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Project leader:	Dr Adrian Fox, Fera Science Ltd
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Key staff:	Dr Becky Howard, PGRO Shona Duffy, PGRO Aimee Fowkes, Fera Science Ltd
Location of project:	York and Peterborough, plus multiple field sites
Industry Representative:	Philip Langley, G's produce
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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Dr Adrian Fox

Senior Plant Virologist

Fera Science Ltd

Date 7/2/20

Dr Becky Howard

R&D Manager

PGRO

Date 7/2/20

Report authorised by:

Sue Cowgill

Senior Scientist Pests

AHDB

Date 7/2/20

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GROWER SUMMARY

Headline

Pea crops were surveyed using a novel approach to investigate the presence, incidence and impact of virus infections. Initial results suggest that although the expected pea viruses, such as pea enation mosaic virus were present, an unexpected virus, turnip yellows virus, was present in more crops and at greater incidence.

Background

Pea (*Pisum sativum)* is an important legume crop which is grown worldwide for consumption by humans and animals. Pea plants are also grown in rotation with cereals to help manage disease and improve fertility of the soil (Congdon et al., 2017, Coutts et al., 2008). Using peas, or other legumes, in rotation can reduce the need for application of pesticides and synthetic nitrogen fertilizer (Cernay et al., 2015). Peas can be infected with a number of viruses, and while Plant Virus Online lists 124 viruses which can infect pea, only 43 viruses were found to naturally infect pea (Brunt, 1996). Of the viruses with the potential to infect pea 27 have been previously recorded in the UK, but only seven (7) have ever been recorded from UK pea crops (Source: UK Virus Checklist, unpublished Fera data). However, there have been few surveys of viruses in pea crops in the United Kingdom (UK). In the late 1950's a survey was conducted covering England, the Netherlands, Sweden, and the former West Germany (Hagedorn, 1958). The UK aspect of this work covered 14 fields in England and reported the presence of 'enation mosaic' (14/14 fields affected), 'mosaic' (4/14), 'streak' (10/14), 'top yellows' (6/14) and 'stunt' (1/14). Although these reports were based purely on observed symptoms, and virus symptoms may be confused with other biotic and abiotic stresses (Latham & Jones, 2001), they give an indication of the prevalence of virus diseases in pea crops at the time. Most other pea viruses recorded in the UK have been the result of testing small numbers of samples, again as the result of diagnostic testing following symptom observation. The current virus health status of UK pea crops in unknown.

Recent surveys of leguminous crops in Europe have identified a new genus of virus, the genus *Nanovirus*, Family *Nanoviridae*. Viruses from this genus have been reported from legumes including clover, black medic, milk vetch, faba bean and pea. Several of these viruses have been reported to infect peas, including pea necrotic yellow dwarf virus (PNYDV), pea yellow stunt virus (PYSV), and faba bean necrotic stunt virus (FBNSV and black medic leaf roll virus (BMLRV) (Grigoras et al., 2014, Grigoras et al., 2010). Of these pea necrotic yellow dwarf virus has been shown to have spread throughout Germany and into the

Netherlands and Denmark (Gaafar et al., 2017, Gaafar et al., 2018). However, to date nanoviruses have not been recorded from UK crops.

The aim of this work is to develop a generic survey technique, which could be applied in any crop, but using pea as an initial model crop. The approach uses an integrated diagnostics approach linking screening of large bulked samples using a non-targeted approach (high-throughput sequencing; HTS) to ascertain the presence/absence of viruses, and this is supported with back testing using a targeted approach (real-time RT-PCR) to ascertain the incidence of viruses in fields which were detected in the initial screening tests. At the end of the season a sub-set of fields were sampled to assess the impact of virus infection on crops.

Summary

20 pea crops were identified for sampling representing a broad geographic spread across UK pea growing regions. Samples were collected from 100m x 100m grid, with a plant sampled at random, regardless of symptom status of each plant, at each grid intersection, giving 121 sampling points. These plants were combined to make a large bulk sample. On arrival at the laboratory these samples were sub-divided to allow for two different testing approaches. One whole-crop bulk sample was prepared and nucleic acids (RNA) extracted. This sample was then screened for the presence of viruses using High-throughput sequencing. This technique analyses all the nucleic acid present in a sample and should, in theory, detect the presence of any virus present in the sample. The other part of the sample was divided into random sub-samples, consisting of 15 individual leaves, and 15 lots of 7 leaves. RNA was extracted from these samples and stored for subsequent testing for the viruses indicated to be present in the initial screening work. The combined results of both these tests are presented in table 1.

Table1. Results of both HTS screening and real-time RT_PCR testing showing presence and incidence of viruses from 20 pea fields. Estimate results are a calculated % mean virus content based on the number of bulk samples positive for virus, nt = Not Tested

Site	HTS result	TuYV Result Estimate	PEMV1 Result Estimate	SbDV Result Estimate
1	Negative	nt	nt	nt
2	Negative	nt	nt	nt
3	Negative	nt	nt	nt
4	Negative	nt	nt	nt
5	Negative	nt	nt	nt
6	TuYV	12.46	nt	nt
7	Negative	nt	nt	nt

8	PEMV1 PEMV2	nt	27.44	nt
9	TuYV	1.71	nt	nt
10	TuYV	6.76	nt	nt
11	TuYV	60.62	nt	nt
12	TuYV PEMV2	9.7	0.85	nt
13	TuYV PEMV2	21.8	0	nt
14	TuYV PEMV2 SbDV	93.33	nt	1.71
15	TuYV PEMV1 PEMV2 SbDV	2.64	0.85	4.53
16	TuYV PEMV1 PEMV 2 PEMV Sat	8	3.72	nt
17	TuYV	6.98	nt	nt
18	TuYV PEMV Sat	93.33	nt	nt
19	TuYV PEMV 2	80.01	nt	nt
20	TuYV PEMV1 PEMV 2 PEMV Sat	14.29	30.09	nt

13 of the 20 crops tested were positive for virus infections ranging in incidence from 0.85% to 93.33% estimated infection. One of the pea viruses which has been historically reported as being present in the UK, pea enation mosaic virus, was shown to be present in 5 crops, ranging from 0.85% to 30.09% virus infection. Pea enation mosaic is actually a complex infection of two viruses (PEMV1 and PEMV2), however, this was only present as a 'single' infection in one crop, at 27.44% infection. More commonly detected, and present at higher incidence, was turnip yellows virus. This virus ranged in incidence from 1.71% to 93.33% virus and was present in 12 of the 20 crops tested. This finding represents a first report of TuYV in peas in the UK, although the virus has been reported in pea crops elsewhere in Europe. Additionally, the virus soybean dwarf virus was also detected in two of the 20 crops tested. This represents a first record of this virus in the UK. Where detected the virus was present at low incidence, and further testing for this virus will be conducted in the second and third year of the project.

