Detection of Environmental Sources of Infectious Diseases in Groundwater Networks (DESIGN)

DESIGN

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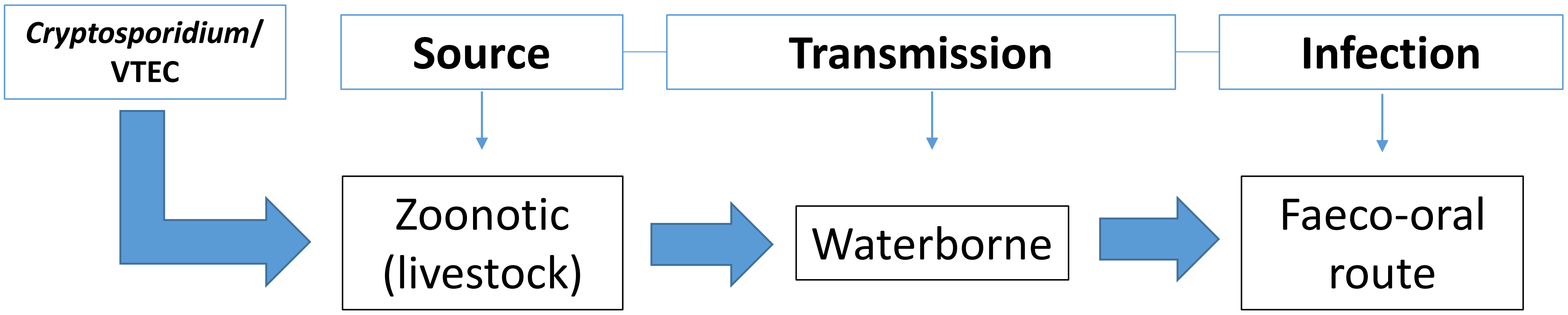
epa Research Climate - Water - Sustainability Identifying pressures • Informing policy • Developing solutions

DESIGN – Background

- 1-year project funded by Republic of Ireland (ROI) Environmental Protection Agency (EPA).

- Focus on incidence of pathogens Verotoxigenic *Escherichia coli* (**VTEC**) and ***Cryptosporidium*** in private (unregulated) groundwater wells in the ROI and public-health risks associated with drinking water consumption:



DESIGN – Rationale

- *Cryptosporidium*/VTEC = most **prevalent** enteric infectious diseases in the ROI[1].

- Manifested in gastroenteritis, diarrhoea, haemorrhagic colitis, fever, etc. – Infants, elderly and immunocompromised at particular risk[2].

- ROI = **highest incidence** of notified VTEC infections in European Union (EU) and increasing (2004-2016)[3,4].

- ROI = *Cryptosporidium* Galway outbreak (2007): 242 cases and ~ €19 million cost[5] – High impact.

DESIGN – Rationale

- Contamination sources, pathways (i.e., ingress mechanisms), and spatio-temporal patterns for *Cryptosporidium*/VTEC groundwater supply contamination remain largely **uncertain**.

- Groundwater traditionally attributed as "safe" source of drinking water owed to "presumed" pollutant attenuation capacity of (sub-)soil layers.

- ROI = Evidence for groundwater being a pathway for enteric infection[4].

- ROI = Geostatistical link between groundwater contamination and rural areas[6,7]. High private well reliance (~ **750K in ROI**) and density of (zoonotic) environmental reservoirs of both pathogens (i.e., livestock).

DESIGN – Objectives

DESIGN = improved understanding of *Cryptosporidium*/VTEC prevalence in private wells within the ROI through three (interrelated) key objectives:

i. Production of **'scoping' literature reviews** focusing on VTEC/*Cryptosporidium* incidence in 'domestic' groundwater supplies and (extra-)local contamination risk factors.

ii. Characterise VTEC/*Cryptosporidium* contamination status of private wells in the ROI through spatio-temporal (seasonal) **sampling campaigns**.

iii. Eventual development of Quantitative Microbial Risk Assessments (**QMRA**), Environmental Fate models (VTEC) and **risk factor analysis** (*Cryptosporidium*) based on seasonal (temporal) incidence data.

