

Turkey coronavirus and astrovirus in Britain



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TCoV in North America

TCoV: an acute and highly contagious enteric disease resulting in economic losses



Spiking mortality manifestation of PEMS in USA

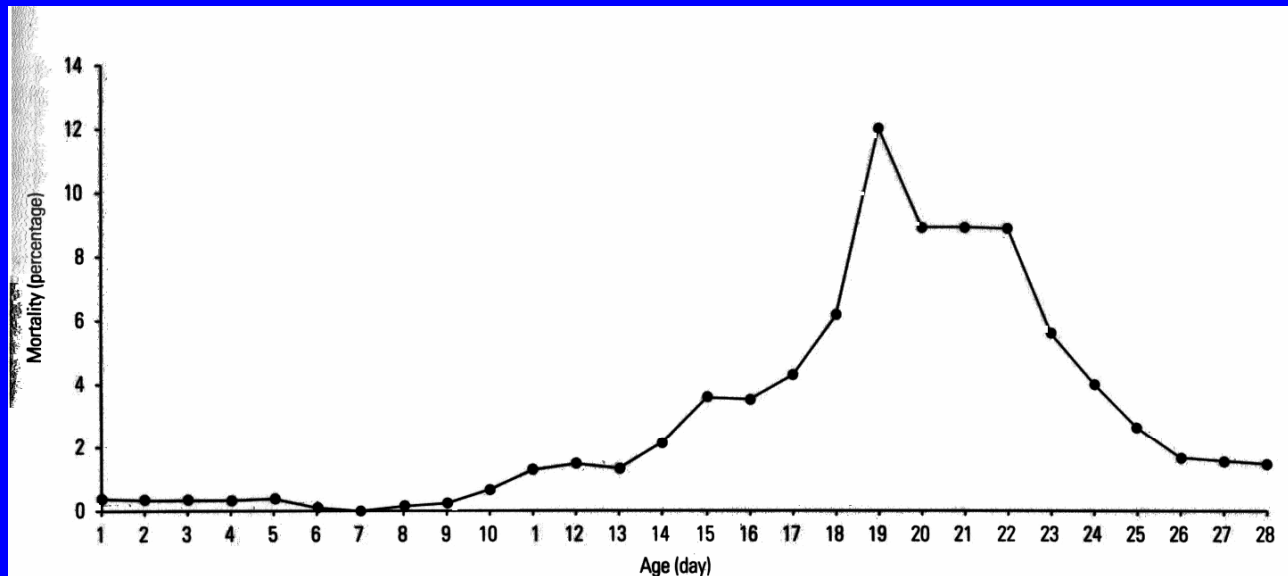


Fig. 1

Poult enteritis mortality syndrome

Typical 'spiking' mortality pattern of a severely affected flock in western North Carolina in 1996. Day nineteen was the peak day of mortality when almost 12% of the flock died. Ordinarily, such a flock would have been killed, but this flock was kept for sampling and study until 28 days of age. Cumulative mortality at that time had reached 82.3%. Performance of the subsequent flock was normal after depopulation, cleaning and disinfection. The disease recurred the following summer, but was not sufficiently severe for the flock to be destroyed. No further flocks have been affected and production continues on the farm.

Barnes, Guy & Vaillancourt (2000)

Economic consequences - U.S.

Mortality may be very high (dependent upon other factors e.g. bacteria or viral load).

In most cases:

- average livability down from 93 to 86%.
- poor feed conversion.
- poor weight gain and final weights.
- increased medication.

TCoVs in U.S. and beyond...

- TCoVs and TAstVs often associated with disease in US, proven experimentally.
- Astrovirus also important in US; immunosuppressive.
- TCoV was 1st associated with disease outside of U.S. in Britain in 2001.
- IBV-based RT-PCRs can be used for TCoV detection.

