Received: 30 November 2021 | Revised: 9 March 2022 | Accepted: 24 March 2022

DOI: 10.1111/tbed.14539

RESEARCH NEWS

Transboundary and Emerging Diseases WILEY

Investigation into an outbreak of Border disease virus in pigs in England

Akbar Dastjerdi[1] | Rebecca Strong[1] | S. Anna La Rocca[1] | Mark Wessels[2] | Julie Wessels[3] | Kate Whitaker[2] | Ben Strugnell[4] | Susanna Williamson[2]

[1]Virology Department, Animal and Plant Health Agency (APHA)-Weybridge, Addlestone, Surrey, UK

[2]APHA-Preston, Animal Health Centre, Barton, Preston, UK

[3]APHA-Bury St. Edmunds, Suffolk, UK

[4]APHA-Thirsk, West House, Thirsk, UK

Correspondence
Akbar Dastjerdi, Virology Department, Animal and Plant Health Agency (APHA)-Weybridge, Addlestone, Surrey KT15 3NB, UK.
Email: akbar.dastjerdi@apha.gov.uk

Present address
Mark Wessels, Finn Pathologists, Mayflower Way, Harleston, Norfolk IP20 9 EB, UK
Julie Wessels, Crowshall Veterinary Services LLP, Crowshall Lane, Attleborough, Norfolk NR17 1AD, UK
Kate Whitaker, Town and Country Veterinary Group, Whalley Road, Clayton le Moors, Accrington, BB5 5ED, UK
Ben Strugnell, Farm Post Mortems Ltd, Hamsterley House, Hamsterley, Bishop Auckland, County Durham DL13 3QF, UK

Funding information
Department of Environment, Food and Rural Affairs

Abstract

Border disease (BD) was first reported in 1959 in lambs from the border region of England and Wales. The causative virus (BD virus; BDV) has since been identified in several other ruminant species and pigs. The virus is prevalent in sheep flocks of UK, Europe and USA and has potential to inflict substantial economic losses. Natural BDV infection of pigs was first reported in the UK in 1992 from pigs with haemorrhagic lesions and more recently from healthy pigs in Spain and Japan. Here, a persistent problem of poor growth and anaemia in a small proportion of growing pigs on a mixed pig and sheep holding was investigated and tissues were tested in a pan viral microarray. The microarray detected BDV RNA in several tissues which was further confirmed by sequencing, specific BDV PCR and immunohistochemistry. Phylogenetically, the virus clustered with other BDVs in the sub-genotype 1b. This investigation highlights likely interspecies transmission of pestiviruses and their impact on pestivirus detection and eradication programs.

KEYWORDS
Border disease virus, congenital infection, microarray, pestivirus, pigs

1 | INTRODUCTION

Pestiviruses, in the family Flaviviridae, infect pigs and ruminants, including cattle, sheep, goats and wild suids (Tautz et al., 2015) resulting in substantial economic loss. Moreover, pestivirus sequences have been detected in samples from bats and rats by next-generation sequencing (NGS), although no infectious pestivirus has yet been isolated from these host species (Firth et al., 2014; Wu et al., 2018). Virus species under the *Pestivirus* genus have been reclassified recently as *Pestivirus A* (Bovine viral diarrhea virus 1), *Pestivirus B* (Bovine viral diarrhea virus 2), *Pestivirus C* (Classical swine fever virus, CSF), *Pestivirus D* (Border disease virus) (BDV), *Pestivirus E* (pronghorn pestivirus), *Pestivirus F* (Bungowannah virus), *Pestivirus G* (giraffe pestivirus), *Pestivirus H* (Hobi-like pestivirus), *Pestivirus I* (Aydin-like pestivirus), *Pestivirus J* (rat pestivirus) and *Pestivirus K* (atypical porcine pestivirus) (Smith et al., 2017). A bat-derived virus and pestiviruses identified from sheep and

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
© 2022 Crown copyright. *Transboundary and Emerging Diseases* published by Wiley-VCH GmbH. This article is published with the permission of the Controller of HMSO and the Queen's Printer for Scotland.

goat (Tunisian sheep pestiviruses), which lack complete coding region sequences, may represent two additional species.

The pestivirus genome is a single-stranded positive-sense RNA of approximately 12.3 kb in length, coding for a single open reading frame that is flanked by untranslated regions (UTR). The genome codes for four structural proteins, the capsid (C) and three envelope proteins (Erns, E1 and E2), plus several non-structural proteins (https://talk.ictvonline.org/ictv-reports). The 5′-UTR or N^{pro} sequences have routinely been used for phylogenetic analysis of pestiviruses and accordingly BDV isolates have been divided into six phylogenetic groups; BDV1 has been detected in sheep from the USA (Sullivan et al., 1997), the UK (Vilcek et al., 1997), Australia (Becher et al., 1994) and New Zealand (Vilcek et al., 1998); BDV2 in ruminants in Germany (Becher et al., 2003); BDV3 in Switzerland (Stalder et al., 2005) and Austria (Krametter-Froetscher et al., 2007); BDV4 in Spain (Valdazo-González et al., 2007) and BDV5 and 6 in France (Dubois et al., 2008). Isolates from Turkey form a possible seventh group, BDV7, that remain to be fully characterised (Oguzoglu et al., 2009) and a BDV8 from Alpine chamois was identified in Italy (Caruso et al., 2017).

Pestiviruses were originally differentiated according to the host species from which they were isolated, for example, CSFV for swine, BVDV1 and 2 for bovine and BDV for ovine. However, it has been shown that these three pestiviruses have the ability to infect certain other animal species both experimentally and naturally (Paton, 1995; Paton & Done, 1994). The interspecies transmission of the pestiviruses among domestic and wild artiodactyla has been previously reported. CSFV is able to experimentally infect cattle, sheep, goats and deer (Biró et al., 1966; CFSPH, 2015; Loan & Storm, 1968; Shimizu & Kumagai, 1989). BVDV1 and 2 also infect pigs, goats, sheep, deer, yak, buffalo and wild ruminants (Casaubon et al., 2012; Deng et al., 2015; Ridpath et al., 2010) and BDV has been isolated from goat, cattle and pigs (Becher et al., 1997).

