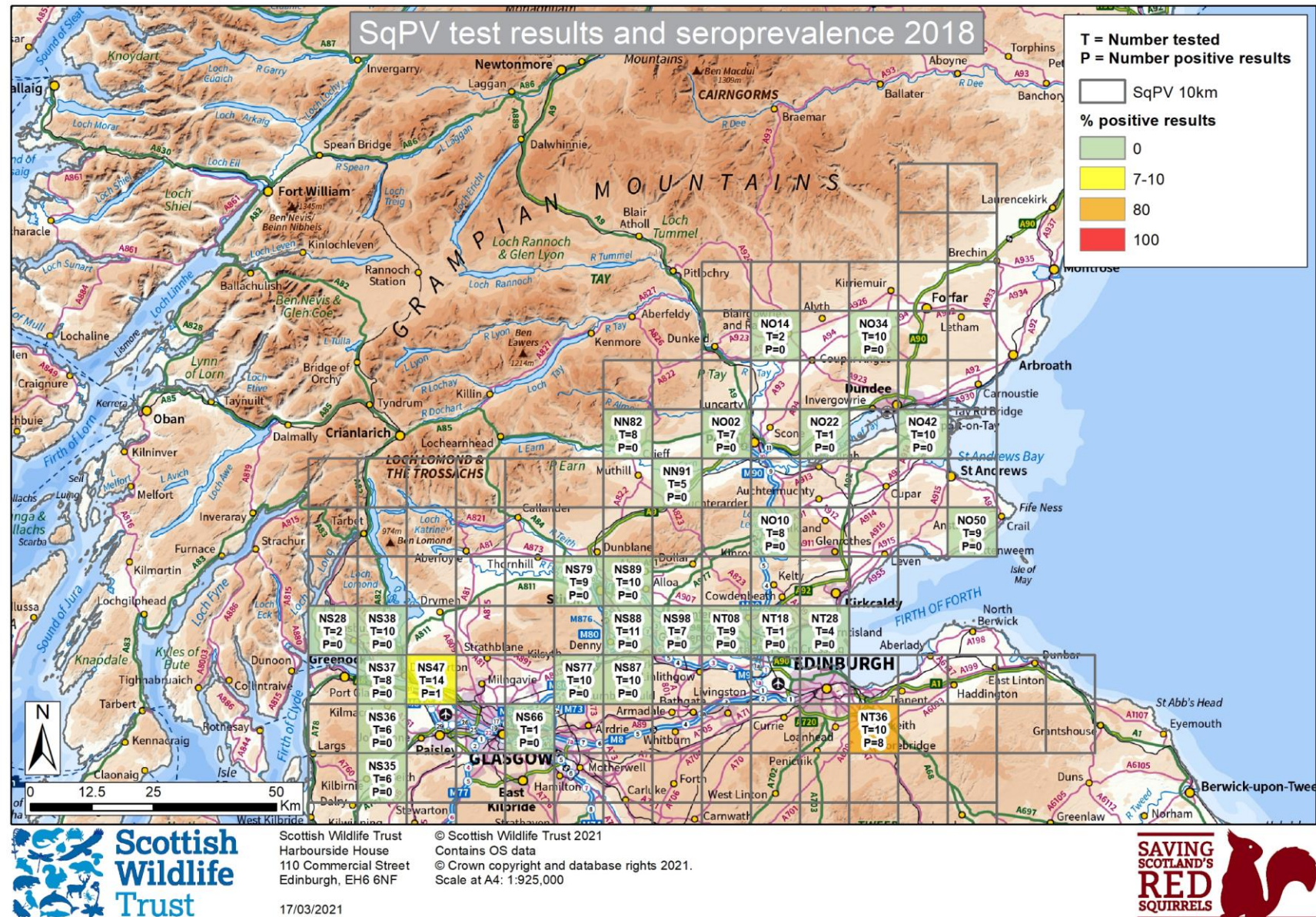
However, we did receive another anomalous result in 2020 from a red squirrel found dead in Comrie (NN72). This squirrel had apparently died in a state of acute malnourishment, according to the post mortem report, but the carcass was sent for squirrelpox electron microscopy as a routine check.

² PCR is *polymerase chain reaction*, a technique to amplify the trace of viral DNA (or RNA) and detect presence even when it occurs at very low levels.