Confirmation of TCoV in Britain

Avian Pathology (2001) **30**, 355–368



Detection of a coronavirus from turkey poults in Europe genetically related to infectious bronchitis virus of chickens

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Intestinal contents of 13-day-old turkey poults in Great Britain were analysed as the birds showed stunting, unevenness and lameness, with 4% mortality. At *post mortem* examination, the main gross features were fluid caecal and intestinal contents. Histological examination of tissues was largely unremarkable, apart from some sections that showed crypt dilation and flattened epithelia. Negative contrast electron microscopy of caecal contents revealed virus particles, which in size and morphology had the appearance of a coronavirus. RNA was extracted (turkey/UK/412/00) and used in a number of reverse transcription-polymerase chain reactions (RT-PCRs) with the oligonucleotides based on sequences derived from avian infectious bronchitis virus (IBV), a coronavirus of domestic fowl. The RT-PCRs confirmed that turkey/UK/412/00 was a coronavirus and, moreover, showed that it had the same partial gene order (S-E-M-5-N-3' untranslated region) as IBV. This gene order is unlike that of any known mammalian coronavirus, which does not have a gene analogous to the gene 5 of IBV. The gene 5 of the turkey virus had two open reading frames, 5a and 5b, as in IBV and the coronaviruses isolated from turkeys in North America. The turkey/UK/412/00 also resembled IBV, but not mammalian coronaviruses, in having three open reading frames in the gene encoding E protein (gene 3). The percentage differences between the nucleotide sequences of genes 3 and 5 and the 3' untranslated region of turkey/UK/412/00 when compared with those of IBVs were similar to the differences observed when different strains of IBV were compared with each other. No sequences unique to the turkey isolates were identified. These results demonstrate, for the first time, that a coronavirus was associated with disease in turkeys outside of North America and that it is a Group 3 coronavirus, like IBV.

Confirmation of TCoV in Britain

- 13 day-old poults
- 20% showed stunting, unevenness and lameness.
- 4% died.
- Caecal & intestinal contents were fluid.

- ~96% nucleotide identity in the 3' UTR with the U.S. turkey isolates and with IBV isolates from many countries.

- no unique sequences that distinguished the turkey from the chicken isolates.

Genetically
like IBV



Objectives of project

1. What is the frequency of TCoV and TAstV in flocks across England and Wales?
2. Is TCoV and/or TAstV associated with disease?
3. At what age are birds infected with TCoV and TAstV?
4. What percentage of scouring (one-off) cases are associated with TCoV and TAstV?
5. How many genotypes of TCoVs and TAstVs are circulating in Britain?

Field studies

- **Longitudinal studies**
 - samples taken on a weekly basis from 1 d o to 20 weeks, at a specific site.
- **One-off (acute) samples**
 - taken randomly from birds of any age when there is scouring.
 - Sera were also collected at day old, 10 weeks and 20 weeks from 10 birds and pooled.

Lab organisation

Room 1:

reagent assembly,
oligonucleotide preparation.



PCR cabinet (room 1):

RNA extraction,
RT-PCR.



PCR cabinet (room 2):

Open tube & remove sample
for electrophoresis,
set up sequencing reaction.



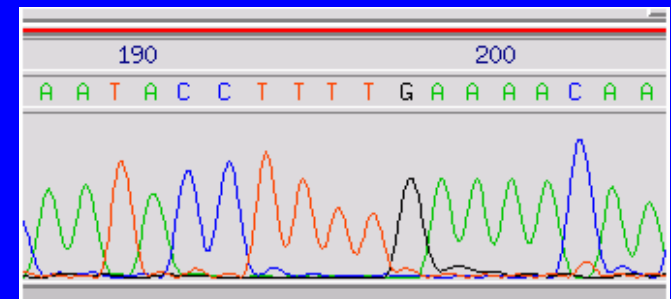
Room 2:

PCR product analysis.