BDV was first reported in 1959 in lambs from the border region of England and Wales, hence the name (Hughes et al., 1959). The virus is now prevalent in sheep in the UK, Europe and USA and can result in substantial economic losses in sheep flocks particularly through its adverse effect on reproduction and disease in neonatal lambs following in utero infection. Postnatal infection is usually sub-clinical with transient viremia; however, in pregnant animals, the virus has a tropism for hair follicles, foetal lymphoid tissues and the central nervous system. It causes infertility, abortion or the birth of weak 'hairy shaker' lambs; lambs born with hair rather than wool and cerebellar hypoplasia (Toplu et al., 2011). If the ewe is infected in the first-third of gestation, immunotolerant lambs will be born with no apparent clinical signs but are persistently infected (PI).

Roehe et al. (1992) first reported natural infection with BDV in pigs with haemorrhagic lesions in the UK. The virus has also been described in pigs more recently in Spain and Japan (Kawanishi et al., 2014; Rosell et al., 2014). In both cases, infection was detected following serological cross reactions found during routine CSFV sero-surveillance on farm; no clinical signs were seen in the infected pigs including no detectable effect on growth or productivity.

We investigated a persistent problem of poor growth and anaemia in a small proportion of growing pigs on a mixed pig and sheep holding. Microarray, BDV-specific PCRs and pestivirus immunohistochemistry (IHC) confirmed presence of BDV, nearly 20 years after the first recorded case in the UK (Roehe et al., 1992). Clinical presentations of the progeny of sows showed similarity to those which might be seen in litters of breeding pigs infected during pregnancy with a CSFV of moderate to low virulence (Brown & Bevins, 2018).

2 | MATERIALS AND METHODS

2.1 | Animals

Six commercial hybrid pigs within three submissions were sent from a single pig farm to an APHA Veterinary Investigation Centre in England between September 2012 and March 2013 for post-mortem examination and full diagnostic investigation.

2.2 | Sample processing and microarray analysis

Kidney, spleen and heparinised blood collected from one pig were processed for pan viral microarray analysis. TRIzol® Reagent (Fisher Scientific) and QIAamp viral RNA mini kit (Qiagen) were used to extract nucleic acid from various tissues. Approximately 25 mg of tissue was added to 1 ml of TRIzol Reagent inside an M tube (Miltenyi Biotec) and homogenised using the gentleMACS™ Dissociator. The M tube was then centrifuged at 2000×*g* for 3 min and the content supernatant was transferred to an Eppendorf tube and processed as per TRIzol manufacturer protocol. An aliquot (140 μl) of the aqueous phase or blood was used in the QIAamp viral RNA mini kit (Qiagen) to isolate the nucleic acid according to the kit protocol. A 16 μl volume of the extracted nucleic acid was treated with 2 μl DNase I (1 U/μl, Fisher Scientific) and prepared for analysis on a pan viral microarray (GEO accession number GPL8185) as described (Dastjerdi et al., 2016).

2.3 | Molecular analysis and phylogeny

Extracted RNA was converted to cDNA using SuperScript III Reverse Transcriptase (Fisher Scientific) and random hexamers following the manufacturer's protocol. A 285 bp DNA fragment of the 5′ UTR region was amplified from cDNA using primer 324 and primer 326 (Easton et al., 1994), corresponding to the positions of 98–118 and 362–382 on the sequence NC_003679.1. The PCR using the Taq Gold polymerase (Promega) was carried out at 95°C for 5 min, followed by 40 cycles of 95°C for 45 s, 57°C for 1 min, 72°C for 1 min and 72°C for 7 min. A 736 bp DNA fragment from N^{pro} and a part of the C coding region was amplified using primer 320F (position in NC_003679.1, 308–328) and primer 1040R (1021–1043) (Strong et al., 2010) using KOD Hot Start DNA polymerase (Sigma) at 95°C for 2 min, followed by 40 cycles of 95°C for 20 s, 52°C for 10 s, 72°C for 45 s and 72°C for 5 min. A 967 bp DNA fragment of the E2 gene was amplified from cDNA using primer

BDV-F (5′-AGRRCCTGACAACAACYTG-3′; position 2513–2531 on the NC_003679.1) and BDV-R (5′-CWGAGTTCATCGTGCTRGC-3′; 3461–3479) using the KOD Hot Start DNA polymerase at 95°C for 2 min, followed by 40 cycles of 95°C for 20 s, 50°C for 10 s, 70°C for 15 s and 70°C for 1 min. The amplicons were sequenced in house at the APHA Central Sequencing Unit. The sequence data were assembled and aligned using SeqMan, EditSeq and MegAlign software (DNASTAR Inc.). Sequences used for comparison were obtained from GenBank and the accession numbers are given in the phylogenetic trees. The evolutionary history of CLUSTAL W aligned sequences inferred by the Maximum Likelihood method and Tamura–Nei model (Tamura & Nei, 1993). Initial tree(s) for the heuristic search was obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach, and then selecting the topology with superior log likelihood value. Bootstrap resampling (500 replicates) was carried out to demonstrate robustness of groupings (Felsenstein, 1985). The nucleotide sequences of this BDV isolate were deposited in the NCBI GenBank under accession numbers MT432532-4.

2.4 | Other viral and bacterial tests

Spleen and/or lung samples from all six animals were collected and processed for BDV and BVDV RT-qPCRs. Ruminant pestivirus RT-qPCR assays were carried out as described by La Rocca and Sandvik (2009) and McGoldrick et al. (1999). The assays detect and differentiate between BDV and BVDV (BVDV1 and 2). A RT-qPCR was also used for the detection of PRRSV in blood and spleen or lung tissue samples (Kleiboeker et al., 2005). The ORF7 gene encoding the nucleocapsid protein of the virus is detected in this test. The swine influenza A virus real time RT-qPCR was carried out on a pool of tonsil, trachea and cranial lung from each pig as described (Hoffmann et al., 2010). Primary bacterial culture and identification, *Mycoplasma* species detection (including *M. suis*) and *Salmonella* culture were carried out using in-house standard operating procedures.

2.5 | Histopathology and IHC

Tissues for histopathology were fixed in formalin and processed routinely for histological examination and the 5 μm sections stained with Harris's haematoxylin and eosin (H&E). The presence of pestivirus antigen in selected tissue sections was investigated by IHC as described previously (Otter et al., 2009) using the pan-pestivirus monoclonal antibody (15C5) which recognises an epitope on the 48-kD Erns glycoprotein (Donis et al., 1988).

3 | RESULTS

3.1 | Clinical history and gross pathological examination

This disease investigation involved pigs from a single mixed livestock holding including a 170-sow indoor breeder-finisher unit and a 250-ewe breeding flock. Over the course of 2 years, between 20 and 30 pigs were affected with poor growth and pallor (anaemia), with all cases dying or requiring euthanasia. Clinical signs were seen from 8-weeks-old. The submitted pigs were of mixed sex (four male, two female), aged between 12 and 16 weeks and weighed 10–18 kg. Pigs 4–6 were submitted as part of the ongoing investigation into poor growth and anaemia and the farmer also reported a recent outbreak of pneumonia.