DESIGN – *Cryptosporidium* 'Scoping' Review

DESIGN

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Identifying pressures • Informing policy • Developing solutions

Key trends – based on (pooled) groundwater samples (*n* = 3070) and supplies (*n* = 1797):

- 31/37 (83.8%) *Cryptosporidium* positive investigations (Fig. 1) with sample (10.4-13.3%) and supply (19-19.1%) detection rates indicating ***Cryptosporidium* is common** in domestic groundwater; estimates highly applicable as '**baseline**' figures (e.g. QMRA).
- Private wells showed **higher** sample/supply (16.6%; 16%) *Cryptosporidium* incidence than public groundwater infrastructure (9.2%; 11.8%).
- Animal waste (71.3%) and groundwater recharge (92.3%) most common contamination source and ingress mechanism reported.
- Lack of standardized reporting and critical knowledge gaps identified as key concerns.
- Results recently published in **Water Research** (https://doi.org/10.1016/j.watres.2020.115726).

DESIGN – *Cryptosporidium* 'Scoping' Review

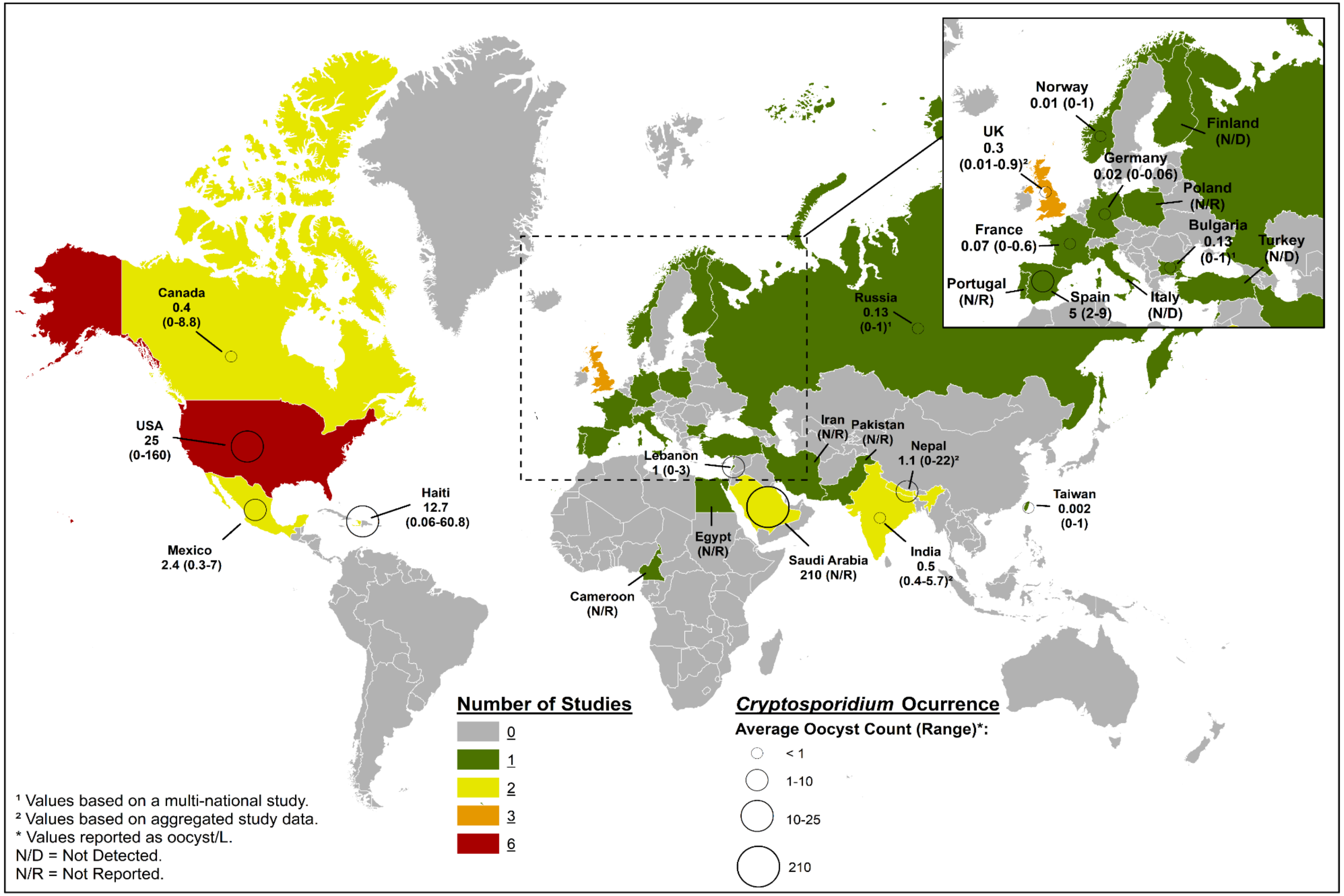


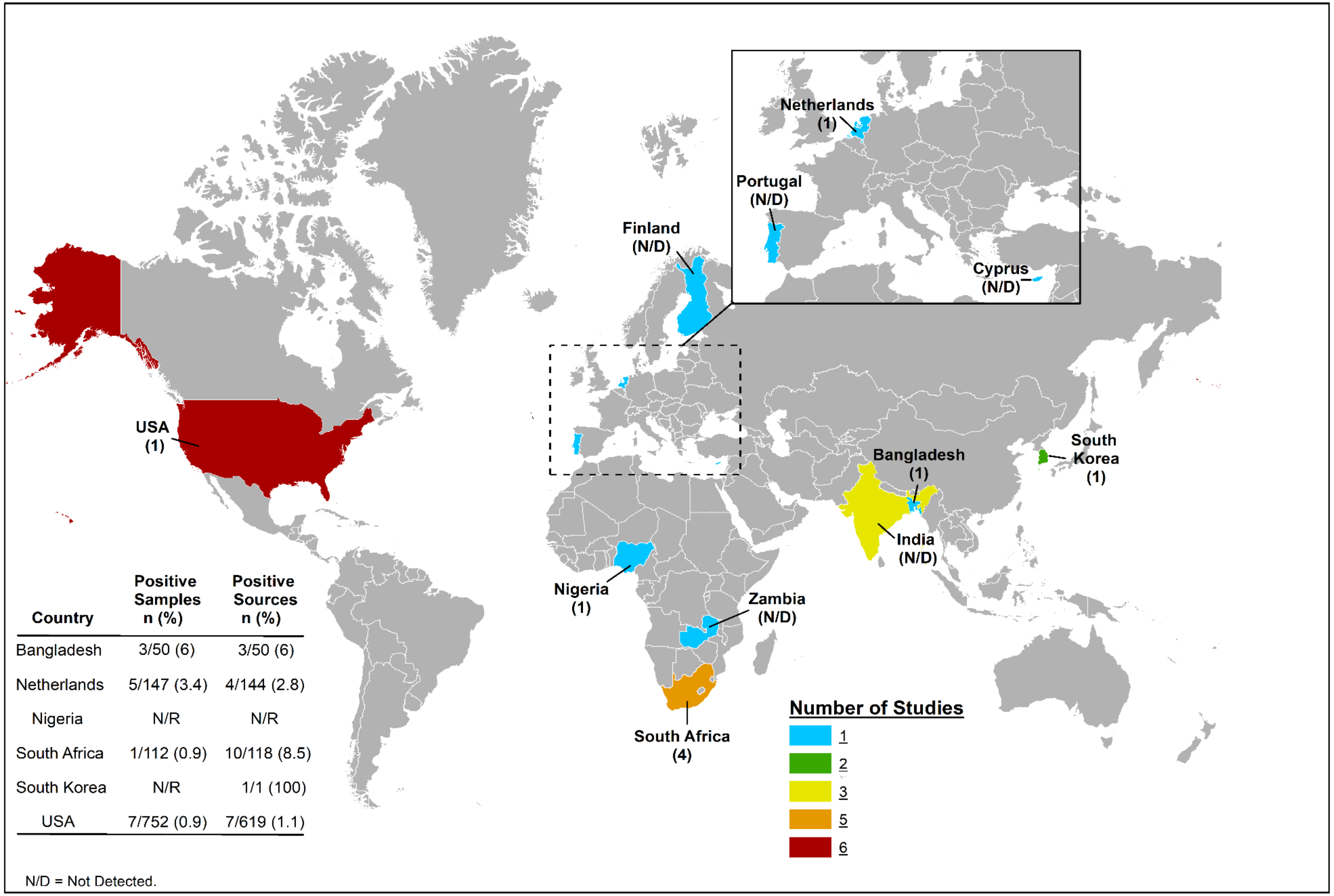
Figure 1. *Map illustrating the global distribution of identified studies in the literature review including Cryptosporidium oocyst counts per country. Average counts (oocyst/L) and range values were aggregated at a country level where applicable.*