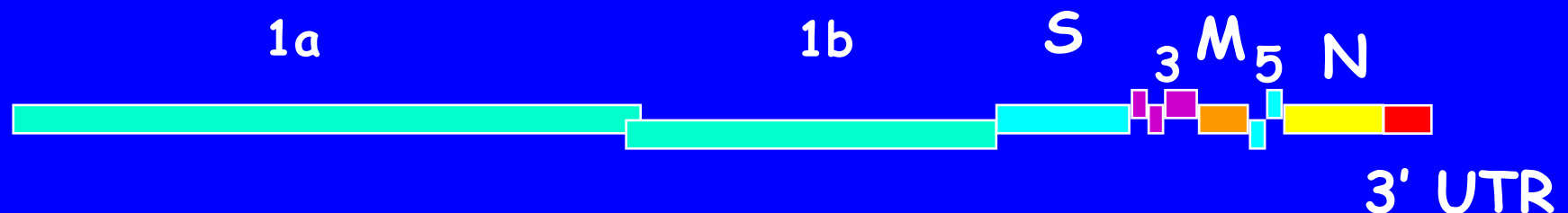
RT-PCR approach

- Processing of samples (300 +)
 - RNA extraction (Qiagen Stool extraction kit)
 - RT-PCR - universal oligos
 - Sensitivity of RT-PCR
 - Internal standard control
-
- Sequencing of all field-positive samples



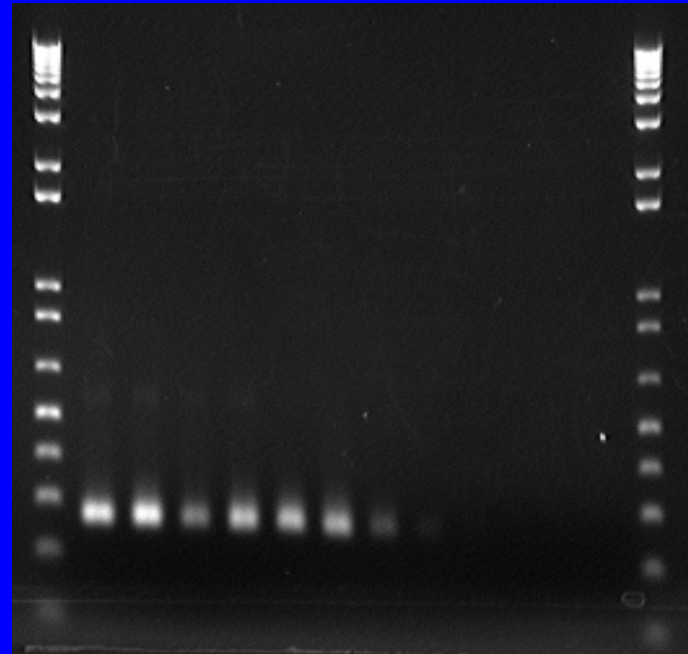
RT-PCR for TCoV detection

- The chosen oligonucleotides are able to detect TCoV in faeces.
- Oligonucleotides for 3' UTR are based on many IBV serotypes and published TCoV seqs.
- Oligonucleotides for gene 1 are universal CoV oligos.