Table 1 summarises clinical details and gross pathology seen in each pig. The most consistent findings were underweight pigs which were all small for their age, with severe anaemia and variable degrees of generalised lymph node enlargement. There was visible thymic atrophy in two pigs. One pig (pig 1) had marked and widespread haemorrhages. Anaemia was confirmed by haematology in all six pigs with leucopenia in pig 2 and neutropenia in pigs 5 and 6 (Table 2). Pig 4 had a relative neutrophilia and shift to the left, likely reflecting the necrotising pneumonia identified. Platelet clumping prevented platelet counts in most pigs. However, there was no thrombocytopaenia in pig 5 (platelets 222×10^9/l, reference $100—900 \times 10^9$/l). Where haemorrhages were visible, these involved the subcutis, kidney cortices, lymph nodes, mucosal and serosal surfaces of the small intestine, liver, thymus and, to a lesser extent, other tissues such as salivary glands.

Mild cranioventral pulmonary consolidation in pig 3 was associated with *Haemophilus parasuis* infection. Pasteurellosis due to *Pastefurella multocida* was diagnosed in pig 4 which had a multifocal necrotising pleuropneumonia while *Bordetella bronchiseptica* with *Mycoplasma hyopneumoniae* infection was found in the cranioventral pneumonia in pig 5. No other pathogens were identified, including *Mycoplasma suis* (by microscopy, DGGE and PCR on EDTA blood), PRRSV, and swine influenza virus. Serum iron concentrations in two pigs were 20.0 and 24.2 μmol/l, both within the reference range of 11–32 μmol/l.

3.2 | Histopathological findings and IHC

Histopathology revealed reactive lymphadenosis (four out of six pigs), thymic atrophy (two out of three), extra-medullary haematopoiesis (in the spleen, lymph node, liver and kidney in six out of six), bone marrow hyperplasia with erythroid shift (six out of six), focal, segmental to global glomerulonephritis (five out of six), mild plasmalymphocytic gastritis (three out of three), minimal plasmalymphocytic colitis (one out of three) and multifocal, minimal, angiocentric, non-suppurative encephalitis (one out of three) (Figure 1).

Pestivirus antigen was detected by IHC in neurons, glia and pericytes (Figure 2). Intense antigen labelling in the cerebrum and hippocampus was also observed in all three pigs (pigs 4–6). Labelling was noted particularly in the lacunar layer of the hippocampus and ependymal cells together with smaller numbers of glial cells with morphology suggestive of astrocytes, mostly in the hippocampus. Rare labelling of a similar population of cells was also seen in cerebral cortex.

The brain was not fixed for histopathology from pig 1. The spleen of this pig showed sparse to moderate punctate labelling of approximately half of the megakaryocyte population involving maturation stages 1–3 (counts were not performed). Kidney had extensive granular to diffuse labelling of many tubular epithelial cells, also punctate labelling of

TABLE 1 Clinical details and gross pathology in submitted pigs

Pig no.	Age (weeks)	Weight (kg)	Sex	LN	Thymic atrophy	Haemorrhages	Other pathologies
1	13	17	M	3+	Not noted	Subcutis, kidney, LN, small intestine, liver, thymus, salivary glands	
2	16	10	F	1+	Not noted	LN	
3	16	13	M	1+	Not noted	LN	Cranioventral lung consolidation.
4	14	14	M	2+	Yes	LN	Multifocal severe necrotising pleuropneumonia. Cranioventral lung consolidation.
5	14	13	M	2+	Yes	LN	Cranioventral lung consolidation.
6	14	18	F	2+	No	LN	Cranioventral lung consolidation.

Wasting was the main sign observed in the six pigs.
M, male; F, female; LN, lymph nodes enlargement.
Lymph node enlargement scores: 0 = none, 1 = mild, 2 = moderate, 3 = marked.

TABLE 2 Haematological findings in the six pigs infected with BDV

Blood indices	Ref. range	Pig 1	Pig 2	Pig 3	Pig 4	Pig 5	Pig 6
Hb	11–17 g/dl	2.7	2.5	2.3	6.3	3.0	6.2
MCH	15–25 pg	15.8	14.5	17.0	15.9	14.6	18.5
MCHC	28–36 g/dl	20.8	20.5	23.7	23.4	21.9	26.1
MCV	55–67 fl	76.0	70.5	71.9	67.9	66.8	70.8
PCV	0.37–0.5 L/L	0.13	0.12	0.10	0.27	0.14	0.24
RBC	$6–9 \times 10^{12}$/L	1.70	1.70	1.40	4.00	2.00	3.40
WBC	$10–23 \times 10^{9}$/L	5.7	12.7	2.1	12.6	7.4	11.2
Neutrophils	$3.1–10.3 \times 10^{9}$/L	0.5 (9%)	5.6 (44%)	0.5 (24%)	6.3 (50%)	1.4 (19%)	2.1 (19%)
Band neutrophils	Rare $\times 10^{9}$/L	(0%)	(8%)	(4%)	(3%)	(0%)	(0%)
Lymphocytes	$4.3–13.6 \times 10^{9}$/L	5.2 (91%)	7 (55%)	1.6 (76%)	6.3 (50%)	6 (81%)	9.1 (81%)
Eosinophils	$0–0.4 \times 10^{9}$/L	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Monocytes	$0.2–1 \times 10^{9}$/L	0 (0%)	0.1 (1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Basophils	$0–0.2 \times 10^{9}$/L	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Hb, haemoglobin; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cell volume; RBC, red blood cells; WBC, white blood cells.

