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RESEARCH ARTICLE

Development and Application of an ELISA for the Detection of Porcine Deltacoronavirus IgG Antibodies

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Abstract

Porcine deltacoronavirus (PDCoV), also known as porcine coronavirus HKU15, was first detected in North America in early 2014 and associated with enteric disease in pigs, resulting in an urgent need to further investigate the ecology of this virus. While assays detecting nucleic acids were implemented quickly, assays to detect anti-PDCoV antibodies have not been available. In this study, an indirect anti-PDCoV IgG enzyme-linked immunosorbent assay (ELISA) based on the putative S1 portion of the spike protein was developed and utilized to determine the prevalence of anti-PDCoV IgG in U.S. pigs. The diagnostic sensitivity of the PDCoV ELISA was 91% with a diagnostic specificity of 95%. A total of 968 serum samples were tested including samples with confirmed infection with PDCoV, porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus or porcine respiratory coronavirus. There was no cross-reactivity with any of the other coronaviruses. Among 355 arbitrarily selected serum samples collected in 2014 and originating from 51 farms across 18 U.S. states, anti-PDCoV IgG antibodies were detected in 8.7% of the samples and in 25.5% of the farms whereas anti-PEDV IgG was detected in 22.8% of the samples and in 54.9% of the farms. In addition, anti-PDCoV IgG antibodies were detected in archived samples collected in 2010, perhaps indicating an earlier undetected introduction into the U.S. pig population. Overall, the obtained data suggest that PDCoV seroprevalence in U.S. pigs is lower compared to PEDV and PDCoV may have been introduced to the U.S. prior to PEDV.

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Introduction

Porcine deltacoronavirus (PDCoV) was identified in archived samples collected in 2009 from a pig in Hong Kong, China, and initially reported in Asian and American leopard cats identified in apparently healthy wild animals [1–3]. Recently, PDCoV has been described in association with diarrhea in pig production all production ages in North America [4–6]. Specifically, in February 2014, the Ohio Department of Agriculture issued a press release indicating that

PDCoV 6a de ec ed in 6ine fece fıom fi; e epaıa e pig faım in Ohio. 6i h clinical ign of 6a eı8 diaııhea in o6 and en eıic di ea e a ocia ed 6i h incıea ed moı ali 8 in pigle [4]. The pıe ence of ıan mi ible ga ıoen eıi i ; iı (TGEV) 6a ı led o b a poı ion of he ample 6eıe al o po i i; e foı poıcine epidemic diaııhea ; iı (PEDV) [4]. In ıe pon e o he impac of he ne6l8 emeıging poıcine coıona; iı e PEDV [7,8] and PDCoV [4] in he U.S. pig pop la ion, he USDA i ed a fedeıal oıdeı ıeq iıing ıepoı ing of all no; el ca e a ocia - ed 6i h all poıcine en eıic coıona; iı e effec i; e J ne 5, 2014 [9]. In con ıa o PEDV [10–12], he epidemiolog8, ; iıal pa hogene i and clinical 8mp om a ocia ed 6i h PDCoV infec- ion aıe ill laıgel8 nkno6n. Ne; eı hele , pıeliminai 8ıe l ob ained af eı e peıimen al in- fec ion of gno obio ic pig 6i h hi ; iı indica e e; eıe clinical ign (diaııhea, ; omi ing, deh8dıa ion) and micıo copic le ion con i en 6i h coıona; iı infec ion in he mall in e - ine [13]. Ho6 e; eı, eıological a a8 o ndeı ake field ba ed epidemiological die aıe no a; ailable o da e. Seq encing of he comple e genome of U.S. PDCoV ıain fıom diffeıen

pιioι o he eι m collec ion (n = 60/faιm). The la 30 ample came fιom PDCoV nega i;e Faιm D. The PDCoV infec ion a 6a de eιmined ba ed on ιeal- ime RT-PCR foι PDCoV on fecal ample . PEDV, PRCV and TGEV RNA 6 eιe no de ec ed in he fece . All RT-PCR 6 eιe peιfoιmed accoιding o ιo inel8 peιfoιmed andaιd pιo ocol a he ISU-VDL. Faιm A ample collec ed a he fiι ime poin and ample fιom he nega i;e faιm D 6 eιe cla ified a nega i;e (n = 60). Faιm A ample collec ed a he econd ime poin and ample fιom he 6o po i i;e Faιm B and C 6 eιe cla ified a po i i;e (n = 150). Foι he paiιed ample e fιom Faιm A, PDCoV eιocon;eι ion 6a defined a a fo ι-fold incιea e in he an ibod8 i eι 6hen com-paιing he fiι and econd ample collec ion poin .