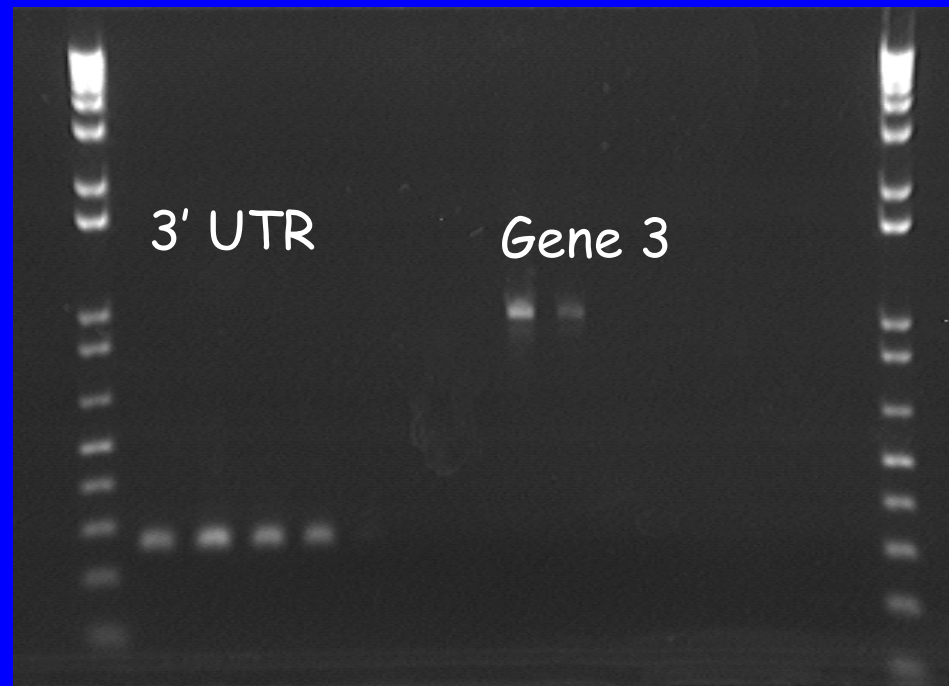
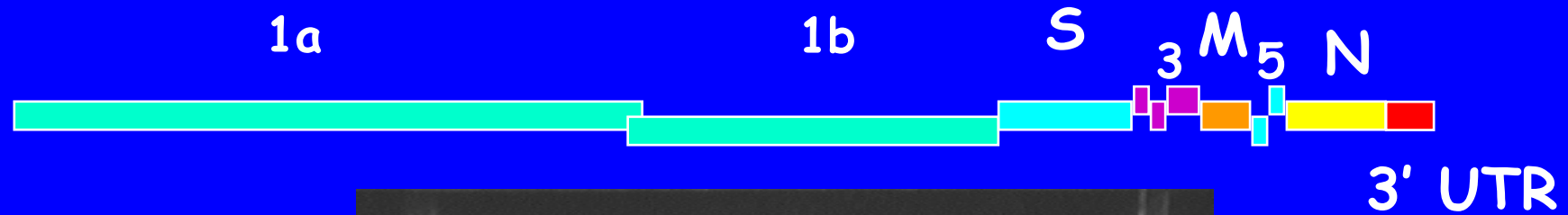


Sensitivity of RT-PCR

- By adding various dilutions of known amount of infectious virus (determined by plaque assays) to faeces
 - RNA extraction
 - RT-PCR.
- Approx. 1 plaque forming unit of virus can be detected.



3'UTR vs gene 3 oligos



Oligonucleotides in 3' UTR are more sensitive.

Generation of internal standard (IS RNA)

PCR product (250 nt)



Deletion (50 nt) introduced by PCR mutagenesis



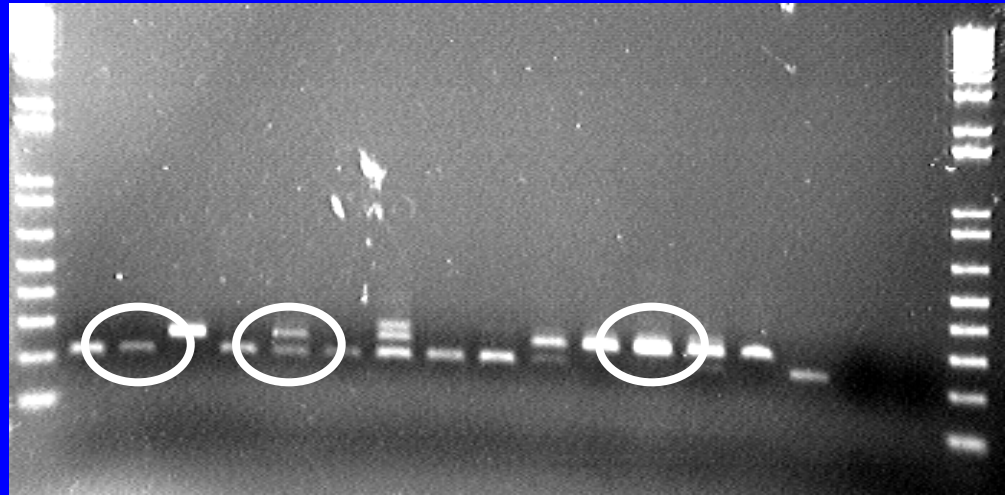
DNA (200 nt) inserted into plasmid vector