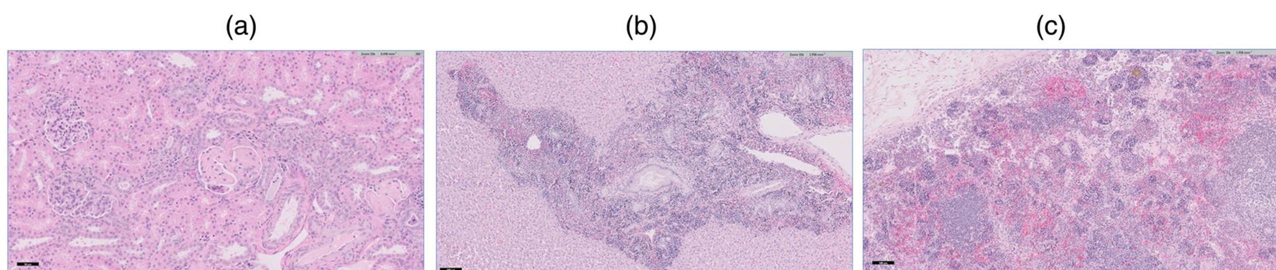

FIGURE 1 Histopathology of formalin-fixed tissues stained with haematoxylin and eosin (H&E). (a) Kidney with segmental to global membranous glomerulonephritis (in five out of six pigs). (b and c) Liver and lymph node, respectively, with marked extramedullary haematopoiesis (in four out of six pigs). Bar = 100 μm

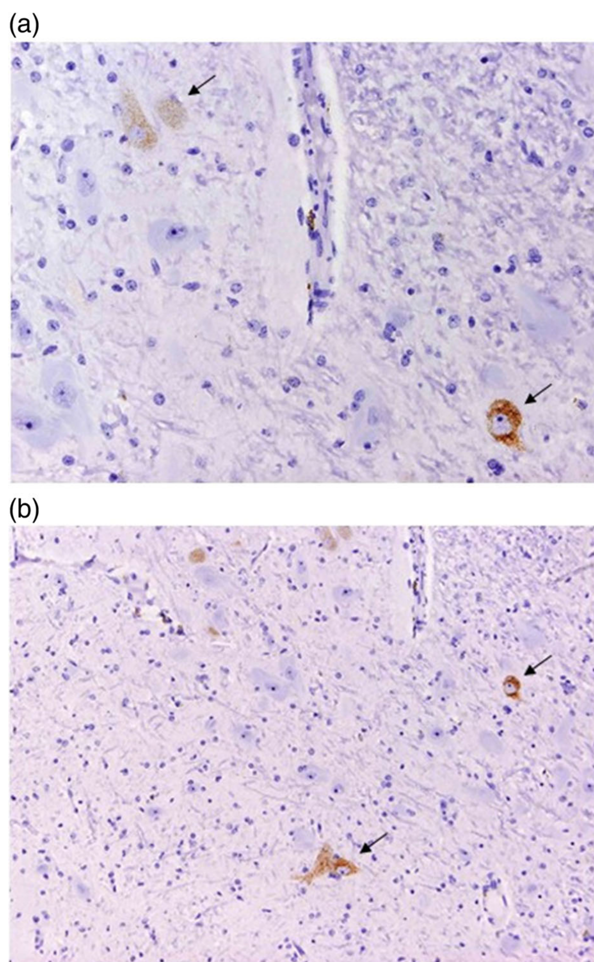

FIGURE 2 Representative images of BDV immunohistochemistry of the brain. Intense neuronal labelling (arrows) was particularly observed in lacunar layer of hippocampus together with smaller numbers of glial cells, morphology suggestive of astrocytes, mostly in hippocampus (a). Rare labelling of a similar population of cells was also seen in cerebral cortex (b). Similar pattern of immunostaining was observed in the brain of other two piglets examined from this submission

occasional cells in Bowmans capsules as well as moderate labelling of vascular smooth muscle and connective tissue cells. Glomerular tuft labelling was not detected. Liver had extensive labelling of sinusoidal lining cells and moderately extensive labelling of portal cells including connective tissue/fixed tissue macrophage-like cells. Lung presented intense labelling of chondrocytes and in a reticular pattern in germinal centres in BALT, moderate labelling of bronchiolar epithelium, together with labelling of varying intensity of occasional clusters of macrophages. Small intestine (ileum) also showed intense labelling in a reticular pattern in germinal centres in GALT, and moderate labelling of villus cores including lacteals.

3.3 | Microarray and pestivirus RT-qPCRs

Analysis of kidney, spleen and heparinised blood from one pig by the microarray indicated presence of a BDV RNA in the three samples. This microarray result was confirmed by the ruminant pestivirus RT-qPCR assays with only BDV detected in kidney and spleen samples. The presence of BDV nucleic acid in heparin blood was not confirmed, possibly due to the inhibitory property of heparin in the RT-qPCR (Schrader et al., 2012). BVDV1 and 2 were not detected. The virus was also isolated in cell culture from kidney, spleen and blood.

Spleen and blood samples and, in some, lung from the five other pigs were tested by the pestiviruses RT-qPCR. BDV RNA was detected in all pigs (though not all samples in any one pig) except pig 2 and all were negative for BVDV1 and 2 RNA.

3.4 | Sequence similarity and phylogenetic analysis

The BDV detected from pig 1 was subjected to genomic characterisation through sequencing of 5′ UTR, N^{pro} and E2 genes. For the three genes, 243, 662 and 866 nucleotide sequences, respectively, were compared with representative of other BDV sequences from GenBank. The BDV sequences shared the highest nucleotide identity with viruses in the sub-genotype BDV1b especially those detected in the UK from ovine and bovine species, an identity of 99.6% to both the bovine strain 1505744 and the ovine strain K1729/3 for the 5′UTR, 98.4% to the bovine strain 1505744 for N^{pro} and 94.8% to ovine strain V2536/2 for E2. In the phylogenetic analysis, the virus also clustered with viruses in the sub-genotype 1b (Figure 3 and Supplementary figure). The virus from this outbreak was therefore classified as a BDV1b sub-genotype.

4 | DISCUSSION

This is the first outbreak of BD in commercial pigs diagnosed in England since the 1990s. A sheep flock on the same site was suspected as the likely source of BDV infection. This serves as a reminder of the potential for cross species transmission of ruminant pestiviruses to pigs which is especially relevant for small-holder situations where direct or indirect contact between mixed species is more likely. In this case, the pig and sheep enterprises involved on the farm were commercial in nature with biosecurity gaps which provided opportunity for indirect contact and introduction of BDV infection from the sheep into pregnant sows. The farm was visited as part of the investigation. Sheep handling facilities were found to be immediately adjacent to the dry sow yard with no separation of air space and shared staff. This would have provided the opportunity for transmission of BDV between sheep and pregnant sows. Several management practices were identified which were likely to have favoured continued transmission and propagation of BDV in the pig herd without further introduction of infection from sheep being necessary. These practices included keeping in-pig gilts in the fattener shed with a shared scrape-through dung channel and using empty boar pens in the service house for growing pigs with ill thrift. Advice was given to change these practices, as well as preventing direct or indirect contact between pigs and sheep. Further diagnostic investigations were recommended, but no diagnostic submissions associated with the BD outbreak were subsequently received from the premises.