To compare the novel survey approach being used with a visual approach focused on symptomatic samples, further symptomatic samples were submitted for testing from a range of pea crops both within the above survey and from other crops. These were treated as individual samples, but were subjected to the same testing regime as previously described, with an initial screen for the presence of viruses using HTS, and subsequent confirmatory testing carried out using real-time RT-PCR. The results of this testing can be seen in table 2.

Table 2. Single symptomatic samples submitted for screening from UK pea fields

Location	Variety	PEMV1	PEMV2	PEMV Satellite	PSbMV	TuYV	BYMV	Novel CABY associated-like
A	TBC							+
B	TBC	+	+			+		
B	TBC		+			+		
C	TBC	+	+		+	+		+
D	TBC	+	+		+	+		
D	TBC		+			+		
E	TBC	+	+	+		+	+	
F	TBC	+	+		+	+		
F	TBC	+	+	+				
G	Ashton		+			+		
G	Ashton	+	+			+		
G	Ashton	+	+			+		
G	Ashton	+	+			+		
G	Ashton	+	+			+		
G	Ashton	+	+			+		
G	Ashton	+	+			+		
G	Ashton	+	+			+		
H	Oasis	+	+	+		+		
H	Oasis	+	+	+		+		
H	Oasis	+	+	+		+		
H	Oasis	+	+	+		+		
H	Oasis	+	+	+		+		
H	Oasis	+	+	+		+		
I	Kimberley		+			+		
I	Kimberley		+			+		
I	Kimberley		+			+		
J	Oasis					+		
J	Oasis					+		

The results of this testing (Table 2) support the conclusions from the general field survey that TuYV is present in a greater number of crops than PEMV. Further viruses were found to be present in these symptomatic samples, including expected viruses such as pea seed-borne mosaic virus and bean yellow mosaic virus. One further novel virus-like sequence was also detected from two samples, which appears to be genetically most closely related to cucurbit aphid-borne yellows associated RNA. This sequence will be the subject of some further work in the coming year of the project to ascertain the nature of this finding. Given these samples

were taken on the basis of expressing symptoms, work would need to be carried out to investigate which of these viruses, or which combination of viruses was causing the observed symptom in the plant.

Further work was also conducted to investigate the potential impact of virus infection in crops. In each sampled crop an area was marked out and this was left 'untreated', compared to the rest of the field which received treatment to mitigate against aphids, the vectors of many of the viruses causing issues in pea crops. From the HTS screening work, five of these crops were identified for further study, and at the end of the growing season these were sampled and assessments of yield were made in both the treated and untreated areas of the field. From these data a statistical analysis (linear regression) was performed to estimate the impact of virus infection (Figure 1).

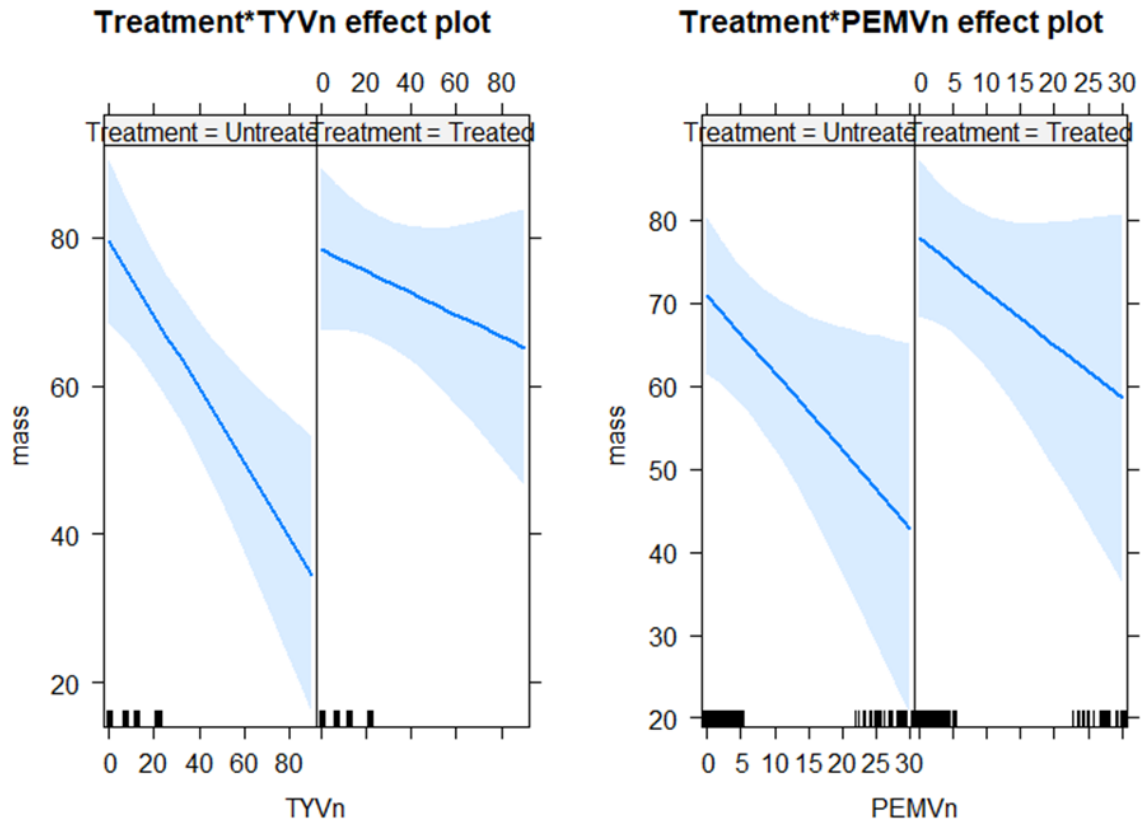

Figure 1. Estimated effects of virus prevalence of TuYV (labelled TYVn) and PEMV1 (labelled PEMVn) and treatment on productivity.

This analysis indicates that TuYV could impact yield of a crop by an estimated 44% against an uninfected crop (CL 19%-67%), and "treatment" would ameliorate this to around 81% of the yield of an uninfected crop. PEMV was also estimated to have a potential yield impact, but neither the impact of virus or the effect of treatment was statistically significant. It is vital

to note that these effects are based on limited dataset from a single year of a three-year study and will be further investigated in the next two growing seasons.

Financial Benefits

As this is the first year of a three-year study there are no financial benefits to be reported at this stage.

Action Points

As this is a first year, interim report, action points for growers will be formulated following the results of year two of the study.