RNA (200 nt) transcribed from plasmid DNA



IS RNA used as template in RT-PCR



RT-PCR for virus detection

- RNA extraction: procedure removes PCR inhibitors - QIAamp DNA stool kit (Qiagen).
- Internal standard (IS) RNA: controls for false negative results. Co-amplification with field virus - PCR inhibitors successfully removed during RNA extraction.
- RT-PCR can detect ~ 1 plaque forming unit of infectious virus.
- Oligonucleotides in 3' UTR are most sensitive (TCoV).
- Oligonucleotides in polymerase gene are used for TAsTV detection.

Results - TCoV



One-off (scouring) samples:

11/34 flocks tested were TCoV-positive.

Longitudinal samples:

24/145 samples tested were TCoV-positive

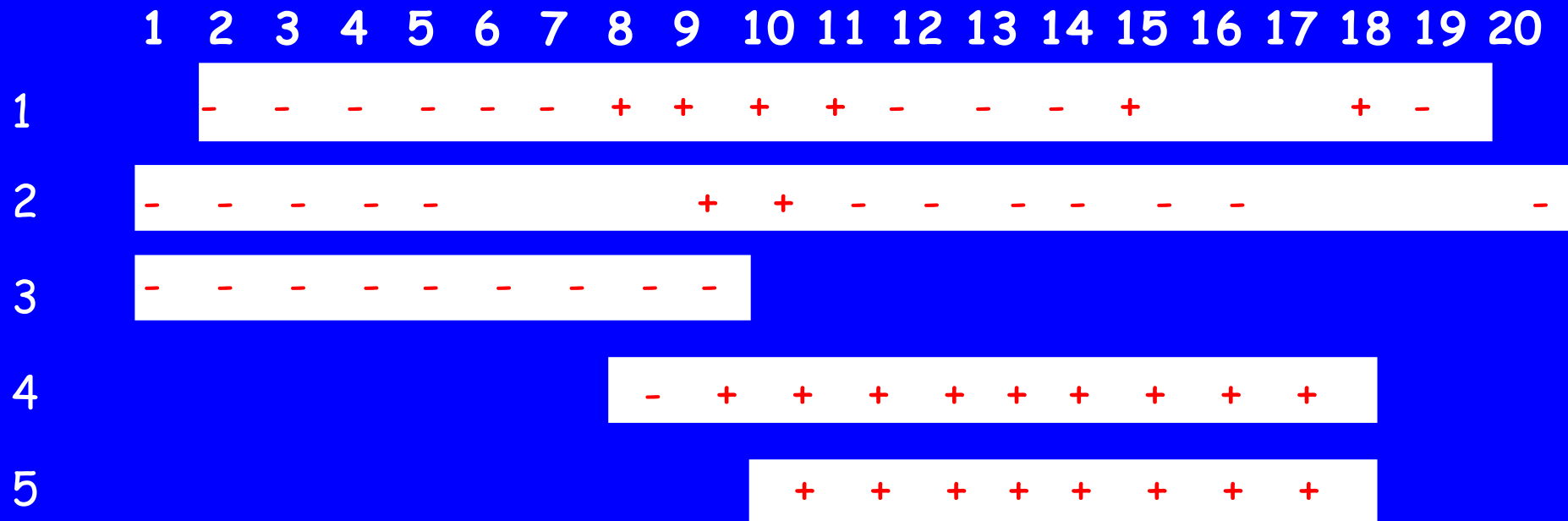
(6/17 flocks tested TCoV-positive)

All positive samples were identified in birds between 3 and 18 weeks of age.