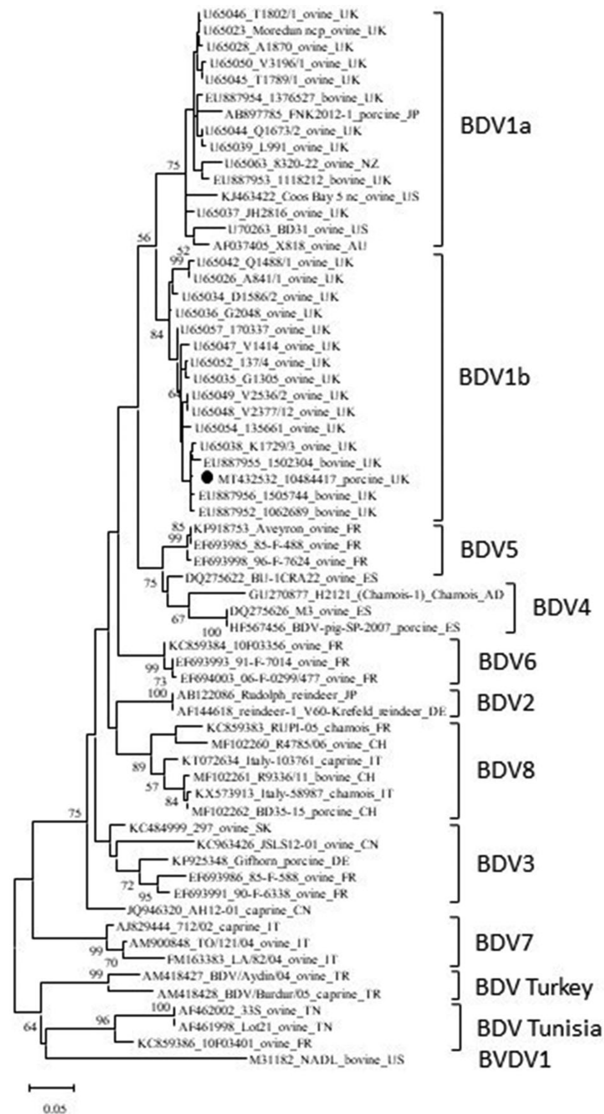

FIGURE 3 Phylogenetic analysis of partial 5′ UTR sequence of porcine Border disease viruses (BDV). The evolutionary analyses were conducted in MEGA X (Kumar et al., 2018) using the Maximum Likelihood method and Tamura–Nei model (Tamura & Nei, 1993). The tree with the highest log likelihood (−9353.20) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Representative BDV strains of the different sub-genotypes are included in the tree and denoted by GenBank accession number, strain or isolate name, the animal species of origin and the country of isolation. BVDV-1 NADL isolate was used as outgroup. The partial 5′ UTR sequences is corresponding to nucleotides 136 to 358 (223 nucleotides) of reference strain X818 (accession number NC_003679.1)

The clinical presentation of disease investigated here was of poor growth, pigs being small for their age and anaemic (seen on farm as pale pigs) and affected pigs were reported to either die or require euthanasia on welfare grounds. Of the six typical cases submitted for investigation, one (pig 1) had haemorrhagic lesions similar to those seen in cases of mucosal disease in cattle PI with BVDV, possibly representing a similar terminal event. This disease presentation might also occur in the progeny of breeding pigs infected during gestation with CSFV in countries where CSF is endemic, or elsewhere if the CSFV is of low virulence and wider disease is not apparent. When the first case was submitted, disease had been ongoing for several weeks on farm, the possibility of swine fever was considered and ruled out on clinical grounds. Ruminant pestivirus infection should be borne in mind as a differential diagnosis of low morbidity ill thrift and anaemia in pigs, where there is potential contact with sheep or cattle. Where haemorrhagic disease occurs in pigs, the possibility of swine fever should be considered and, if suspected, such cases must be reported to the Animal Health authorities. Where swine fever is not suspected, differentials include vasculopathies and coagulopathies caused by viraemia; (non-CSFV pestiviruses, PRRSV, PCV2-associated disease, PCV3-associated disease); bacterial septicaemia (disseminated intravascular coagulation, for example due to *Streptococcus suis, Klebsiella, Erysipelothrix, Escherichia coli, Glaesserella (Haemophilus) parasuis*); immune-mediated disease (porcine dermatitis and nephropathy syndrome, thrombocytopenia purpura, acquired megakaryocyte aplasia); neoplasia (e.g. lymphoma, myeloma); vitamin K deficiency (acquired or anticoagulant toxicity) and mycotoxicosis. In sporadic cases of haemorrhagic disease in anaemic individual pigs, in the absence of disease on other pigs, the main differentials include ruminant pestivirus infection, septicaemia, anticoagulant toxicity and acquired immune-mediated thrombocytopaenia (Bidewell et al., 2013).

Natural infection of pigs with ruminant pestiviruses, BVDV, BDV and even with APPV, usually results in mild clinical or sub-clinical signs so long as in utero infection does not occur and thus usually causes minimal economic loss. Such infections, however, may interfere with serological surveillance and diagnosis of CSFV and other pestivirus infections, due to cross-reactivity among this group of viruses (Kawanishi et al., 2014). Therefore, in-depth characterisation of existing and emerging non-CSFV pestiviruses is important to ensure specificity of pestiviruses detection assays. This case also illustrates the usefulness of a pan viral microarray in investigation of unusual disease incidents. In this case, histopathology and IHC findings combined with the results of virological molecular assays confirmed infection with BDV.

The pronounced neuronal tropism and pestivirus antigen distribution demonstrated by IHC in tissue sections were very similar to those observed in persistent pestivirus infection in immunotolerant ruminants and likely to indicate congenital infection of the pigs (Fernandez et al., 1989; Hewicker et al., 1990; Montgomery, 2007; Potts et al., 1985; Waldvogel et al., 1995; Wilhelmsen et al., 1991; Wöhrmann et al., 1992). Previous studies have also demonstrated that fetal neuronal tissues are a primary target of congenital pestiviral infection in cattle, sheep, goat, white-tailed deer and alpacas (Duncan et al., 2008; Henningson et al., 2013; Hewicker et al., 1990; Hewicker-Trautwein et al., 1995; Passler et al., 2012, 2014). It also has been shown that experimentally infected pigs with BVDV at the age of 6–10 weeks lack pestivirus antigen labelling in the brain tissue (Makoschey et al., 2002) which further suggests congenital infection with BDV in these pigs.