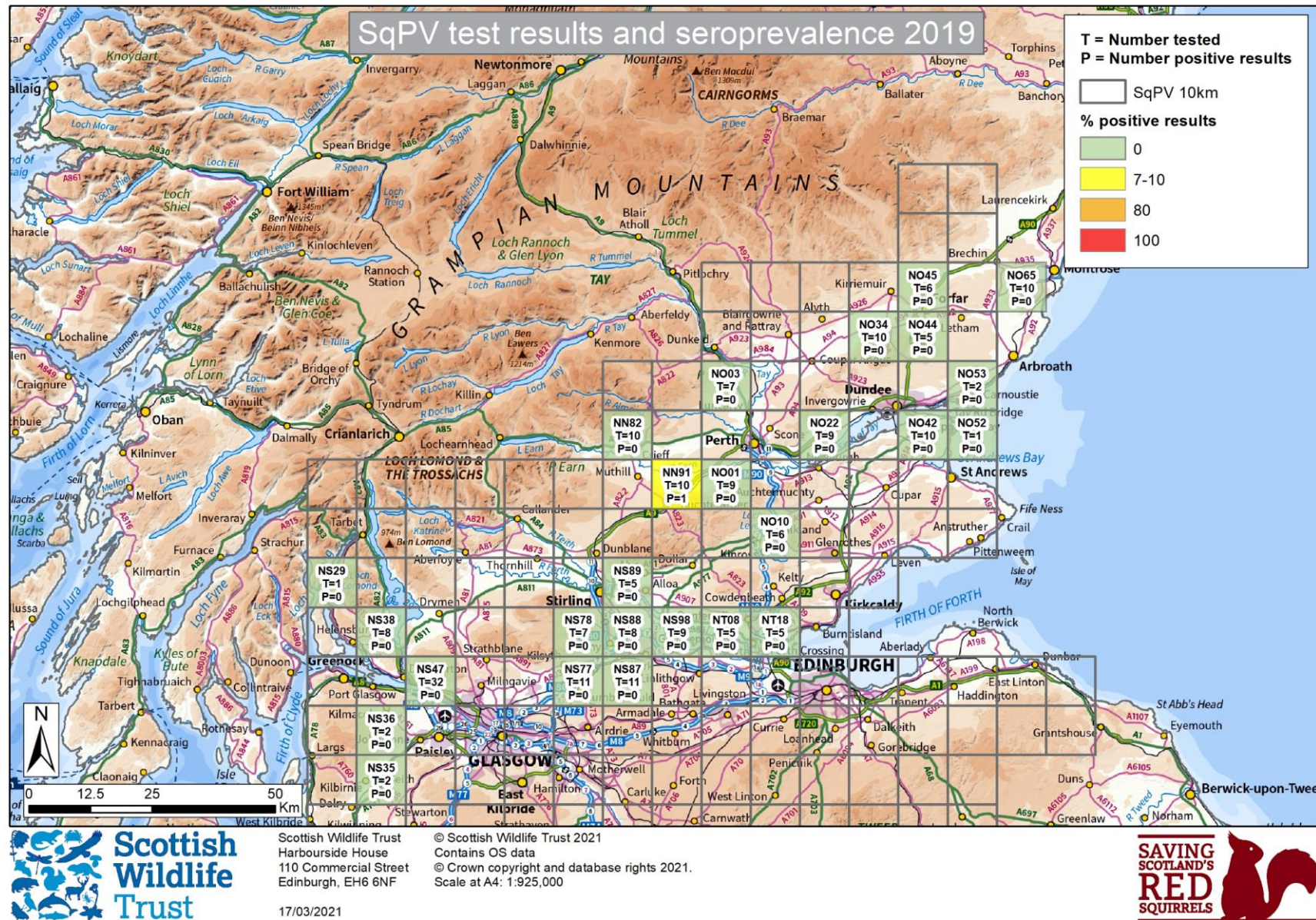
Map 2



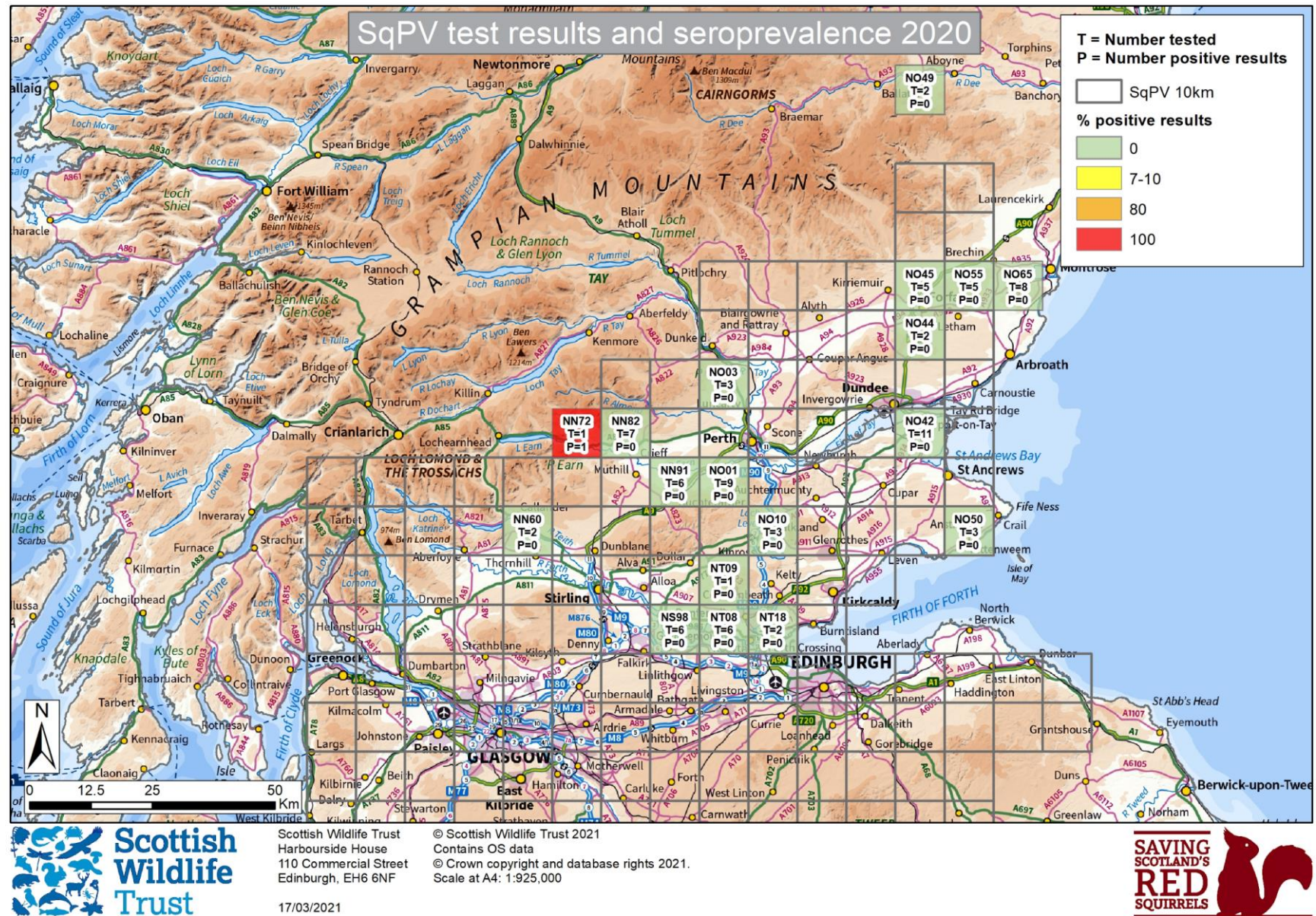
Map 3



Map 4



Map 5



The microscopy was inconclusive, so samples of whisker and tail hair were tested for squirrelpox DNA using PCR. Both samples came back as positive. This result is again very difficult to interpret. It would seem that the squirrel had at least been in contact with squirrelpox virus, but there was so far no other trace of it in the immediate landscape, the nearest previous trace having been another questionable result from NN91, about 20km away. Only further testing will allow us more confidence in interpretation. For the moment it is only possible to say that the level of threat of squirrelpox virus in Comrie, or elsewhere along the Highland Line, is likely very low.

Discussion

Squirrelpox testing over the last nine years in the Central Lowlands has been imperfect. However, all the results taken together suggest that squirrelpox virus is not being spread rapidly through the Central Lowlands grey squirrel population, as had been expected. The few sample squares that returned positive results to the testing did so at a low seroprevalence, and was not detectable at all in some years in each of these sites. It is difficult to know how to account for this. The rapid spread predicted by the mathematical modelling of White et al (2016) was based on the distribution of squirrel habitat of differing carrying capacity for grey squirrels taken from detailed forest inventory mapping by the then Forestry Commission Scotland, which ought to have been a reasonable reflection of the density of grey squirrels present, as it was in southern Scotland, where, if anything, the spread of disease was faster than predicted.