SCIENCE SECTION

Introduction

Pea (*Pisum sativum)* is an important legume crop which is grown worldwide for consumption by humans and animals. Pea plants are also grown in rotation with cereals to help manage disease and improve fertility of the soil (Congdon et al., 2017, Coutts et al., 2008). Using peas, or other legumes, in rotation can reduce the need for application of pesticides and synthetic nitrogen fertilizer (Cernay et al., 2015). Peas can be infected with a number of viruses, and while Plant Virus Online lists 124 viruses which can infect pea, only 43 viruses were found to naturally infect pea (Brunt, 1996). Of the viruses with the potential to infect pea 27 have been previously recorded in the UK, but only seven (7) have ever been recorded from UK pea crops (Source: UK Virus Checklist, unpublished Fera data). However, there have been few surveys of viruses in pea crops in the United Kingdom (UK). In the late 1950's a survey was conducted covering England, the Netherlands, Sweden, and the former West Germany (Hagedorn, 1958). The UK aspect of this work covered 14 fields in England and reported the presence of 'enation mosaic' (14/14 fields affected), 'mosaic' (4/14), 'streak' (10/14), 'top yellows' (6/14) and 'stunt' (1/14). Although these reports were based purely on observed symptoms, and virus symptoms may be confused with other biotic and abiotic stresses (Latham & Jones, 2001), they give an indication of the prevalence of virus diseases in pea crops at the time. Most other pea viruses recorded in the UK have been the result of testing small numbers of samples, again as the result of diagnostic testing following symptom observation. The current virus health status of UK pea crops in unknown.

Recent surveys of leguminous crops in Europe have identified a new genus of virus, the genus *Nanovirus*, Family *Nanoviridae*. Viruses from this genus have been reported from legumes including clover, black medic, milk vetch, faba bean and pea. Several of these viruses have been reported to infect peas, including pea necrotic yellow dwarf virus (PNYDV), pea yellow stunt virus (PYSV), and faba bean necrotic stunt virus (FBNSV and black medic leaf roll virus (BMLRV) (Grigoras et al., 2014, Grigoras et al., 2010). Of these pea necrotic yellow dwarf virus has been shown to have spread throughout Germany and into the Netherlands and Denmark (Gaafar et al., 2017, Gaafar et al., 2018). However, to date nanoviruses have not been recorded from UK crops.

Further afield, Australian researchers have had a greater focus on pea crops. This has largely focussed on mitigating the effects of pea seed-borne mosaic virus (Congdon et al., 2017, Coutts et al., 2008). However, some survey work had been carried out, which suggested that luteoviruses may be present in pea crops at a higher incidence than previously recognised (Wilson et al., 2012). Although viruses from this family, such as turnip yellows virus (TuYV)

and Bean leaf roll virus (BLRV) have been recorded from peas, the incidence of these viruses in UK and EU crops is unknown.

To date most virus surveillance work carried out on any crop follows a general formulaic approach, namely: Identify a suite of viruses likely to be present in the crop; collect samples from a number of fields based on likely symptoms; test these for the pre-selected suite of viruses using targeted diagnostics such as ELISA or PCR based methods. This approach gives limited information as it can only report on the known viruses, it leaves open questions about the identity of the causal agent of symptoms where a plant is sampled but tests negative for virus, and arguably, on this any test which is 'negative' could be considered to be wasted diagnostic resource. High-Throughput sequencing (HTS) is a technique that has been in development for plant pathology applications for around ten years. It gives a method for testing for the presence of the total genetic sequence contained in a sample, which can then be compared to known sequences to give an indication of the presence of a suspected pathogen. Thus far it has been primarily used for either screening germplasm or for single sample diagnosis where conventional diagnostics has failed to give a result, but is now being applied to landscape-scale ecology studies (Maree et al., 2018, Adams et al., 2018). However, it has not yet been applied in a plant health surveillance scenario. The aim of this project is to use an integrated approach linking HTS and conventional diagnostic methods to give a generic method for carrying out a survey for the presence of viruses in a crop, using UK pea crops as model system, where the final result is both a measure of the presence and incidence of viral pathogens. Additionally, the aim is to investigate the impact of these viral pathogens on crop production.

Materials and methods

1. *Presence and incidence of viruses in pea crops - Sampling*
    1.1. 20 pea crops were sampled for the presence of pea-infecting viruses. Sampled crops are shown in table 1. Samples were taken c. 6 weeks prior to harvest to give a measure of viruses from seed-sources and to account for those likely to be present following early aphid migrations.
    1.2. 120 individual plants were sampled at random along a 100m x 100m grid at 10m intervals (Fox et al., 2017). Additional meta-data was also recorded including location, variety, etc.
    1.3. In addition to the random sample, up to three (3) individual plants exhibiting symptoms consistent with virus infection were submitted for confirmatory testing of virus presence.

Table 1. Sites of pea crops sampled during Summer 2019

Site	Location	Variety
1	Ancaster	Anubis
2	Low Habberly	SV1022
3	Long Sutton	Tomahawk
4	Lincoln	Anubis
5	Woodbridge	Bartesa (PP)
6	Adisham	Combining Pea
7	Brechin	Tomahawk
8	Broughton	Combining Pea
9	East Riding	Swallow
10	Ulceby	EBBA
11	Chatteris	Vidor
12	Langtoft	Amalfi
13	Louth	Realm
14	Market Weighton	Ashton
15	Perth	TBC
16	Wainfleet All Saints	Oasis
17	Stoneleigh	Vidor/Ambassador
18	Bedingfield	Kimberley
19	Langton	Oasis
20	Chirnside Borders	Boogie

2. *Presence and incidence of viruses in pea crops – Laboratory testing*

2.1. On submission to the laboratory, the 120 randomly sampled plants were sub-sampled, and a composite bulked sample of all the sampled plants in each field was tested by HTS (Whole crop bulk). This initial non-target screen identified candidate pathogens for subsequent incidence testing.

2.2. Whole crop bulk samples were tested by HTS, with appropriate quality procedures, and resulting data were analysed in accordance with previously published methods (Adams et al., 2014, Fox et al., 2019)

2.2.1. Viruses inferred from HTS data were assigned provisional taxonomic placement and identified as candidate viruses for confirmation testing through mixed bulk testing (See 2.3)

2.3. Concurrently, whole crop samples were sub divided into smaller bulks for downstream confirmation testing by real-time RT-PCR. This sub-division consisted of 15 lots of 7 leaves, and a further 15 individual leaves (Mixed bulks)

2.3.1. RNA was extracted from mixed bulks using Kingfisher magnetic bead extraction in accordance with manufacturer's instructions

2.3.2. Mixed bulks from crops shown to contain virus infection were tested for the specific candidate viruses indicated to be present in those crops by real-time RT-PCR, using existing published diagnostic assays where possible. These will be used as part validated tests and validation was at the systems level with multiple methods being used to confirm the presence of candidate viruses (Roenhorst et al., 2018).

2.3.3. The virus incidence in a sample was inferred from interpretation of bulked sample test results (see table in Appendix 1).