Widespread haemorrhages were seen in one pig for which a platelet count was not possible, although platelet anisocytosis was noted at blood smear examination. Nucleated RBCs were seen on the blood film indicating a regenerative anaemia and histology showed the bone marrow was reactive. Haemorrhagic disease in BVDV1 infection in cattle is associated with thrombocytopenia (Colloff et al., 2012) and that may be the explanation in this pig.

In conclusion, using a pan viral microarray, BDV PCRs and pestivirus IHC, we have confirmed infection with BDV in this commercial pig herd as the cause of their chronic ill thrift and anaemia. The virus was classified in the BDV sub-genotype 1b. This investigation highlights potential interspecies transmission of pestiviruses and their impact on disease surveillance and eradication programs as well as on health, welfare and productivity.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the farmer and veterinary surgeon for providing the material for diagnosis and farm and clinical information. We are also grateful to Cynthia Kathir and Emma Pulman with their help in sequencing the virus. This work was funded by the Department of Environment, Food and Rural Affairs (DEFRA) through the 'Scanning surveillance for Pig Diseases in England and Wales' (ED1200) project at APHA.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. All samples used for this study were collected for clinical purposes under the Veterinary Surgeons Act 1966, the United Kingdom.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in GenBank at https://www.ncbi.nlm.nih.gov/nuccore/, accession number MT432532-4.

ORCID

Akbar Dastjerdi https://orcid.org/0000-0001-6251-6588

REFERENCES

Becher, P., Avalos Ramirez, R., Orlich, M., Cedillo Rosales, S., König, M., Schweizer, M., Stalder, H., Schirrmeier, H., & Thiel, H. J. (2003). Genetic and antigenic characterization of novel pestivirus genotypes: Implications for classification. *Virology*, *20*, 311(1), 96–104. https://doi.org/10.1016/s0042-6822(03)00192-2

Becher, P., Orlich, M., Shannon, A. D., Horner, G., König, M., & Thiel, H. J. (1997). Phylogenetic analysis of pestiviruses from domestic and wild ruminants. *Journal of General Virology*, *78* (Pt (6),), 1357–66.

Becher, P., Shannon, A. D., Tautz, N., & Thiel, H. J. (1994). Molecular characterization of border disease virus, a pestivirus from sheep. *Virology*, *198*(2), 542–551. https://doi.org/10.1006/viro.1994.1065

Bidewell, C., Williamson, S. M., Whitaker, K., Wessels, M. E., Barnett, J., Scholes, S. F. E., & Gaudie, C. M. (2013). Sporadic cases of multiple haemorrhages in pigs. *Pig Journal*, *68*, 90–94.

Biró, J., Oláh, P., & Palatka, Z. (1966). Attempt to adapt the lapinized hog cholera virus strain "Suvac" to sheep. *Acta Veterinaria Academiae Scientiarum Hungaricae*, *16*(3), 293–299.

Brown, V. R., & Bevins, S. N. (2018). A review of classical swine fever virus and routes of introduction into the United States and the potential for virus establishment. *Front Vet Sci*, *5*, 31. https://doi.org/10.3389/fvets.2018.00031

Caruso, C., Peletto, S., Cerutti, F., Modesto, P., Robetto, S., Domenis, L., Masoero, L., & Acutis, P. L. (2017). Evidence of circulation of the novel border disease virus genotype 8 in chamois. *Archives of Virology*, *162*(2), 511–515. https://doi.org/10.1007/s00705-016-3112-4

Casaubon, J., Vogt, H. R., Stalder, H., Hug, C., & Ryser-Degiorgis, M. P. (2012). Bovine viral diarrhea virus in free-ranging wild ruminants in Switzerland: Low prevalence of infection despite regular interactions with domestic livestock. *BMC Vet Res*, *8*, 204.

Centre for Food Security and Public Health (CFSPH). (2015). Classical Swine Fever. *Factsheets*. Available via www.cfsph.iastate.edu

Colloff, A., Watson, P., Duff, P. J., & Scholes, S. (2012). Haemorrhagic disease in cattle with genotype 1 bovine viral diarrhoea virus infection. *The Veterinary Record*, *171*, 530.

Dastjerdi, A., Fooks, A. R., & Johnson, N. In (C. E. Rupprecht, A. R. Fooks, & B. Abela-Ridder Eds.), (2016). *Laboratory techniques in rabies diagnosis, research and prevention* (5th edn., Vol., 1).

Deng, M., Ji, S., Fei, W., Raza, S., He, C., Chen, Y., Chen, H., & Guo, A. (2015). Prevalence study and genetic typing of bovine viral diarrhea virus (BVDV) in four bovine species in China. *Plos One*, *10*(4), e0121718. https://doi.org/10.1371/journal.pone.0121718

Donis, R. O., Corapi, W., & Dubovi, E. J. (1988). Neutralizing monoclonal antibodies to bovine viral diarrhoea virus bind to the 56K to 58K glycoprotein. *Journal of General Virology*, *69* (Pt (1),), 77–86. https://doi.org/10.1099/0022-1317-69-1-77

Dubois, E., Russo, P., Prigent, M., & Thiéry, R. (2008). Genetic characterization of ovine pestiviruses isolated in France, between 1985 and 2006. *Veterinary Microbiology*, *130*(1-2), 69–79. https://doi.org/10.1016/j.vetmic.2008.01.002

Duncan, C., Ridpath, J., Palmer, M. V., Driskell, E., & Spraker, T. (2008). Histopathologic and immunohistochemical findings in two white-tailed deer fawns persistently infected with Bovine viral diarrhea virus. *Journal of Veterinary Diagnostic Investigation*, *20*(3), 289–96. https://doi.org/10.1177/104063870802000305

Easton, L. A., Vilcek, S., & Nettleton, P. F. (1994). Evaluation of a 'one tube' reverse transcription-polymerase chain reaction for the detection of ruminant pestiviruses. *Journal of Virological Methods*, *50*(1-3), 343–8. https://doi.org/10.1016/0166-0934(94)90189-9

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution; International Journal of Organic Evolution*, *39*, 783–791.

Fernandez, A., Hewicker, M., Trautwein, G., Pohlenz, J., & Liess, B. (1989). Viral antigen distribution in the central nervous system of cattle persistently infected with bovine viral diarrhea virus. *Veterinary Pathology*, *26*, 26–32.