DESIGN – VTEC ‘Scoping’ Review

Key trends – based on (pooled) groundwater samples (*n* = 2230) and supplies (*n* = 1949):

- 9/23 (39.1%) VTEC positive investigations (Fig. 2). VTEC groundwater sample/supply detection rates (0.7-1%; 1.3-4%) and ‘generic’ (or FIO) *E*. *coli*:VTEC ratios (7.5-16.7%) (Fig. 3) applicable in (bespoke) groundwater management including QMRA.
- Evidence of selective supply targeting (bias) identified potentially affecting results.
- <u>Sub</u>-<u>Saharan</u> <u>Africa</u> characterized by **high** VTEC source detection rates (6.6%) – Potential links with settlement patterns and (regional) socio-economic drivers.
- Again, <u>heterogeneous</u> and <u>vague</u> <u>reporting</u> **prevalent** among reviewed studies. Relatively **low number** of investigations and **limited** geographical distribution constrain data applicability.
- Results being collated for peer-review submission ASAP.

DESIGN – VTEC ‘Scoping’ Review



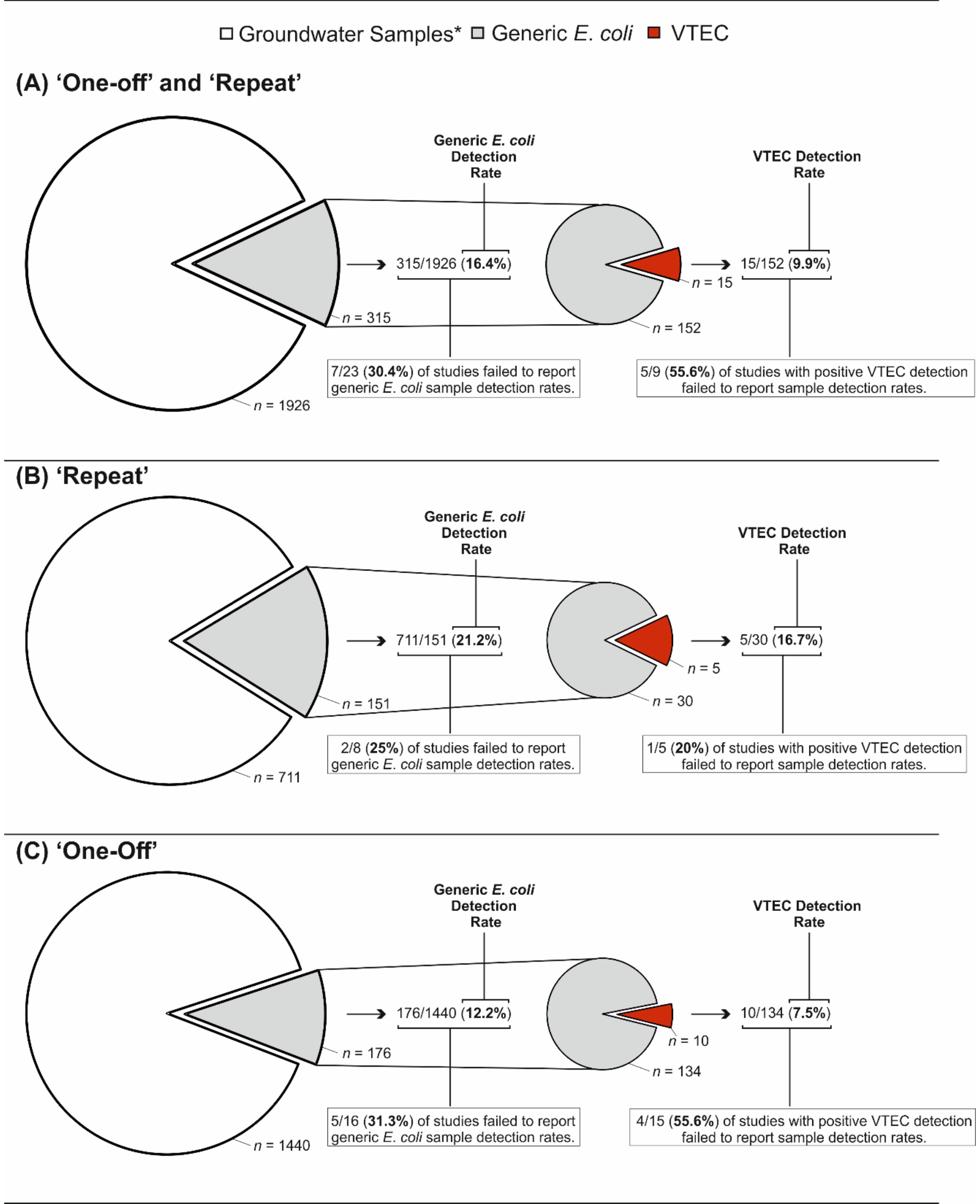
Country	Positive Samples n (%)	Positive Sources n (%)
Bangladesh	3/50 (6)	3/50 (6)
Netherlands	5/147 (3.4)	4/144 (2.8)
Nigeria	N/R	N/R
South Africa	1/112 (0.9)	10/118 (8.5)
South Korea	N/R	1/1 (100)
USA	7/752 (0.9)	7/619 (1.1)