2.4. Individual symptomatic samples will be tested in parallel to the bulk samples detailed above to give additional intelligence on the viruses present in pea crops. These were extracted and tested by HTS as detailed above.

3. Impact assessment of pea infecting viruses

3.1. Using the information obtained through incidence testing detailed above, 5 crops were identified for further study. Crops were assessed at harvest to give estimates of yield loss due to High/Moderate/Low levels of virus incidence in the crop.

3.2. At all sites an area 10m x 10m was marked within the sampling area. The area remained untreated, with no aphicides applied, to allow maximum potential yield loss from virus infection to be measured. The remaining crop was treated with standard insecticide applications by the grower.

3.3. At five selected sites 16 small plots, 1m x 1m were harvested from within the 10m x 10m area, and 16 from outside the area to compare yield from the commercial crop against yield from the untreated area. Plants from those small plots were returned to PGRO and threshed using a static vining machine or combine harvester. Five (5) pea sites were harvested. Yield was measured for all 5 sites.

3.3.1. Maturity was measured for vining peas using a tenderometer machine to give a TR score, and moisture content recorded for peas that were combined.

3.3.2. Vining peas were size graded to give an additional measure of quality. Additional sub-samples of seeds were taken from all sites and assessed for symptoms of PSbMV, a virus that produces tissue scarring on the surface of the seed.

Results

Combining the results for the single sample and whole crop bulk HTS indicated the presence of six (6) viruses and a satellite RNA. Five of these were expected and are known to be common in peas in the UK as indicated by the previous literature review (AHDB FV 453). These were:

- Pea enation mosaic virus 1 (PEMV1), genus *Enamovirus*
- Pea enation mosaic virus 2 (PEMV2), genus *Umbravirus*
- Pea enation mosaic virus satellite
- Pea seed-borne mosaic virus (PSbMV), genus *Potyvirus*
- Bean yellow mosaic virus (BYMV), genus *Potyvirus*

Two viruses which had not been previously recorded in pea crops in the UK were also detected in both single symptomatic samples and from bulked field samples, and these were:

- Turnip yellows virus (TuYV), genus *Polerovirus*
- Soybean dwarf virus (SbDV), genus *Luteovirus*

Additionally, sequence fragments of a potentially novel virus like associated RNA were also detected, listed here as cucurbit aphid-borne yellows associated RNA-like (CABY-like).
Pea seed-borne virus and Bean yellow mosaic virus were not detected during the randomised field survey, but only from testing individual symptomatic plants.

Presence and Incidence in Pea crops

The viruses inferred in samples from the whole crop bulk HTS analysis are listed in table 2, along with the relative incidence of the three viruses which were tested for in mixed bulk samples: turnip yellows virus, soybean dwarf virus and pea enation mosaic virus 1. In total 13 crops out of 20 had detectable levels of virus (65%). Total virus content ranged from 1.7% up to 93.3%. Five of 20 crops contained a single virus (25%), whereas 8 crops contained multiple virus infections (40%). Generally, there was a pattern of lower virus incidence and content earlier in the season. Five (5) crops out of 20 were found to contain PEMV1, where detected the mean incidence of PEMV1 was 12.59% (0.85% - 30.09%). More commonly detected, and present at a higher incidence, was the virus TuYV, present in 12 of 20 crops (60%). The incidence of TuYV ranged from 1.71% - 93.33% (avg. where detected 34.3%). Soybean dwarf virus was present at low incidence in two crops with an incidence of 1.71% and 4.53%.

Table 2. Viruses inferred from whole crop bulk HTS data and mean estimated virus content from the accompanying mixed bulk testing. nt=not tested

Site	Variety	HTS result	TuYV Result Estimate	PEMV1 Result Estimate	SbDV Result Estimate
1	Anubis	Negative	nt	nt	nt
2	SV1022	Negative	nt	nt	nt
3	Tomahawk	Negative	nt	nt	nt
4	Anubis	Negative	nt	nt	nt
5	Bartesa (PP)	Negative	nt	nt	nt
6	Combining Pea (TBC)	TuYV	12.46	nt	nt
7	Tomahawk	Negative	nt	nt	nt
8	Combining Pea	PEMV1 PEMV2	nt	27.44	nt
9	Swallow	TuYV	1.71	nt	nt
10	EBBA	TuYV	6.76	nt	nt
11	Vidor	TuYV	60.62	nt	nt
12	Amalfi	TuYV PEMV2	9.7	0.85	nt
13	Realm	TuYV PEMV2	21.8	0	nt
14	Ashton	TuYV PEMV2 SbDV	93.33	nt	1.71
15	TBC	TuYV PEMV1 PEMV2 SbDV	2.64	0.85	4.53
16	Oasis	TuYV PEMV1 PEMV 2 PEMV Sat	8	3.72	nt
17	Vidor/Ambassador	TuYV	6.98	nt	nt
18	Kimberley	TuYV PEMV Sat	93.33	nt	nt
19	Oasis	TuYV PEMV 2	80.01	nt	nt
20	Boogie	TuYV PEMV1 PEMV 2 PEMV Sat	14.29	30.09	nt

Presence of virus in single symptomatic samples

The viruses detected in single symptomatic samples by HTS are presented in table 3. TuYV was the virus most commonly detected, with PEMV 2 the second most commonly detected virus. PEMV2 was detected in more samples than the virus PEMV1 which is the recognised helper virus for transmission of PEMV2. Pea seed-borne mosaic virus was only detected from samples at three sites, and bean yellow mosaic virus was detected from a single sample. Soybean dwarf virus was not detected from any of the samples submitted under this part of the study, however, small fragments of sequence of a novel virus-like RNA resembling cucurbit aphid-borne yellows associated RNA were detected in two samples.

Table 3. Virus present in single symptomatic samples inferred from HTS data.

Location	Variety	PEMV1	PEMV2	PEMV Satellite	PSbMV	TuYV	BYMV	Novel CABY associated-like
A	TBC							+
B	TBC	+	+			+		
B	TBC		+			+		
C	TBC	+	+		+	+		+
D	TBC	+	+		+	+		
D	TBC		+			+		
E	TBC	+	+	+		+	+	
F	TBC	+	+		+	+		
F	TBC	+	+	+				
G	Ashton		+			+		
G	Ashton	+	+			+		
G	Ashton	+	+			+		
G	Ashton	+	+			+		
G	Ashton	+	+			+		
G	Ashton	+	+			+		
G	Ashton	+	+			+		
G	Ashton	+	+			+		
H	Oasis	+	+	+		+		
H	Oasis	+	+	+		+		
H	Oasis	+	+	+		+		
H	Oasis	+	+	+		+		
H	Oasis	+	+	+		+		
H	Oasis	+	+	+		+		
I	Kimberley		+			+		
I	Kimberley		+			+		
I	Kimberley		+			+		
J	Oasis					+		
J	Oasis					+		

Impact of virus infection

Five crops with a range of virus content were sampled to assess the impact of virus infection both with and without treatment. These crops were crop 6, crop 8, crop 13, crop 17 and crop 18. Estimated effects are shown in Figure 1 and estimates for the effect of virus prevalence and treatments in the population, expressed as the 10-plant pea mass, were gained via a parametric bootstrap of the fitted model.