Firth, C., Bhat, M., Firth, M. A., Williams, S. H., Frye, M. J., Simmonds, P., Conte, J. M., Ng, J., Garcia, J., Bhuva, N. P., Lee, B., Che, X., Quan, P. L., & Lipkin, W. I. (2014). Detection of zoonotic pathogens and characterization of novel viruses carried by commensal Rattus norvegicus in New York City. *mBio*, *5*(5), e01933–14. https://doi.org/10.1128/mBio.01933-14

Henningson, J. N., Steffen, D. J., Topliff, C. L., Kurth, K. L., Dubielzig, R. R., Brodersen, B. W., Bedenice, D., Eskridge, K. M., Callan, R. J., Reggiardo, C., Rupp, G. P., & Kelling, C. L. (2013). Systemic distribution of viral antigen in alpacas persistently infected with bovine pestivirus. *Veterinary Pathology*, *50*(2), 308–17. https://doi.org/10.1177/0300985812447827

Hewicker, M., Wöhrmann, T., Fernandez, A., Trautwein, G., Liess, B., & Moennig, V. (1990). Immunohistological detection of bovine viral diarrhoea virus antigen in the central nervous system of persistently infected

cattle using monoclonal antibodies. *Veterinary Microbiology*, *23*, 203–210.

Hewicker-Trautwein, M., Trautwein, G., Frey, H. R., & Liess, B. (1995). Variation in neuropathogenicity in sheep fetuses transplacentally infected with non-cytopathogenic and cytopathogenic biotypes of bovine-virus diarrhoea virus. *Zentralbl Veterinarmed B*, *42*(9), 557–67. https://doi.org/10.1111/j.1439-0450.1995.tb00748.x

Hoffmann, B., Harder, T., Lange, E., Kalthoff, D., Reimann, I., Grund, C., Oehme, R., Vahlenkamp, T. W., & Beer, M. (2010). New real-time reverse transcriptase polymerase chain reactions facilitate detection and differentiation of novel A/H1N1 influenza virus in porcine and human samples. *Berliner Und Munchener Tierarztliche Wochenschrift*, *123*(7-8), 286–92.

Hughes, L. E., Kershaw, G. P., & Shaw, I. G. (1959). B" or border disease. An undescribed disease of sheep. *The Veterinary Record*, *71*, 313–317.

Kawanishi, N., Tsuduku, S., Shimizu, H., Ohtani, Y., Kameyama, K., Yamakawa, M., Tsutsui, T., Matsuura, K., Ohashi, S., Isobe, T., & Yamada, S. (2014). First isolation of border disease virus in Japan is from a pig farm with no ruminants. *Veterinary Microbiology*, *171*(1-2), 210–4. https://doi.org/10.1016/j.vetmic.2014.03.032

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, *35*, 1547–1549.

Kleiboeker, S. B., Schommer, S. K., Lee, S.-M., Watkins, S., Chittick, W., & Polson, D. (2005). Simultaneous detection of North American and European porcine reproductive and respiratory syndrome virus using real-time quantitative reverse-transcriptase-PCR. *Journal of Veterinary Diagnostic Investigation*, *17*, 165–170.

Krametter-Froetscher, R., Kohler, H., Benetka, V., Moestl, K., Golja, F., Vilcek, S., & Baumgartner, W. (2007). Influence of communal alpine pasturing on the spread of pestiviruses among sheep and goats in Austria: First identification of border disease virus in Austria. *Zoonoses Public Health*, *54*(5), 209–213. https://doi.org/10.1111/j.1863-2378.2007.01049.x

La Rocca, S. A., & Sandvik, T. (2009). A short target real-time RT-PCR assay for detection of pestiviruses infecting cattle. *Journal of Virological Methods*, *161*(1), 122–7. https://doi.org/10.1016/j.jviromet.2009.06.005

Loan, R. W., & Storm, M. M. (1968). Propagation and transmission of hog cholera virus in non-porcine hosts. *American Journal of Veterinary Research*, *29*, 807–811.

Makoschey, B., Liebler-Tenorio, E. M., Biermann, Y. M., Goovaerts, D., & Pohlenz, J. F. (2002). Leukopenia and thrombocytopenia in pigs after infection with bovine viral diarrhoea virus-2 (BVDV-2). *Deutsche Tierarztliche Wochenschrift*, *109*(5), 225–230.

McGoldrick, A., Bensaude, E., Ibata, G., Sharp, G., & Paton, D. J. (1999). Closed one-tube reverse transcription nested polymerase chain reaction for the detection of pestiviral RNA with fluorescent probes. *Journal of Virological Methods*, *79*(1), 85–95. https://doi.org/10.1016/s0166-0934(99)00010-5

Montgomery, D. L. (2007). Distribution and cellular heterogeneity of bovine viral diarrhea viral antigen expression in the brain of persistently infected calves: A new per-spective. *Veterinary Pathology*, *44*, 643–654.

Oguzoglu, T. C., Tan, M. T., Toplu, N., Demir, A. B., Bilge-Dagalp, S., Karaoglu, T., Ozkul, A., Alkan, F., Burgu, I., Haas, L., & Greiser-Wilke, I. (2009). Border disease virus (BDV) infections of small ruminants in Turkey: A new BDV subgroup? *Veterinary Microbiology*, *135*(3-4), 374–379. https://doi.org/10.1016/j.vetmic.2008.09.085

Otter, A., Welchman, D., de, B., Sandvik, T., Cranwell, M. P., Holliman, A., Millar, M. F., & Scholes, S. F. (2009). Congenital tremor and hypomyelination associated with bovine viral diarrhoea virus in 23 British cattle herds. *The Veterinary Record*, *20*, 164(25), 771–8. https://doi.org/10.1136/vr.164.25.771

Passler, T., Riddell, K. P., Edmondson, M. A., Chamorro, M. F., Neill, J. D., Brodersen, B. W., Walz, H. L., Galik, P. K., Zhang, Y., & Walz, P. H. (2014). Experimental infection of pregnant goats with bovine viral diarrhea virus (BVDV) 1 or 2. *Veterinary Research*, *45*(1), 38. https://doi.org/10.1186/1297-9716-45-38

Passler, T., Walz, H. L., Ditchkoff, S. S., van Santen, E., Brock, K. V., & Walz, P. H. (2012). Distribution of bovine viral diarrhoea virus antigen in persistently infected white-tailed deer (Odocoileus virginianus). *Journal of Comparative Pathology*, *147*(4), 533–41. https://doi.org/10.1016/j.jcpa.2012.02.008

Paton, D. J., & Done, S. H. (1994). Congenital infection of pigs with ruminant-type pestiviruses. *Journal of Comparative Pathology*, *111*(2), 151–63.