N/D = Not Detected.

Figure 2. *Map illustrating the global distribution of investigations comprising the review dataset including the number of identified studies per country. The amount of VTEC positive investigations in each country is provided in brackets. Country-specific (pooled) groundwater supply source and sample VTEC detection rates are provided in the lower left. (N/D) = VTEC not detected in groundwater supplies.*

DESIGN – VTEC ‘Scoping’ Review

Figure 3. *Schematic of ‘generic’ E. coli and VTEC detection rates and incidence ratios. VTEC detection rates and ratios specific to sampling strategy categories (‘one-off’ and ‘repeat’) are also provided. The number of investigations failing to report relevant data to calculate detection rates is provided. * Includes (pooled) groundwater samples from all investigation and corresponding sampling strategy categories as indicated.*



DESIGN – ‘Scoping’ Review

- Key insights:

  - *Cryptosporidium*/VTEC contamination does occur in (presumably) “protected” domestic groundwater supplies.

  - Need for improved and comprehensive reporting in almost every aspects of investigations.

  - Multi-disciplinary and integrative research urgently needed – Future studies should amalgamate hydrogeology, microbiology, epidemiology and public-health sub-disciplines.

  - Potential contamination ‘baselines’ which can be employed by relevant stakeholders in the ROI and abroad.

DESIGN – Field Sampling

- **Geo-Referencing and Risk Factor Analysis:**
- Each sampling (well) locations to be linked to multiple variables in GIS environment (**spatiotemporal database**):
    - Agricultural data.
    - Hydrogeological characteristics (vulnerability/permeability).
    - Population density.
    - Land-use (CORINE).
    - Infrastructure (well type, age, depth, on-site wastewater treatment).
    - Meteorological data (geo-referenced to closest synoptic station).
- Analytical/spatial data extrapolated for **Risk Factor Analysis**:
    - Bivariate associations between dependent variable (*Cryptosporidium* presence/absence) and independent variables.
    - Multivariable analyses then undertaken.