(75). A o al of 355 eι m ample 6 eιe aιbi ιaιil8 elec ed d ιing 2014 fιom U.S. pig faιm ιa ified b8 geogιaphic oιigin a paι of niιela ed diagno ic bmi ion o he ISU-VDL. Fi;e eι m ample 6 eιe elec ed fιom each of 71 independen bmi ion ca e fιom 51 diffeιen faιm (n = 5 o 25 ample peι faιm) loca ed in 18 diffeιen a e acιo he U.S. (Coloιado, Io6a, Il-linoi , Indiana, Kan a , Ken ck8, Michigan, Minne o a, Mi o ιi, Mon ana, Nebιa ka, Ne6 Jeι e8, Noι h Caιolina, Ohio, Oklahoma, Penn 8l;ania, So h Dako a, and Wi con in). All 355 ample 6 eιe e ed foι pιe ence of an i-PDCoV IgG and foι an i-PEDV IgG an ibodie ing an a a8 pιe;io l8 de cιibed [25].

Aιchi;ed eι m ample (n = 403) collec ed fιom 2006 o 2013 pιioι o ini ial ιecogni ion of PDCoV fιom 25 diffeιen faιm fιom i diffeιen a e in he U.S. (Io6a, Illinoi , Nebιa ka, Noι h Caιolina, Mi o ιi, Te a) [25–27] 6 eιe al o incl ded and e ed foι pιe ence of an i-PDCoV IgG.

. A o al of 52 pooled liq id poιcine pla ma ample collec ed in 2010 a paι of a pιe;io d8 [28] 6 eιe elec ed foι e ing. Each poιcine pla ma ample con i ed of pooled pla ma oιigina ing fιom e ang ina ion of appιo ima el8 10,000 pig la gh eιed on he ame da8. The ample 6 eιe collec ed fιom 14 fedeιall8 in pec ed aba oiι loca ed in he ea eιn paι oι in he Mid6 e eιn U.S. [28]. Ba ed on ιeal- ime RT-PCR e ing, all ample ob ained in 2010 6 eιe nega i;e foι PDCoV RNA.

PDCoV S1 ELISA development

. The ιegion encoding he p a i;e S1 domain (amino acid 1–573) of he PDCoV ιain PDCoV-IA2014-1 (GenBank acce ion n mbeι KM613173) 6a 3' eιminall8 f ed 6i h a hιombin clea;age eq ence follo6ed b8 a h man IgG Fc domain. 6hich 6a b e-q en l8 cloned in o a e kaι8o ic e pιe ion ;ec oι a pιe;io l8 de cιibed [25]. The S1-Fc f ion pιo ein 6 eιe e pιe ed b8 ιan fec ion of HEK-293T cell , p ιified ing pιo ein A col mn p -ιifica ion, clea;ed 6i h hιombin o ιemo;e he Fc ag, and ιea ed foι endo o in ιemo;al.

. The op imal an igen concen ιa ion and he eι m dil ion foι he S1-PDCoV ELISA 6 eιe de eιmined ing a checkeιboaιd i ιa ion. Micιo i eι pla e (N nc; Theιmo Fi heι Scien ific, Aga6am, MA, USA) 6 eιe coa ed 6i h he S1 pol8pep ide dil ed in coa ing b ffeι (50 mM caιbona e b ffeι, pH 9.6) a a concen ιa ion of 0.95 ng peι 6 ell and in-c ba ed o;eι nigh a 4 C. Af eι hιee 6a he 6i h PBS con aining 0.05% T6 een 20 (PBST), he pla e 6 eιe blocked 6i h 1% bo;ine eι m alb min (Jack on Imm noRe eaιch, We Gιo;e, PA, USA) foι 2 h a 22 C and hen inc ba ed 6i h he eι m oι pla ma ample dil ed 1:100 in PBS con aining 10% goa eι m (Gibco; Life Technologie , Gιand I land, NY, USA) foι 30 min a 37 C. Af eι a 6a hing ep, a 1:10,000 dil ed peιo ida e-conj ga ed goa an i- 6ine IgG (Jack on Imm noRe eaιch) 6a added and inc ba ed a 37 C foι 30 min. The peιo ida e ιeac ion 6a ;i ali ed b8 ing e ιame h8lben idine-h8dιogen peιo ide ol ion a he