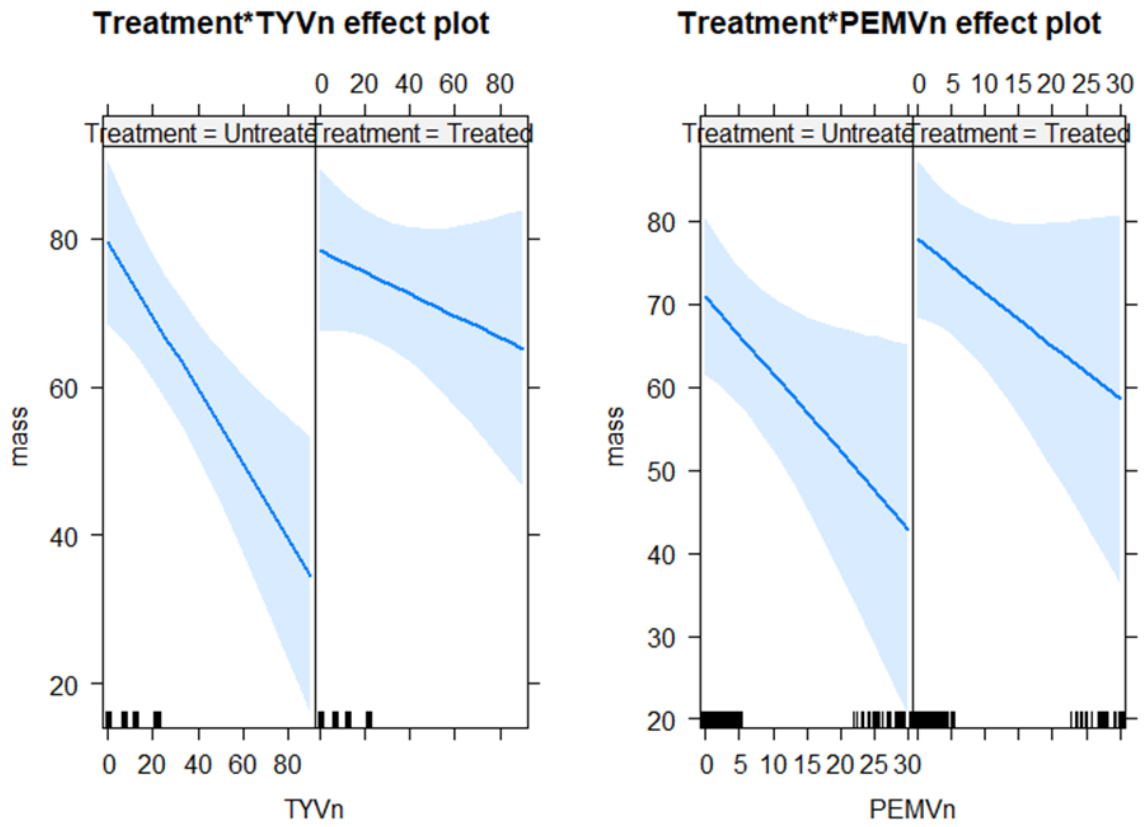

Figure 2. Estimated effects of virus prevalence of TuYV (labelled TYVn) and PEMV1 (labelled PEMVn) and treatment on productivity.

Estimates of effect size of virus infection and treatment are shown in Table 4. TuYV was found to significantly reduce productivity ($p<0.001$) in fields, and this effect appears to be ameliorated by treatment ($p>0.001$). PEMV1 was also found to significantly reduce productivity ($p<0.011$) however treatment did not appear to ameliorate this effect ($p<0.18$). However, it must be stressed that this analysis is preliminary based on an extremely limited data set and further analysis of these effects will be investigated throughout the course of the project.

Table 4. Estimates of effect sizes and significance of treatment and TuYV and PEMV1

Value	Estimated effect	95% C.I.		Significance
10-plant pea mass in clean untreated fields	85g	73g	97g	
Effect of treatment[a]	97%	88%	106%	0.240[b]
Effect of TuYV[a]	44%	19%	67%	<0.001[c]
Effect of TuYV and treatment[a]	81%	55%	108%	<0.001[d]
Effect of PEMV[a]	70%	47%	96%	0.011[e]
Effect of PEMV and treatment[a]	76%	53%	100%	0.180[f]

[a] Expected population average 100-pea mass in this type of field expressed as a proportion of expected 10-plant pea mass in equivalent (same sites) virus free untreated fields

[b] Null hypothesis: treatment increases the 10-plant pea mass

[c] Null hypothesis: TUYV presence increases the 10-plant pea mass

[d] Null hypothesis: treatment reduces the 10-plant pea mass when TUYV is present

[e] Null hypothesis: PEMV presence increases the 10-plant pea mass

[f] Null hypothesis: treatment reduces the 10-plant pea mass when PEMV is present

Discussion

The traditional approach to carrying out crop surveys would involve carrying out a literature search for potential candidate viruses that may be detected including a suite of common, unusual and those not yet present. From this list of candidate pathogens a suite of 'target' viruses would be selected and these would be tested for using a range of conventional, targeted diagnostic tests such as ELISA, PCR and real-time PCR. The generic applicability of this approach is then limited by the range of viruses 'in test' with no information given on novel or unexpected viruses not previously reported from the host. In a crop where there is limited information about the viruses likely to be encountered it is challenging to develop the suite of potential candidate viruses for testing. For example, if designing a panel of diagnostic tests for surveying pea crops, would the list of targets include the seven viruses previously recorded in the UK on peas, the 27 viruses from the UK record which have been reported on pea elsewhere, or some of the non-UK pea viruses? Each of these decisions would incur an additional diagnostic cost. The aim of this work was to investigate the feasibility of using HTS as generic virus screen to identify candidate viruses, which could then be confirmed through downstream testing by conventional diagnostic methods.

Using HTS revealed the presence of turnip yellows virus (TuYV) in pea crops in the UK. This virus has been previously reported to infect peas (Graichen & Rabenstein, 1996, Stevens *et al.*, 2008), but not from the UK. It was surprising that this virus was present in more crops, and at higher incidence, than pea enation mosaic virus 1 (PeMV1). It was also surprising that

soybean dwarf virus (SbDV) was found to be present, although this was only in a limited number of crops and at a low incidence where recorded. This is a first finding from the UK, from a recent report from Germany (Gaafar & Ziebell, 2019). From discussions with the authors of this report it is likely that other luteoviridae and associated viruses may be found in pea crops throughout this survey. The overall approach of sequencing a large bulk sample to identify pathogen candidates therefore appears to be sound, in that the pathogens identified through the sequencing work were then confirmed through follow up testing, validating the findings at the systems level (Roenhorst *et al*., 2018).