DESIGN – *Cryptosporidium* Sampling

- Initial C*ryptosporidium* sampling phase (*n*=20) (Fig. 4) based in south-east Ireland (County Cork) did not identify *Cryptosporidium* (oocyst) contamination.
- Oocyst analysis based on > 20 L water samples, cartridge concentration and immunofluorescent assay (IFA) analysis.
- Well locations characterized by high elevation/topographical relief (Fig. 5); potentially linked to lower contamination risk.
- A majority of groundwater wells had “good” structural protection; well-suited to withstand *Cryptosporidium* supply ingress.

DESIGN – *Cryptosporidium* Sampling

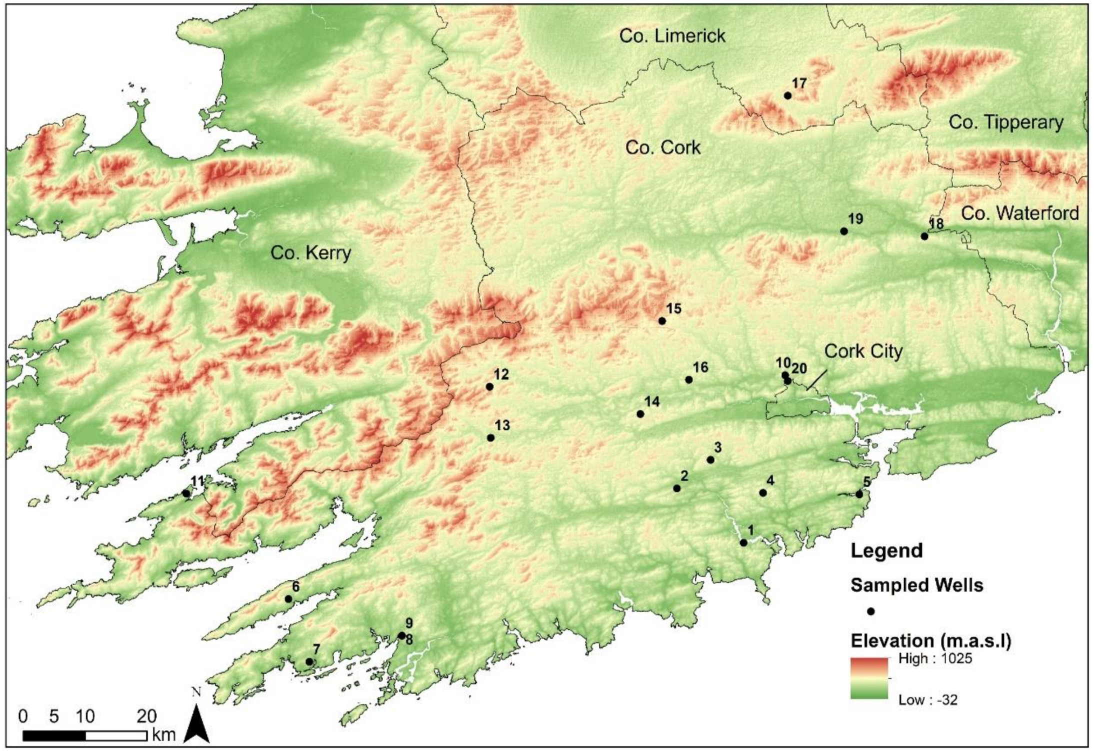


Figure 4. *Elevation map and location of private wells sampled for Cryptosporidium prevalence in initial sampling survey (October, 2019).*