Plans by SSRS-DCA to deliver an intensive communications campaign in 2018 aimed at alerting the relevant communities in the Central Lowlands of the imminent arrival of squirrelpox among local grey and – where they occur – red squirrels, were delayed partly by other events within the project, and partly because there seemed to be no evidence of rapid disease spread, or even consistent gradual disease spread. There was a perceived danger of arousing public anxiety too far in advance of any actual increasing risk, such that vigilance might have declined by the time squirrelpox arrived in local red squirrels. However, in the last year of the SSRS-DCA project it was decided to press ahead with the information campaign as this was the last opportunity before the end of the project.

One possibility to account for the slower spread of squirrelpox north of Edinburgh-Glasgow could be that grey squirrels in the region are perhaps not reaching the carrying capacity of the local habitat, achieving only low densities, which would make it more difficult for the virus to continue circulating freely. Unfortunately, we have no figures on grey squirrel population densities, but suggest it might be possible that grey squirrels are being prevented from reaching the habitat carrying capacity due to the continuing reestablishment of the native pine marten, a variable which was not factored into the modelling of White et al (op. cit.). A report by Croose et al (2014) shows pine marten range has expanded right into the range into which we would expect squirrelpox to spread in the counties of East and West Dunbartonshire, North Lanarkshire and Falkirk. Research by Sheehy et al (2018) showed that pine marten predation reverses the well-documented outcome of resource- and apparent disease-mediated competition between red and grey squirrels. It is thus possible that grey squirrels in areas accessible to pine martens exist at densities which are too low to sustain actively circulating virus in the local population, thus slowing its spread through the landscape. Sightings on the SSRS website <https://scottishsquirrels.org.uk/squirrel-sightings/> show an increasing presence of red squirrels from 2015 to 2021 in areas like Larbert, the landscape to the north of Milngavie, and parts of coastal Fife around Dalgety Bay, which used to be home only to grey squirrels, which may also point to the impact of pine martens on grey squirrels here. Sheehy et al (op.cit.) contemplate the possibility that grey squirrels may continue to exist in urban refugia, while – with pine marten assistance – red squirrels regain former haunts in the more rural areas accessible to pine martens.

The importance of this native predator to the fortunes of the native squirrel species is already being investigated in new modelling work by Professor Andrew White and colleagues supported by the Red Squirrel Trust Wales to look at the potential impact of pine marten on the red/grey squirrel interaction in North Wales. Some preliminary results suggest that as pine martens spread, they reduce grey density to levels that cannot support squirrelpox virus (A. White, pers. comm.) and we

look forward to seeing the final results of the work and the valuable light it sheds on this critical relationship.

The mathematical models, and the scientific community in Britain at large, continue to face a large gap in our knowledge of how grey squirrels function in urban areas, a factor which will also help to determine the likely spread of squirrelpox northwards, particularly if pine marten presence in non-urban areas is shaping squirrel species presence.

Other questions exist about how we should interpret low-level squirrelpox positive results such as we have seen in the grey squirrel at Strathallan (NN91) in 2019, in the red squirrel at Comrie (NN72) in 2020 and in the grey at Loch Lomond (NS38) in 2016. The project has taken a cautious view up till now, awaiting further testing information before any publicity about them is released.

Finally, with a natural boom in grey squirrel numbers in 2020 coming at a time when the Coronavirus pandemic caused a temporary cessation of grey squirrel management work just at the most important season for trapping, squirrelpox testing results from 2021 are awaited anxiously as we may see an effect of increased grey squirrel densities on SQPV spread.

The DCA phase of SSRS is now entering its final year and the project partners are considering alternative approaches to facilitate the squirrelpox surveillance in the Central Lowlands in the future. One possibility is that the squirrelpox testing of grey squirrels could be taken over by potential future research interests investigating the rate of spread of squirrelpox in the Central Lowlands and the interaction between the squirrels species, the pine marten and the disease.

The SSRS project approach to squirrelpox in the Central Lowlands could then become more like the model currently operating in South Scotland. Here, detection is limited to public vigilance for the disease in red squirrels. The project has developed protocols to help communities and land managers respond to such disease outbreaks to minimise the impact on local red squirrels in ways that have successfully enabled healthy red squirrel populations to persist after the end of an outbreak.

We are indebted to the Moredun Research Institute for their continued contribution to the SSRS project through provision of the ELISA squirrelpox antibody testing, and to all the volunteers who assisted in collecting blood samples for us, without whom this information could not have been gathered.

Mel Tonkin
Saving Scotland's Red Squirrels Project Manager
SSRS Partnership Report
2021

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