Paton, D. J. (1995). Pestivirus diversity. *Journal of Comparative Pathology*, *112*, 215–236.

Potts, B. J., Laurence, J., Berry, L. J., Osburn, B. I., & Johnson, K. P. (1985). Viral persistence and abnormalities of the central nervous system after congenital infection of sheep with border disease virus. *The Journal of Infectious Diseases*, *151*, 337–343. https://doi.org/10.1093/infdis/151.2.337

Ridpath, J. F. (2010). Bovine viral diarrhea virus: Global status. *The Veterinary Clinics of North America. Food Animal Practice*, *26*(1), 105–121. https://doi.org/10.1016/j.cvfa.2009.10.007

Roehe, P. M., Woodward, M. J., & Edwards, S. (1992). Characterisation of p20 gene sequences from a border disease-like pestivirus isolated from pigs. *Veterinary Microbiology*, *33*(1-4), 231–238. https://doi.org/10.1016/0378-1135(92)90051-t

Rosell, R., Cabezón, O., Pujols, J., Domingo, M., Muñoz, I., Núñez, J. I., & Ganges, L. (2014). Identification of a porcine pestivirus as a border disease virus from naturally infected pigs in Spain. *The Veterinary Record*, *174*(1), 18. https://doi.org/10.1136/vr.101920

Schrader, C., Schielke, A., Ellerbroek, L., & Johne, R. (2012). PCR inhibitors - occurrence, properties and removal. *Journal of Applied Microbiology*, *113*(5), 1014–26. https://doi.org/10.1111/j.1365-2672.2012.05384.x

Shimizu, M., & Kumagai, T. (1989). Experimental infection of pregnant goats with swine fever virus. *Vet. Microbiol*, *20*, 207–214.

Smith, D. B., Meyers, G., Bukh, J., Gould, E. A., Monath, T., Scott Muerhoff, A., Pletnev, A., Rico-Hesse, R., Stapleton, J. T., Simmonds, P., & Becher, P. (2017). Proposed revision to the taxonomy of the genus Pestivirus, family Flaviviridae. *Journal of General Virology*, *98*(8), 2106–2112. https://doi.org/10.1099/jgv.0.000873

Stalder, H. P., Meier, P., Pfaffen, G., Wageck-Canal, C., Rüfenacht, J., Schaller, P., Bachofen, C., Marti, S., Vogt, H. R., & Peterhans, E. (2005). Genetic heterogeneity of pestiviruses of ruminants in Switzerland. *Preventive Veterinary Medicine*, *72*(1-2), 37–41. https://doi.org/10.1016/j.prevetmed.2005.01.020

Strong, R., La Rocca, S. A., Ibata, G., & Sandvik, T. (2010). Antigenic and genetic characterisation of border disease viruses isolated from UK cattle. *Veterinary Microbiology*, *141*(3-4), 208–15. https://doi.org/10.1016/j.vetmic.2009.09.010

Sullivan, D. G., Chang, G. J., & Akkina, R. K. (1997). Genetic characterization of ruminant pestiviruses: Sequence analysis of viral genotypes isolated from sheep. *Virus Research*, *47*(1), 19–29. https://doi.org/10.1016/s0168-1702(96)01402-5

Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, *10*, 512–526.

Tautz, N., Tews, B. A., & Meyers, G. (2015). The molecular biology of pestiviruses. *Advances in Virus Research*, *93*, 47–160. https://doi.org/10.1016/bs.aivir.2015.03.002

Toplu, N., Oguzoglu, T. Ç., Epikmen, E. T., & Aydogan, A. (2011). Neuropathologic study of border disease virus in naturally infected fetal and neonatal small ruminants and its association with apoptosis. *Veterinary Pathology*, *48*(3), 576–583. https://doi.org/10.1177/0300985810371309

Valdazo-González, B., Alvarez-Martínez, M., & Sandvik, T. (2007). Genetic and antigenic typing of border disease virus isolates in sheep from the Iberian Peninsula. *Veterinary Journal*, *174*(2), 316–324. https://doi.org/10.1016/j.tvjl.2006.10.002

Vilcek, S., Björklund, H. V., Horner, G. W., Meers, J., & Belák, S. (1998). Genetic typing of pestiviruses from *New Zealand*. *New Zealand Veterinary Journal*, *46*(1), 35–7. https://doi.org/10.1080/00480169.1998.36048

Vilcek, S., Nettleton, P. F., Paton, D. J., & Belák, S. (1997). Molecular characterization of ovine pestiviruses [published correction appears in *J Gen Virol* 78(Pt 8), 2129]. *Journal of General Virology*, *78*(Pt (4)), 725–735. https://doi.org/10.1099/0022-1317-78-4-725

Waldvogel, A. S., Ehrensperger, F., Straub, O. C., & Pospischil, A. (1995). An immunohistochemical study of the distribution of border disease virus in persistently infected sheep. *Journal of Comparative Pathology*, *113*, 191–200.

Wilhelmsen, C. L., Bolin, S. R., Ridpath, J. F., Cheville, N. F., & Kluge, J. P. (1991). Lesions and localization of viral antigen in tissues of cattle with experimentally induced or naturally acquired mucosal disease, or with naturally acquired chronic bovine viral diarrhea. *American Journal of Veterinary Research*, *52*, 269–275.

Wöhrmann, T., Hewicker-Trautwein, M., Fernandez, A., Moennig, V., Liess, B., & Trautwein, G. (1992). Distribution of bovine virus diarrhoea viral antigens in the central nervous system of cattle with various congenital manifestations. *Zentralbl Veterinarmed B*, *39*(8), 599–609. https://doi.org/10.1111/j.1439-0450.1992.tb01211.x

Wu, Z., Liu, B., Du, J., Zhang, J., Lu, L., Zhu, G., Han, Y., Su, H., Yang, L., Zhang, S., Liu, Q., & Jin, Q. (2018). Discovery of diverse rodent and bat pestiviruses with distinct genomic and phylogenetic characteristics in several Chinese provinces. *Front Microbiol*, *9*, 2562. https://doi.org/10.3389/fmicb.2018.02562

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Dastjerdi, A., Strong, R., La Rocca, S. A., Wessels, M., Wessels, J., Whitaker, K., Strugnell, B., & Williamson, S. (2022). Investigation into an outbreak of Border disease virus in pigs in England. *Transboundary and Emerging Diseases*, *69*, 1698–1706. https://doi.org/10.1111/tbed.14539