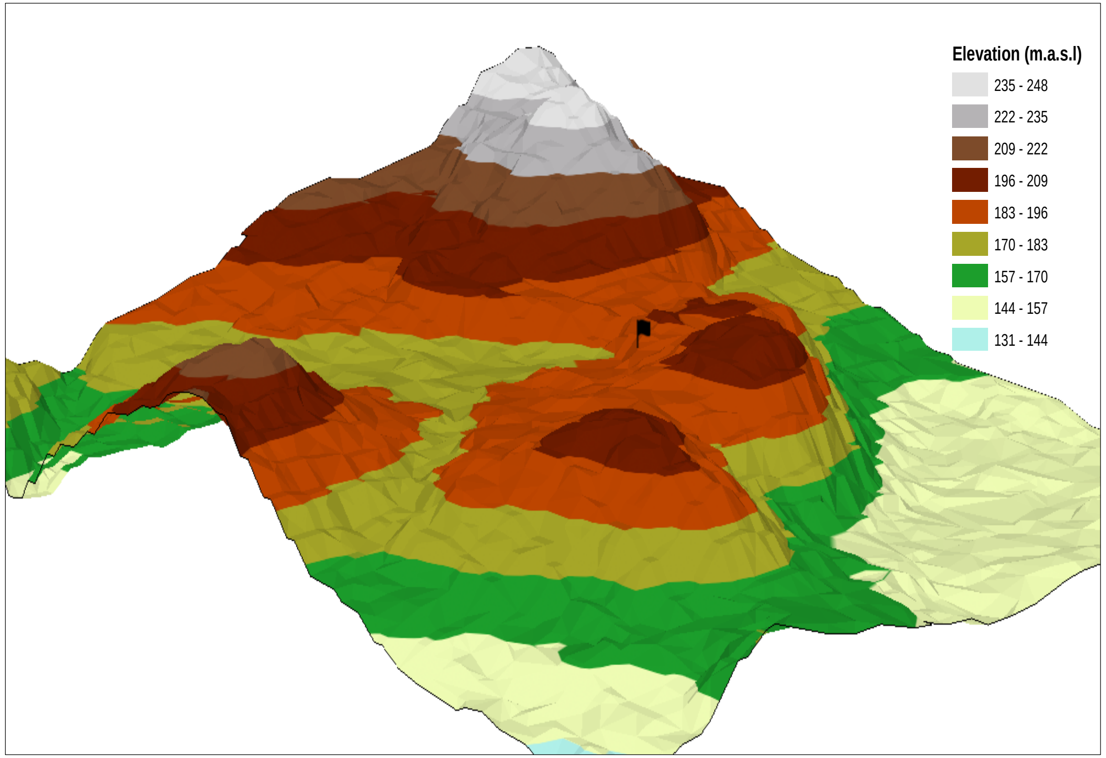


Figure 5. *3D rendering of local topography (5 km^2) of well (17) sampled during the first Cryptosporidium survey (Fig. 2).*

DESIGN – VTEC Sampling

- Initial VTEC sampling phase (*n*=21) (Fig. 6) based in western Ireland (County Galway).

- Analysis based on "CapE" method*, (30 L) water samples concentrated using membrane filtration with VTEC genes analyzed using multiplex Polymerase Chain Reaction (PCR) (*eae*, *vtx1*, *vtx2*).

- VTEC genes detected in 9/21 (**42.8%**) wells.

- Preliminary results indicate very high detection rates.

- No significant association noted with groundwater vulnerability or livestock statistics (sample number still relatively small).