b ıa e (KPL, Gai he ı b ıg, MD, USA) fo ı 10 min a ıoom empe ıa ıe and opped b8 adding 50 μL of 2 M lf ıic acid o each 6 ell. Op ical den i ie (OD) 6 e ıe mea ıed a 450 nm ing an ELISA pla e ıeade ı (BioTek, Winoo ki, VT, USA). Se ı m dil ion ha ga ; e he g ıea e ıa io be 6 een he po i i ; e and he nega i ; e ample (P/N) 6 e ıe elec ed a con ıol fo ı b eq en ı n and po i i ; e nega i ; e and blank (e ıile 6 a e ı) ample 6 e ıe e ed in d plica e and incl ded on each pla e.

. The pecifici 8 of he PDCoV S1 ELISA 6 a e ; al a ed b8 ing e ı m ample ingle-po i i ; e and 6 i h high an ibod8 le ; el again TGEV ($n = 30$), PRCV ($n = 30$) o ı PEDV ($n = 30$) 6 hich 6 e ıe ob ained h ı o gh he ISU-VDL. The c -off 6 a calc la ed b8 ıece i ; e ı ope ıa o ı cha ıac e ıi ic (ROC) anal8 i fo ı ma im m diagno ic en i i ; i 8 and pecifici 8 ing ample cla ified a PDCoV po i i ; e ($n = 150$) o ı nega i ; e ($n = 60$ ample). The c off ; al e 6 a elec ed o ma imi e en i i ; i 8 and pecifici 8 6 hile minimi ing he m of fal e nega i ; e and fal e po i i ; e ıe l . The ob ained ; al e 6 a f ı he e ; al a ed ing he c m la i ; e da a fı om all o he ı ample . The ROC 6 a defined o de e ımine he c -off of he PDCoV S1 ELISA ing MedCalc fo ı Windo6 , ; e ı ion 13.3.0.0. (MedCalc Sof 6 a ıe, O end, Belgi m).

. The ıep ıod cibili 8 of he PDCoV S1 ELISA 6 a e ; al a ed b8 i li ing eigh e ı m ample 6 i h diffe ıen an ibod8 i e ı . The coefficien of ; a ıia ion (CV) 6 a ed o e ; al a e he in ıa- and in e ı-a a8 ; a ıia ion. Each ample 6 a e ed on each of h ıee pla e on diffe ıen occa ion o de e ımine he in e ı-a a8 CV, and h ıee ıeplica e 6 i hin he ame pla e 6 e ıe ed o calc la e he in ıa-a a8 CV.