However, it is difficult to assess what may be "missed" in this process. Missed infections may arise from two sources, sampling, and analytical sensitivity. Using a sample size of 120 leaves would give a 95% confidence of detecting approximately 3% virus incidence in the crop. This means that any finding with an incidence below this level would be detected by chance in the sampling. The consequence of this would be that setting the level of sampling would be dependent upon the surveillance/research question. For quality pathogens, likely to be present at moderate to high incidence, this low-intensity sampling is adequate, however, for emerging pathogens likely to be present at lower incidence, then higher intensity sampling should be considered. For example, a 3000-leaf sample would give an estimated 95% confidence of detecting a 0.1% infection level. Analytical sensitivity is more difficult to quantify with HTS. Recent work has suggested around 1 million sequence reads should be adequate to detect a whole viral genome (Visser *et al.*, 2016). Other research has suggested that as few as 50,000 sequence reads may be enough to detect the presence of a virus in a sample (Pecman *et al*., 2017). However, due to the nature of the extraction and sample enrichment approaches used in HTS this does not equate simply to 'number of samples' bulked in a sequencing library. The sample preparation process used at Fera involves a step to remove plant ribosomal RNA, enriching the sample for viral RNA. However, the presence of SbDV as fragments of sequence rather than whole genomes, and the possible miss of a low concentration PEMV1 in a single sample suggests that the process used here is near to the limit of detection for low levels of virus. Again, dependent upon the research question being asked (and the available budget) greater sequencing depth per sample may reveal a greater diversity of low concentration viruses. This will be investigated further during year 2 of this project.

The testing of single symptomatic samples revealed many of the same viruses as detected through the bulk field sampling. However, pea seed-borne mosaic virus and bean yellow mosaic virus, two viruses which could have been expected to be present in crops, were detected in few individual samples, suggesting these viruses are present a low incidence of the viruses, but not a major issue in crops as a whole.

Preliminary data were also gathered on the potential impacts of infection on crops. Given the design of the surveillance aspect of the project, a 'true' controlled experiment to measure yield reduction could not be carried out. Within the project therefore, areas of crop were demarcated and 'treatment' for aphid vectors was not carried out within these areas. At the end of the season multiple plants were sampled from treated and untreated areas and the yields from these compared. These preliminary data, from a limited number of sites, suggest an ameliorating effect on yield from 'treated' areas in crops infected with TuYV. Some limited ameliorating effect was also measured in relation to the presence of PEMV1. However, due to the limited number of sample sites these data should be considered preliminary and conclusions should not be drawn based on the current data set. However, these data have been used to develop a working hypothesis that treatment to control vectors will have an ameliorating effect on yield in relation to virus infected crops.

Conclusions

- This generic approach to surveillance work appears to be effective, and peas appear to be a successful model crop for this work
- Two viruses, turnip yellows virus and soybean dwarf virus, have been reported to infect UK pea crops for the first time as a result of these data.
- Treatment to limit virus vector aphids may have an ameliorating effect on the yield impact of pea infecting viruses
- These conclusions will be further tested in future years of the project

Knowledge and Technology Transfer

The following activities have been undertaken as KE activity:

Presentations:

- 08/02/2019 – Becky Howard presented an introduction to the project to the HMC Peas grower group and requested volunteers to provide sites
- 13/02/2019 – Becky Howard presented an introduction to the project to the Dengie Crops grower group meeting and requested volunteers to provide sites
- 08/03/2019 – Becky Howard presented an introduction to the project to the Swaythorpe Growers group meeting and requested volunteers to provide sites
- 12 and 13/06/2019 – Becky Howard presented a poster describing the project objectives at the Cereals 2019 event

- 02/07/2019 – Becky Howard presented the same poster at the PGRO Pulse Open Day at Stubton, Lincolnshire
- 10/10/2019 – Adrian Fox presented the work as part of a broader talk on new diagnostic technologies to the BCPC Workshop on pathogens, NIAB, Cambridge.
- 5/11/2019 – Adrian Fox presented the year 1 project results at the Pea and Bean growers conference, Peterborough.
- 19/11/2019 – Becky Howard presented an update of the project to Velcourt Farming managers
- 21/01/2020 – Adrian Fox presented the work as part of a broader talk on diagnostic technologies to Hutchinsons vegetable conference

Publications:

- Pulse magazine (Winter 2019)

Glossary

BYMV	Bean yellow mosaic virus
CABY	Cucurbit aphid-borne yellows virus
HTS	High throughput sequencing
PEMV	Pea enation mosaic virus
PSbMV	Pea seedborne mosaic virus
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase Polymerase chain reaction
SbDV	Soybean dwarf virus
TuYV	Turnip yellows virus

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Appendix 1. Table for interpretation of bulk test results

Bulk size1	Bulk size2	No. positive1	No. positive2	**estimate of incidence**	lower 95% CI	upper 95% CI
1	7	0	0	0	0	2.47
1	7	1	0	0.83	0.02	3.37
1	7	2	0	1.67	0.21	4.69
1	7	3	0	2.5	0.54	6.18
1	7	4	0	3.33	0.98	7.67
1	7	5	0	p<0.001	p<0.001	p<0.001
1	7	6	0	p<0.001	p<0.001	p<0.001
1	7	7	0	p<0.001	p<0.001	p<0.001
1	7	8	0	p<0.001	p<0.001	p<0.001
1	7	9	0	p<0.001	p<0.001	p<0.001
1	7	10	0	p<0.001	p<0.001	p<0.001
1	7	11	0	p<0.001	p<0.001	p<0.001
1	7	12	0	p<0.001	p<0.001	p<0.001
1	7	13	0	p<0.001	p<0.001	p<0.001
1	7	14	0	p<0.001	p<0.001	p<0.001
1	7	15	0	p<0.001	p<0.001	p<0.001
1	7	0	1	0.85	0.02	4.66
1	7	1	1	1.71	0.21	5.16
1	7	2	1	2.57	0.54	6.25
1	7	3	1	3.42	0.98	7.68
1	7	4	1	4.28	1.5	9.18
1	7	5	1	p<0.001	p<0.001	p<0.001
1	7	6	1	p<0.001	p<0.001	p<0.001
1	7	7	1	p<0.001	p<0.001	p<0.001
1	7	8	1	p<0.001	p<0.001	p<0.001
1	7	9	1	p<0.001	p<0.001	p<0.001
1	7	10	1	p<0.001	p<0.001	p<0.001
1	7	11	1	p<0.001	p<0.001	p<0.001
1	7	12	1	p<0.001	p<0.001	p<0.001
1	7	13	1	p<0.001	p<0.001	p<0.001
1	7	14	1	p<0.001	p<0.001	p<0.001
1	7	15	1	p<0.001	p<0.001	p<0.001
1	7	0	2	1.76	0.24	6.17
1	7	1	2	2.64	0.55	6.81
1	7	2	2	3.52	0.98	7.81
1	7	3	2	4.4	1.5	9.2
1	7	4	2	5.28	2.1	10.73
1	7	5	2	6.17	2.76	12.35
1	7	6	2	p<0.001	p<0.001	p<0.001