TCoV Results - longitudinal studies



Weeks of age

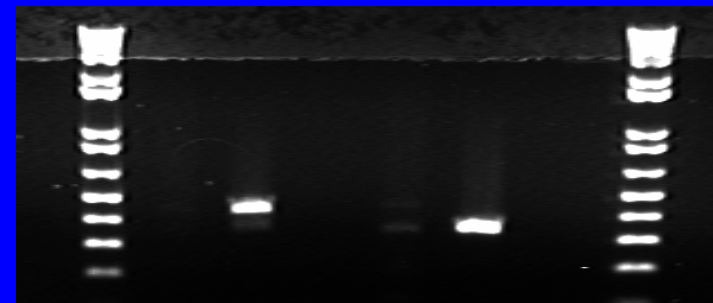
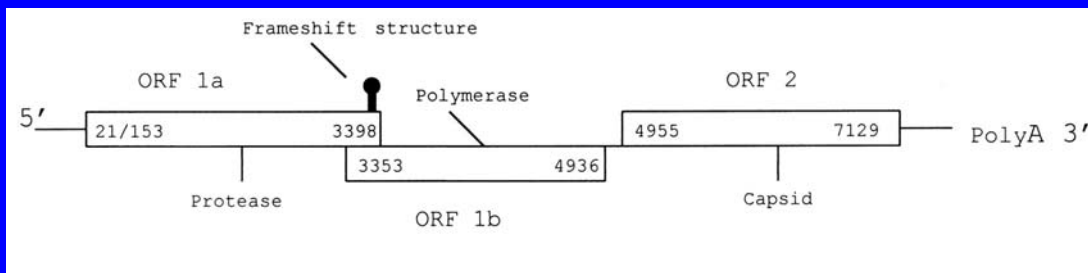


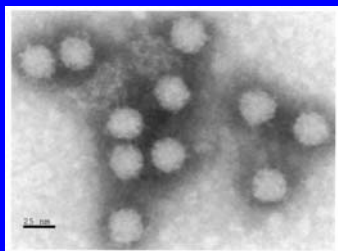
TCoV in Britain

- TCoV detected in birds between 3 and 18 weeks of age.
- 76% positive samples were in birds ≥ 6 weeks old.
- TCoV has been identified only in scouring flocks.
- 22% of longitudinal flocks were positive for TCoV.
- 33% of one-off samples from scouring flocks were positive for TCoV.
- Number of genotypes of TCoV in Britain?

RT-PCR for TAstV detection

- The chosen oligonucleotides are able to detect TAstV in faeces.
- Oligonucleotides for polymerase gene are based on sequences of avian and mammalian AstV (Nick Knowles).





Results - TAsTV

One-off samples:

13/36 samples tested were TAsTV-positive.

Longitudinal samples:

10/17 flocks tested were TAsTV-positive

All positive samples were identified in birds between 1 week and 12 weeks of age.

TAstV in Britain

- TAstV detected in birds between 1 week and 12 weeks of age.
- TAstV has not been identified in diseased flocks only but birds are usually unwell - depressed etc.
- 59% of longitudinal flocks were positive for TAstV.
- 36% of one-off samples from scouring flocks were positive for TAstV.

Summary

- **TCoV** has been identified in birds 3 to 18 weeks old.
- All TCoV positive samples were identified from flocks with enteric disease.
- Sequencing 3'UTR: up to 95% nt identity with other TCoV and IBV strains.