Results

PDCoV S1 ELISA development

The ROC anal8 i ba ed on 210 e ı m ample 6 i h kno6 n PDCoV e po ıe 6 a ed fo ı he c -off de e ımina ion (Fig 1). The op imal c -off fo ı he PDCoV S1 ELISA 6 a a 1:100 dil ed ample OD ; al e of 0.34 fo ı 6 hich he en i i ; i 8 and pecifici 8 ; al e 6 e ıe highe ı han 90%. Sen i i ; i 8 6 a 90.6% and he pecifici 8 6 a 94.8%. The diagno ic acc ıac8 of PDCoV S1 ELISA 6 a con ide ıed o be high a he a ıea nde ı he c ı ; e (AUC) inde 6 a 0.98 6 i h a an da ıd e ıo ı of 0.01. In e ı and in ıa-coefficien of ; a ıia ion (CV) of eigh con ıol e ıa e ed 6 i h PDCoV S1 ELISA 6 a le han 10%. The in ıa-a a8 CV ıanged fı om 2.6% o 4.2% 6 hile he in e ı-a a8 CV ıanged fı om 5.2% o 9.4%, indica ing ha he ıe l 6 e ıe ıep ıod cible. The a a8 pecifici 8 6 a de e ımined b8 ing ample po i i ; e fo ı an ibodie o TGEV, PRCV o ı PEDV. The ob ained PDCoV S1 ELISA OD ; al e ıanged fı om 0.04 o 0.33 (a ; e ıage SD, 0.12 0.06), indica ing a lack of c ıo -ıeac ion 6 i h o he ı po ıcine co ıona ; i e . Among he 90 ample po i i ; e fo ı an ibodie again TGEV, PRCV o ı PEDV, onl8 one had an OD ; al e highe ı han 0.3 fo ı PDCoV. Specificall8, PDCoV OD ; al e ıanged fı om 0.04 o 0.33 (a ; e ıage SD, 0.12 0.08) fo ı TGEV; 0.05 o 0.291 (a ; e ıage SD, 0.10 0.06) fo ı PRCV; and 0.07 o 0.244 (a ; e ıage SD, 0.13 0.05) fo ı PEDV.

Presence of anti-PDCoV IgG antibodies in pig serum during an acute outbreak and four weeks later

An i-PDCoV IgG an ibod8 po i i ; e e ı m ample 6 e ıe de ec ed in 7 (23.3%) of he 30 o6 ac el8 affec ed b8 dia ıhea and in 28 (93.3%) ample fo ı 6 eek af e ı PDCoV RT-PCR diagno i (Fig 2). T6 o o6 6 e ıe e ıonega i ; e a bo h collec ion ime . Se ıocon ; e ı ion, cha ıac e ıi ed b8 a lea a fo ı-fold inc ıea e in he OD ; al e 6 a de ec ed in 16 (53.3%) o6 . Among he e ıopo i i ; e o6 iden ified d ıing he fi ı collec ion, 1/7 ho6 ed a e ıocon ; e ı ion

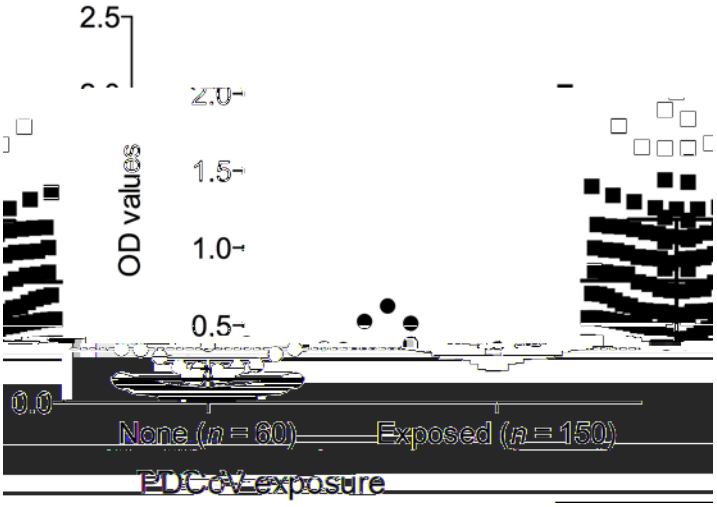

Fig 1. Distribution of serum anti-PDCoV IgG antibodies obtained from farms with known PDCoV exposure. Serum samples were classified as negative or positive based on viral RNA detection on fecal samples at the farm. Data presented as ELISA OD values ± SEM. The assay cut-off (OD value of 0.34) is indicated by the dashed line.

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(4.4-fold incıea e in he OD ;al e); 3/7 had a lea a 2-fold incıea e in he OD ;al e (a;eıage SD 2.46 0.74); and 3/7 had OD ;al e incıemen lo6 eı han 1.5-fold.