DESIGN – VTEC Sampling

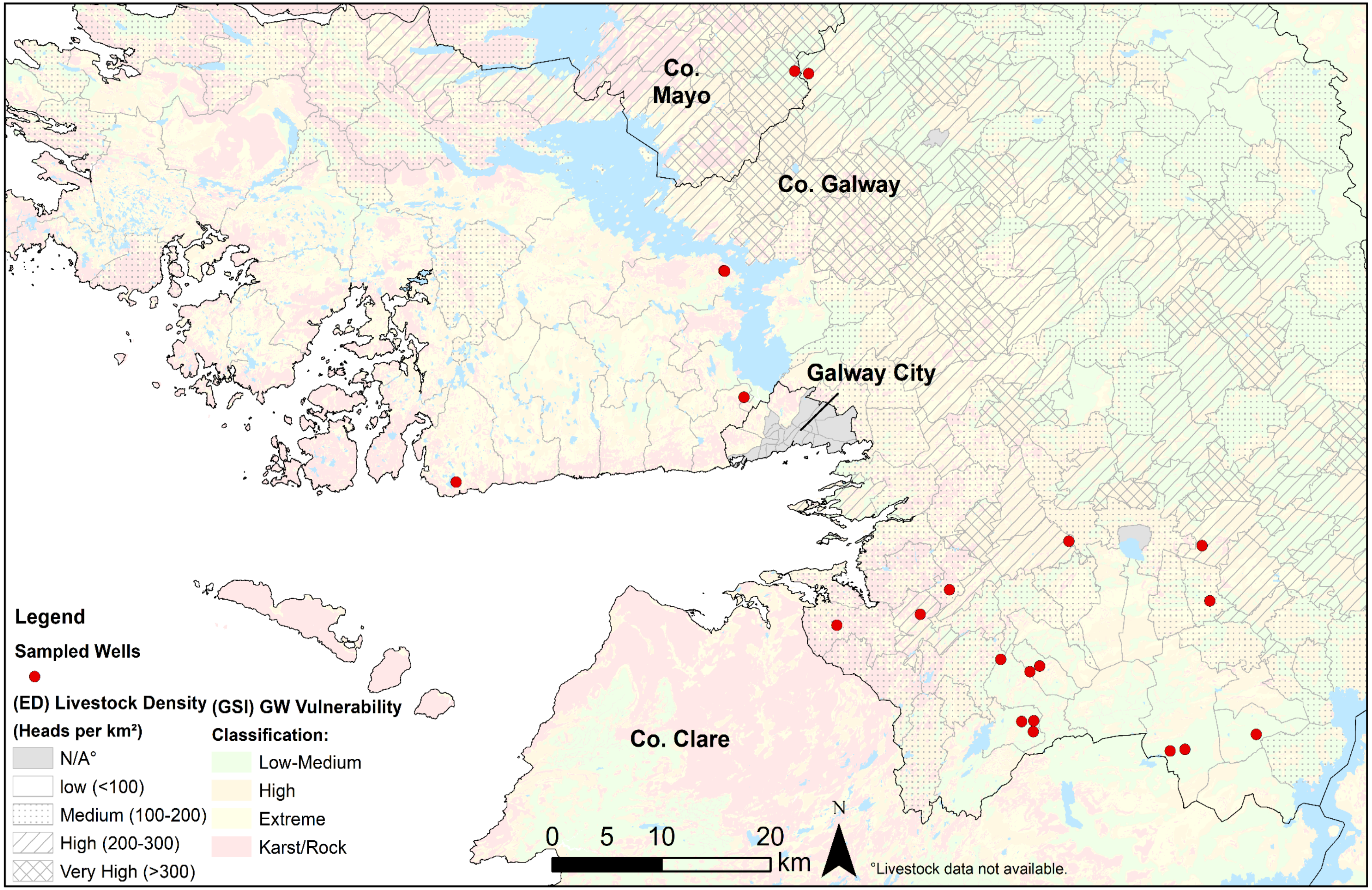


Figure 6. *Location of private wells sampled for VTEC contamination during first sampling survey (October, 2019) in relation to groundwater vulnerability and livestock density.*

DESIGN – Field Sampling

- Second *Cryptosporidium*/VTEC sampling phase due to COVID-19 outbreak.

- Project currently re-assessing situation to complete seasonal sampling.

- Completion of second sampling phase (estimated) in 2021 will enable completion of risk factor analysis, QMRA and environmental fate models.

DESIGN – Outcomes and Applications

- **Stakeholder focus** – Data generated conveyed to policy makers, local authorities and general public.

- Support emerging policy & research needs – **Water Framework Directive** (**WFD**).

- Address National Strategic Outcomes from **Project Ireland 2040**:
    - Strengthened Rural Economies and Communities.
    - Sustainable Water Management.
    - Transition to a Climate Resilient Society.

- DESIGN = One Health Approach – Healthy Ireland.

- Contribute to **bespoke groundwater management** in the ROI.

DESIGN - References

[1] Health Service Executive (HSE) (2017).

[2] Murphy, H.M. et al. 2017. *Hydrogeology Journal*. 25, 981-1001.

[3] Health Service Executive (HSE). 2018. VTEC. https://www.hpsc.ie/a-z/gastroenteric/vtec/.

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[6] OhAiseadha, C. et al. (2017). *Epidemiology* & *Infection*. 145, 95-105.

[7] Brehony, C. et al. (2018). Science for the Total Environment. 637-638, 865-870.