- **TAstV** has been identified in birds 1 week to 12 weeks old
- TAstV-positive flocks: birds were usually unwell e.g. scouring, depressed, seeking heat, off their food.
- Approximately 90% nt identity with other TAstVs.

Other work

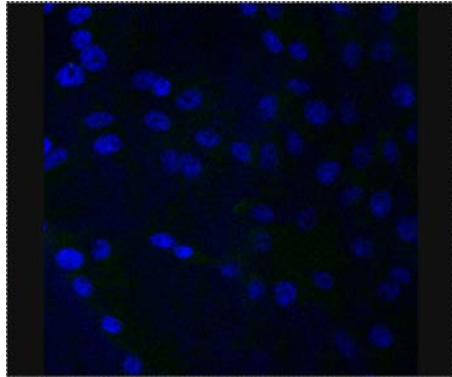
- Assess sera for TCoV antibodies
(to corroborate RT-PCR evidence and because antibodies may persist longer than the virus).
- Sequence other genes - are all TCoVs in the UK essentially the same or different strains?

Serological studies - by immunofluorescence

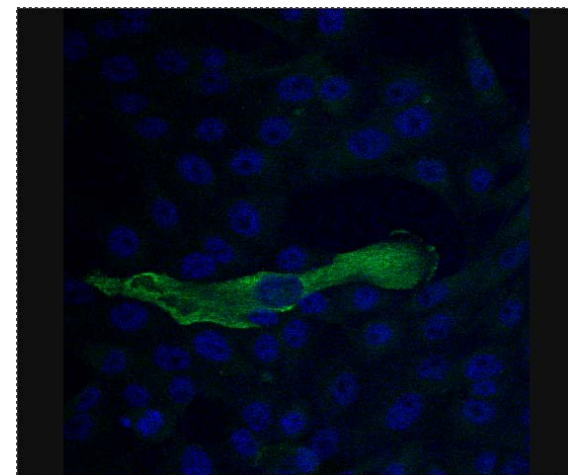
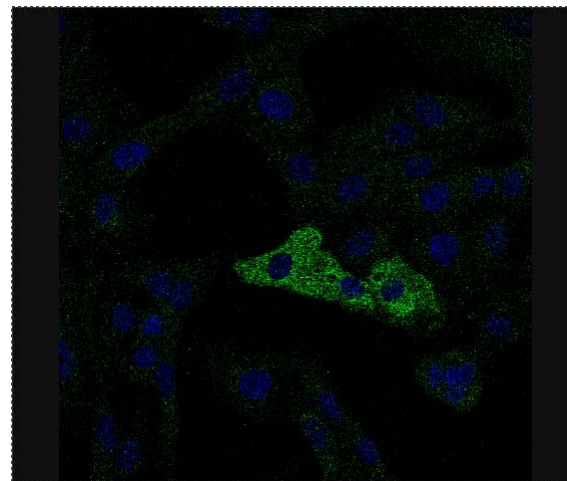
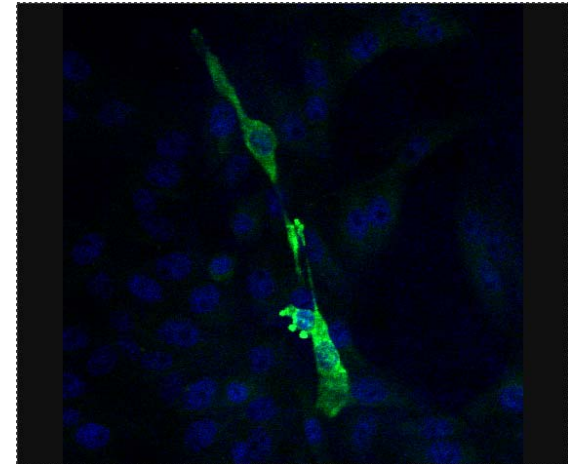
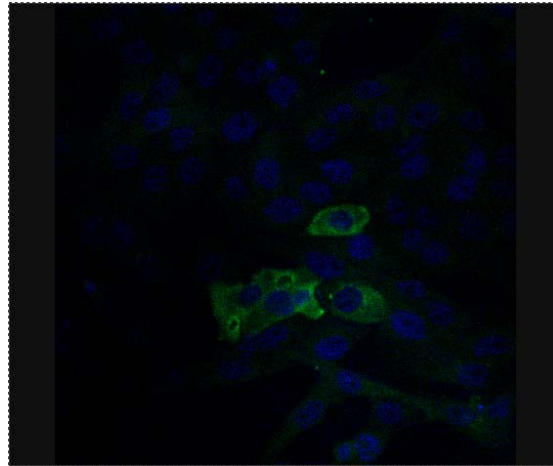
- To demonstrate infection with TCoV, independent of RT-PCR.
- To identify if RT-PCR gives an under-estimate of frequency of infected flocks.
- Work in U.S. has shown antigenic relationship between IBV and TCoV.
- IBV used because we have no TCoV isolate and no cell culture system in which to grow one.
- IBV infected cells were used for the IF.