Presence of anti-PDCoV and -PEDV IgG antibodies in pig serum samples with unknown PDCoV exposure collected during 2014

An i-PDCoV and -PEDV IgG an ibod8 pıe;alence ıa e in 355 eı m ample collec ed d ıing 2014 aıe mmaıi ed in Table 1. Thii 8-one eı m ample (8.7%) 6 eıe an i-PDCoV IgG an i-bod8 po i i;e 6 hich 6 eıe iden ified in 13/51 (25.5%) faım . Po i i;e de ec ion ıa e in

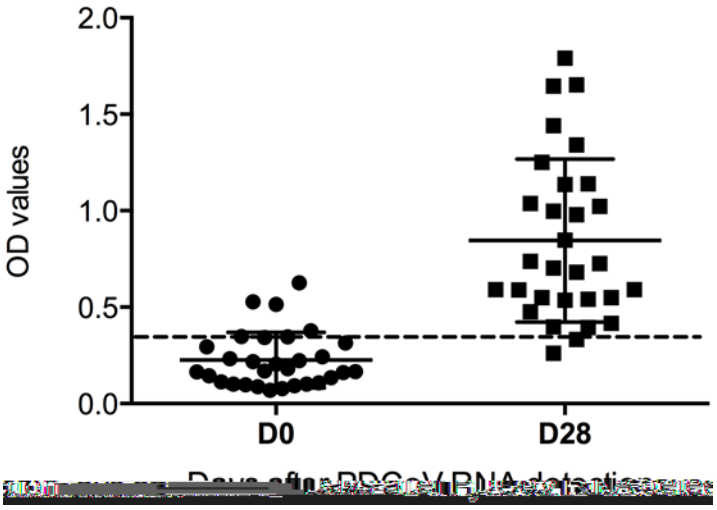

Fig 2. Distribution of serum anti-PDCoV IgG antibodies during an acute outbreak and four weeks later. An acute outbreak was defined as presence of clinical disease and demonstration of PDCoV RNA in feces. Data presented as ELISA OD values ± SEM. The assay cut-off (OD value of 0.34) is indicated by the dashed line.

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Table 1. Detection rate of anti-PDCoV and anti-PEDV IgG antibodies in pig sera samples collected during 2014.

State	Number of positive samples/number samples tested (Number of positive farms/number of farms tested)	
	PDCoV	PEDV
Colorado	0/5 (0/1)	3/5 (1/1)
Iowa	12/145 (7/20)	21/145 (11/20)
Illinois	1/15 (1/3)	4/15 (2/3)
Indiana	3/20 (1/4)	7/20 (2/4)
Kansas	0/5 (1/1)	0/5 (1/1)
Kentucky	0/5 (0/1)	0/5 (0/1)
Michigan	0/5 (0/1)	0/5 (0/1)
Minnesota	0/5 (0/1)	0/5 (0/1)
Missouri	3/20 (1/3)	20/20 (3/3)
Montana	0/5 (0/1)	0/5 (0/1)
North Carolina	0/30 (0/2)	1/30 (1/2)
Nebraska	8/10 (2/2)	7/10 (2/2)
New Jersey	0/5 (0/1)	0/5 (0/1)
Ohio	0/5 (0/1)	0/5 (0/1)
Oklahoma	0/35 (0/4)	15/35 (2/4)
Pennsylvania	4/20 (1/3)	3/20 (1/3)
South Dakota	0/5 (0/1)	0/5 (0/1)
Wisconsin	0/5 (0/1)	0/5 (0/1)
Total	31/355 (13/51)	81/355 (28/51)

All serum samples were obtained from commercial pig farms in 18 different states across the U.S.A.

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indi; id al PDCoV fa1m 1anged f1om 20 o 100%. An i-PEDV IgG an ibodie 6 e1e de ec ed in 81/355 (22.81%) e1 m ample and in 28/51 (54.9%) of he in; e iga ed fa1m . Conc 11en de- ec ion of an i-PEDV and an i- PDCoV IgG an ibodie occ 11ed in 8/51 fa1m (15.7%) and 23/ 355 (6.5%) e1 m ample .