1	7	7	2	p<0.001	p<0.001	p<0.001
1	7	8	2	p<0.001	p<0.001	p<0.001
Bulk size1	Bulk size2	No. positive1	No. positive2	**estimate of incidence**	lower 95% CI	upper 95% CI
1	7	9	2	p<0.001	p<0.001	p<0.001
1	7	10	2	p<0.001	p<0.001	p<0.001
1	7	11	2	p<0.001	p<0.001	p<0.001
1	7	12	2	p<0.001	p<0.001	p<0.001
1	7	13	2	p<0.001	p<0.001	p<0.001
1	7	14	2	p<0.001	p<0.001	p<0.001
1	7	15	2	p<0.001	p<0.001	p<0.001
1	7	0	3	2.71	0.63	7.67
1	7	1	3	3.62	1.01	8.43
1	7	2	3	4.53	1.51	9.41
1	7	3	3	5.44	2.1	10.78
1	7	4	3	6.35	2.76	12.36
1	7	5	3	7.27	3.49	14.05
1	7	6	3	p<0.001	p<0.001	p<0.001
1	7	7	3	p<0.001	p<0.001	p<0.001
1	7	8	3	p<0.001	p<0.001	p<0.001
1	7	9	3	p<0.001	p<0.001	p<0.001
1	7	10	3	p<0.001	p<0.001	p<0.001
1	7	11	3	p<0.001	p<0.001	p<0.001
1	7	12	3	p<0.001	p<0.001	p<0.001
1	7	13	3	p<0.001	p<0.001	p<0.001
1	7	14	3	p<0.001	p<0.001	p<0.001
1	7	15	3	p<0.001	p<0.001	p<0.001
1	7	0	4	3.72	1.14	9.18
1	7	1	4	4.66	1.55	10.07
1	7	2	4	5.6	2.11	11.06
1	7	3	4	6.55	2.76	12.43
1	7	4	4	7.49	3.49	14.07
1	7	5	4	8.44	4.04	15.39
1	7	6	4	9.4	4.61	16.61
1	7	7	4	p<0.001	p<0.001	p<0.001
1	7	8	4	p<0.001	p<0.001	p<0.001
1	7	9	4	p<0.001	p<0.001	p<0.001
1	7	10	4	p<0.001	p<0.001	p<0.001
1	7	11	4	p<0.001	p<0.001	p<0.001
1	7	12	4	p<0.001	p<0.001	p<0.001
1	7	13	4	p<0.001	p<0.001	p<0.001
1	7	14	4	p<0.001	p<0.001	p<0.001
1	7	15	4	p<0.001	p<0.001	p<0.001
1	7	0	5	4.81	1.74	10.73

1	7	1	5	5.78	2.19	11.76
1	7	2	5	6.76	2.79	12.8
Bulk size1	Bulk size2	No. positive1	No. positive2	**estimate of incidence**	lower 95% CI	upper 95% CI
1	7	3	5	7.74	3.5	14.18
1	7	4	5	8.72	4.3	15.89
1	7	5	5	9.71	4.97	17.38
1	7	6	5	10.7	5.33	18.09
1	7	7	5	p<0.001	p<0.001	p<0.001
1	7	8	5	p<0.001	p<0.001	p<0.001
1	7	9	5	p<0.001	p<0.001	p<0.001
1	7	10	5	p<0.001	p<0.001	p<0.001
1	7	11	5	p<0.001	p<0.001	p<0.001
1	7	12	5	p<0.001	p<0.001	p<0.001
1	7	13	5	p<0.001	p<0.001	p<0.001
1	7	14	5	p<0.001	p<0.001	p<0.001
1	7	15	5	p<0.001	p<0.001	p<0.001
1	7	0	6	5.97	2.43	12.35
1	7	1	6	6.98	2.91	13.53
1	7	2	6	8	3.55	14.64
1	7	3	6	9.02	4.31	16.06
1	7	4	6	10.05	4.97	17.43
1	7	5	6	11.09	5.61	18.75
1	7	6	6	12.13	6.38	20.3
1	7	7	6	13.18	7.18	21.98
1	7	8	6	p<0.001	p<0.001	p<0.001
1	7	9	6	p<0.001	p<0.001	p<0.001
1	7	10	6	p<0.001	p<0.001	p<0.001
1	7	11	6	p<0.001	p<0.001	p<0.001
1	7	12	6	p<0.001	p<0.001	p<0.001
1	7	13	6	p<0.001	p<0.001	p<0.001
1	7	14	6	p<0.001	p<0.001	p<0.001
1	7	15	6	p<0.001	p<0.001	p<0.001
1	7	0	7	7.22	3.2	14.05
1	7	1	7	8.28	3.71	15.39
1	7	2	7	9.35	4.39	16.61
1	7	3	7	10.42	5.21	18.08
1	7	4	7	11.5	5.99	19.6
1	7	5	7	12.59	6.72	21.08
1	7	6	7	13.69	7.55	22.75
1	7	7	7	14.8	7.99	23.66
1	7	8	7	p<0.001	p<0.001	p<0.001
1	7	9	7	p<0.001	p<0.001	p<0.001
1	7	10	7	p<0.001	p<0.001	p<0.001