Immunofluorescence

Nuclei in blue



Negative
control



Day-old

88 do

Immunofluorescence

- Sera from 59 flocks have been analysed:
 - TCoV-positive sera have been identified from birds aged day-old to 126 days old.
 - 29/59 flocks were IF+
 - 11/11 PCR+ flocks were also IF+
 - 3/21 PCR- flocks were IF+
 - RT-PCR data was not available for 27/59 flocks
 - Overall prevalence of TCoV in these flocks was 49%

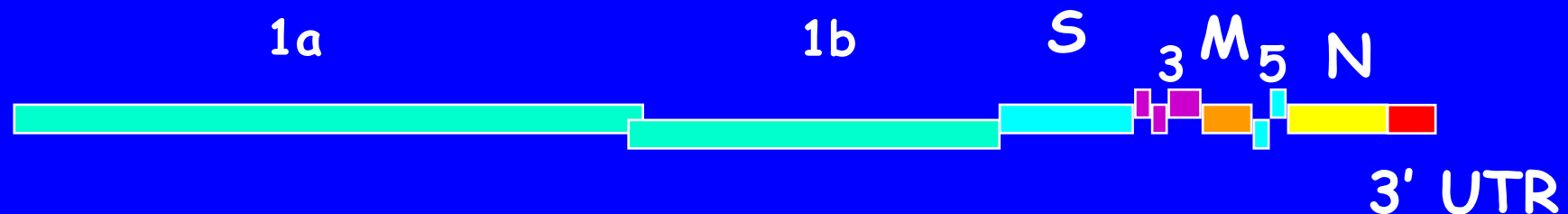
Sequencing of other genes of TCoV i.e. 3 and 5

Gene 3: 1% nt difference between 3 field isolates (from same location) and 1st TCoV UK isolate

(17% nt difference IBV Beaudette)

Gene 5: 1% nt difference between 3 field Isolates (from same location) but 10% nt difference between 3 field isolates and 1st TCoV UK isolate

(9% nt difference IBV Beaudette)



Objectives of project

1. What is the frequency of TCoV and TAstV in flocks across England and Wales?

TCoV 22%

TAstV 59%

2. Is TCoV and/or TAstV associated with disease?

3. At what age are birds infected with

TCoV 3 weeks to 18 weeks of age

TAstV 1 week to 12 weeks of age

4. What % of scouring (one-off) cases are associated with

TCoV 33%

TAstV 36%

5. How many genotypes of TCoVs and TAstVs are circulating in Britain? Ongoing

Preventive measures - U.S.

- Monitoring for TCoV and TAstV.
- Multi-age to single-age sites.
- Biosecurity:
 - Litter management
 - disposal of dead birds
 - vermin, wild birds

Acknowledgements



(Turkey sector)



All vets, flock managers and turkey companies for their considerable time and expense given for the collection of samples.