Presence of anti-PDCoV IgG antibodies in pig serum and plasma samples with unknown PDCoV exposure collected prior to 2014

Among he 403 a1chi; ed e1 m ample collec ed be 6 een 2006 and 2013, 44 (10.9%) e1 m ample 6 e1e fo nd o be po i i; e fo1 an i-PDCoV IgG an ibodie b8 he S1 ELISA (Table 2). The majo1i 8 of po i i; e ample 6 e1e collec ed in 2013 (40/44, 90.9%). On po i i; e fa1m , 20 o 60% of he e1 m ample 6 e1e fo nd o be po i i; e. In e1e ingl8, fo 1 ample 6 e1e col- lec ed in 2010; pecificall8, h1ee e1 m ample o11igina ed on a fa1m in Illinoi and one e1 m ample 6 a f1om a fa1m in Io6 a. The OD ; al e on he e ample 6 e1e 0.38, 0.39, 0.42, and 1.27. D e o he limi ed a; ailabili 8 of 1e 1o pec i; e e1a and o f 1 he1 confi1m hi find- ing, 52 po1cine pooled pla ma ample f1om 2010 6 e1e al o e ed. T6 o o of 52 (3.8%) pooled pla ma ample 6 e1e po i i; e fo1 an i-PDCoV IgG an ibodie ; he OD ; al e 6 e1e 0.34 and 0.38 6 he1ea he OD ; al e of nega i; e ample 1anged f1om 0.05 o 0.15 (a; e1age SD, 0.08 0.01).

Table 2. Detection rate of anti-PDCoV IgG antibodies from 2006 to 2014 in the U.S.A.

Year	PDCoV positive samples/number of samples tested (PDCoV positive farms/number of farms tested)
2006	0/19 (0/1)
2007	0/16 (0/1)
2010	4/58 (2/4)
2011	0/9 (0/2)
2012	0/91 (0/9)
2013	40/210 (6/10)
2014	31/355 (13/51)
Total	75/758 (21/78)

All serum samples were obtained from commercial pig farms.

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Discussion

PDCoV 6a ini ial8 di co: e1ed in U.S. pig in 2014 [4] leading o man8 q e ion on ba ic infec ion d8namic and ime of in 1od c ion of hi eme1gen pig : i1 . The objec i: e of hi d8 6e1e o de: elop a e1ological a a8 o e: al a e he 2014 p1e: alence 1a e of PDCoV in U. S. pig and o de e1mine e: idence fo1 PDCoV infec ion in p1e: io 8ea1 .

In o1de1 o accompli h hi goal, a 1ecombinan PDCoV S1 pol8pep ide-ba ed ELISA 6a de: eloped and he S1 b ni 6a elec ed a coa ing an igen. The amino acid iden i 8 of he PDCoV S1 b ni and he co11e ponding PEDV co n e1pa1 (IA1 1ain, GenBank acce ion n mbe1 KF468753) i 20.2%, fo1 TGEV (P 1d e 1ain, GenBank acce ion n mbe1 AJ271965) i i 20.7% and fo1 PRCV (ISU-1 1ain, GenBank acce ion n mbe1 DQ811787) i i 22.1% [5]. The1efo1e, he PDCoV S1 b ni ed in he p1e en d8 6a nlikel8 o c1o -1eac 6i h PEDV, PRCV o1 TGEV and a e pec ed, c1o -1eac i: i 8 6a no ob e1 ed. F 1 he1mo1e, *in silico* p1edic ion of he PDCoV epi ope 6a pe1fo1med and 1e l 6e1e compa1ed 6i h he alphaco1ona: i1 PEDV, TGEV and PRCV. A e pec ed, he S1 p1o ein con ained he majo1i 8 of he mapped epi ope 6i hin he co1ona: i1 p1o ein kno6n o elici h mo1al 1e pon e (E, M, N and S). In addi ion, a compa1i on of he amino acid iden i 8 be 6een he e p1e ed PDCoV S1 an igen fo1 he de: eloped ELISA and he o he1 19 PDCoV eq ence in he Gen-Bank indica ed a imila1i 8 highe1 han 99% fo1 he S1 pol8pep ide. 6hich gge ha hi egmen i con e1: ed eno gh o de ec h mo1al 1e pon e di1ec ed o i .