1	7	11	7	p<0.001	p<0.001	p<0.001
1	7	12	7	p<0.001	p<0.001	p<0.001
Bulk size1	Bulk size2	No. positive1	No. positive2	**estimate of incidence**	lower 95% CI	upper 95% CI
1	7	13	7	p<0.001	p<0.001	p<0.001
1	7	14	7	p<0.001	p<0.001	p<0.001
1	7	15	7	p<0.001	p<0.001	p<0.001
1	7	0	8	8.59	4.05	15.86
1	7	1	8	9.7	4.61	17.37
1	7	2	8	10.83	5.33	18.73
1	7	3	8	11.96	6.2	20.3
1	7	4	8	13.1	7.1	21.98
1	7	5	8	14.26	7.56	22.81
1	7	6	8	15.43	8.46	24.64
1	7	7	8	16.6	9.39	26.56
1	7	8	8	17.79	9.91	27.65
1	7	9	8	p<0.001	p<0.001	p<0.001
1	7	10	8	p<0.001	p<0.001	p<0.001
1	7	11	8	p<0.001	p<0.001	p<0.001
1	7	12	8	p<0.001	p<0.001	p<0.001
1	7	13	8	p<0.001	p<0.001	p<0.001
1	7	14	8	p<0.001	p<0.001	p<0.001
1	7	15	8	p<0.001	p<0.001	p<0.001
1	7	0	9	10.09	5	17.84
1	7	1	9	11.27	5.62	19.52
1	7	2	9	12.46	6.38	21.05
1	7	3	9	13.67	7.31	22.75
1	7	4	9	14.89	7.99	23.92
1	7	5	9	16.12	8.88	25.61
1	7	6	9	17.38	9.89	27.65
1	7	7	9	18.64	10.49	28.84
1	7	8	9	19.93	11.31	30.51
1	7	9	9	21.23	12.28	32.52
1	7	10	9	p<0.001	p<0.001	p<0.001
1	7	11	9	p<0.001	p<0.001	p<0.001
1	7	12	9	p<0.001	p<0.001	p<0.001
1	7	13	9	p<0.001	p<0.001	p<0.001
1	7	14	9	p<0.001	p<0.001	p<0.001
1	7	15	9	p<0.001	p<0.001	p<0.001
1	7	0	10	11.76	6.03	19.96
1	7	1	10	13.02	6.74	21.85
1	7	2	10	14.29	7.58	23.66
1	7	3	10	15.59	8.46	25.29
1	7	4	10	16.91	9.39	26.94

1	7	5	10	18.25	10.33	28.66
1	7	6	10	19.61	11.27	30.51
Bulk size1	Bulk size2	No. positive1	No. positive2	**estimate of incidence**	lower 95% CI	upper 95% CI
1	7	7	10	20.99	12.27	32.52
1	7	8	10	22.4	13.33	34.75
1	7	9	10	23.83	14.5	37.23
1	7	10	10	p<0.001	p<0.001	p<0.001
1	7	11	10	p<0.001	p<0.001	p<0.001
1	7	12	10	p<0.001	p<0.001	p<0.001
1	7	13	10	p<0.001	p<0.001	p<0.001
1	7	14	10	p<0.001	p<0.001	p<0.001
1	7	15	10	p<0.001	p<0.001	p<0.001
1	7	0	11	13.64	7.18	22.29
1	7	1	11	15	8.09	24.63
1	7	2	11	16.39	8.93	26.56
1	7	3	11	17.8	9.91	28.49
1	7	4	11	19.25	10.98	30.44
1	7	5	11	20.72	12.1	32.5
1	7	6	11	22.23	13.25	34.74
1	7	7	11	23.77	14.46	37.23
1	7	8	11	25.34	14.5	37.25
1	7	9	11	26.95	15.79	40.04
1	7	10	11	28.6	17.23	43.25
1	7	11	11	p<0.001	p<0.001	p<0.001
1	7	12	11	p<0.001	p<0.001	p<0.001
1	7	13	11	p<0.001	p<0.001	p<0.001
1	7	14	11	p<0.001	p<0.001	p<0.001
1	7	15	11	p<0.001	p<0.001	p<0.001
1	7	0	12	15.8	8.77	25.51
1	7	1	12	17.3	9.57	27.62
1	7	2	12	18.84	10.49	29.9
1	7	3	12	20.41	11.56	32.22
1	7	4	12	22.03	12.79	34.63
1	7	5	12	23.69	14.13	37.19
1	7	6	12	25.41	14.5	37.64
1	7	7	12	27.17	15.79	40.2
1	7	8	12	28.99	17.23	43.31
1	7	9	12	30.88	18.46	46.18
1	7	10	12	32.82	19.94	49.53
1	7	11	12	34.83	20.81	51.57
1	7	12	12	p<0.001	p<0.001	p<0.001
1	7	13	12	p<0.001	p<0.001	p<0.001
1	7	14	12	p<0.001	p<0.001	p<0.001

1	7	15	12	p<0.001	p<0.001	p<0.001
1	7	0	13	18.36	10.41	28.83
Bulk size1	Bulk size2	No. positive1	No. positive2	**estimate of incidence**	lower 95% CI	upper 95% CI
1	7	1	13	20.05	11.31	31.14
1	7	2	13	21.8	12.32	33.83
1	7	3	13	23.61	13.51	36.73
1	7	4	13	25.49	14.61	39.07
1	7	5	13	27.44	15.84	41.4
1	7	6	13	29.48	17.25	44.08
1	7	7	13	31.6	18.86	47.4
1	7	8	13	33.83	19.94	49.73
1	7	9	13	36.16	21.6	53.34
1	7	10	13	38.6	23.54	57.63
1	7	11	13	41.16	25.86	62.62
1	7	12	13	43.85	25.86	62.62
1	7	13	13	46.65	28.63	68.04
1	7	14	13	p<0.001	p<0.001	p<0.001
1	7	15	13	p<0.001	p<0.001	p<0.001
1	7	0	14	21.52	12.28	32.64
1	7	1	14	23.5	13.34	35.3
1	7	2	14	25.58	15.19	39.99
1	7	3	14	27.77	16.27	42.99
1	7	4	14	30.09	17.53	46.16
1	7	5	14	32.56	19.03	49.52
1	7	6	14	35.18	20.81	53.27
1	7	7	14	38	22.9	57.61
1	7	8	14	41.02	25.36	62.62
1	7	9	14	44.26	25.86	63.16
1	7	10	14	47.74	28.63	68.23
1	7	11	14	51.46	31.93	73.55
1	7	12	14	55.41	35.86	78.76
1	7	13	14	59.56	35.89	78.77
1	7	14	14	63.87	40.54	83.67
1	7	15	14	68.28	46.02	88.18
1	7	0	15	25.7	15.76	40.03
1	7	1	15	28.2	17.09	43.25
1	7	2	15	30.91	18.47	47.02
1	7	3	15	33.88	20.01	51.57
1	7	4	15	37.15	21.63	56.58
1	7	5	15	40.8	23.55	62.15
1	7	6	15	44.91	26.02	68.04
1	7	7	15	49.57	28.72	73.5
1	7	8	15	54.81	31.98	78.75

1	7	9	15	60.62	35.9	83.67
1	7	10	15	66.87	40.54	88.18
Bulk size1	Bulk size2	No. positive1	No. positive2	**estimate of incidence**	lower 95% CI	upper 95% CI
1	7	11	15	73.38	46.02	92.21
1	7	12	15	80.01	52.38	95.67
1	7	13	15	86.67	59.69	98.34
1	7	14	15	93.33	68.08	99.83
1	7	15	15	100	78.2	100