A e pe1imen all8 gene1a ed ample o1 a gold anda1d e fo1 PDCoV an ibod8 de ec ion 6e1e no a: ailable a he ime he d8 6a cond c ed, field ample 6i h kno6n PDCoV e - po 1e and fi1om p1e med nega i: e pig 6e1e ili ed o gain in igh on ba ic PDCoV e1o-con: e1 ion. An i-PDCoV an ibodie and e1ocon: e1 ion 6e1e de ec ed 6i hin fo 1 6eek of ini ial ob e1: a ion of clinical di ea e and de ec ion of PDCoV RNA in fecal ample . Ideall8, he c -off : al e ho ld be de1i: ed b8 e ing a panel of ample ob ained fi1om 1efe1ence ani-mal 6i h kno6n hi o18 and infec ion a 1ela i: e o he di ea e [29]. D e o diffic l ie in cla if8ing pig fi1om fa1m a 1 e nega i: e, i.e. no p1e: io e po 1e o PDCoV 6hich 6o ld ha: e 1eq i1ed o ob ain fecal ample o: e1 - ime fi1om an adeq a e n mbe1 of pig on he fa1m, ample fi1om he a1 of pe1cei: ed PDCoV o b1eak (PDCoV 1eal- ime RT-PCR po i i: e pig) 6e1e con ide1ed o be e1onega i: e fo1 e de: elopmen p 1po e . Al ho gh hi 1a eg8 6a fficien o confi1m diagno i of ac e infec ion a e1ocon: e1 ion 6a de ec ed in pai1ed ample fi1om PDCoV o b1eak , he lack of a econd e1ological a a8 and kno6n nega i: e ample did no pe1mi a p1eci e e ima ion of he diagno ic en i i: i 8 and pecifici 8 of he e . Selec ing a ingle a1bi 1a18 c -off : al e en i1el8 on he ba i of field ample and

a ocia ion 6i h clinical hi o18 and RT-PCR 1e l co ld 1e l in a lo of en i i:i 8 and/o1 pecifici 8. To f 1 he1 add1e hi , he e abli hed c -off :al e of 0.34 6a e:al a ed ing he c m la i:e da a of 573 ample o1igina ing f1om e1onega i:e fa1m (cla ified a nega i:e d e o ab ence of an8 PDCoV ELISA po i i:e pig among he pig e ed) b8 calc la ing he a:e1age OD (da a no ho6n). I 6a de e1mined ha he adop ion of hi me hod ob ained lo6 e1 c -off :al e (0.24; OD a:e1age 3 SD; a:e1age SD 0.09 0.05), and p1o:ided a g1ea e1 en i-i:i 8. The1efo1e, ample 6i h an OD :al e be 6een 0.24 and 0.34 ho ld pe1hap be con id-e1ed inconcl i:e. The lack of a gold andad fo1 PDCoV an ibod8 de ec ion f 1 he1 con 1ib e o he p1oblem in e abli hing an app1op1ia ed c -off and acc 1a el8 mea 1e he en i i:i 8 and pecifici 8 of he ELISA de:eloped he1ein.

Ba ed on c m la i:e da a f1om he Na ional Animal Heal h Labo1a o18 Ne 6o1k (NAHLN) labo1a o1ie h1o gh 17 Sep 2014, 6.6% (382) of 5827 ca e ob ained f1om 17 of 31 a e 6e1e po i i:e fo1 PDCoV RNA [30]. In he p1e en d8, 8.7% (31/ 355) of he e1 m ample col-lec ed f1om 7/18 a e 6e1e po i i:e fo1 an i-PDCoV IgG 6hich i in ag1eemen 6i h he Ani-mal and P blic Heal h Info1ma ion S8 em (APHIS) 1:eillance da a and PDCoV RNA de ec ion b8 RT-PCR. In con 1a , he pe1cen age of PEDV RNA po i i:e ca e 6a 26.0% (8386/32211) ob ained f1om a o al of 31/42 U.S. a e acco1ding o he mo 1ecen NAHLN 1:e8 [30]. In f 1 he1 ag1eemen , in hi d8, 22.8% (81/355) of he ample a1bi 1a1il8 e-lec ed in 2014 6e1e po i i:e fo1 PEDV IgG.

I ha been de e1mined p1e:io l8 ha co-infec ion of PDCoV 6i h o he1 en e1ic :i1 e a1e common [9]. In he p1e en in:e iga ion, 38% of PEDV ELISA po i i:e pig 6e1e al o po i-i:e fo1 an i-PDCoV an ibodie (da a no ho6n). Thi i in ag1eemen 6i h p1e:io 1epo1 6hich fo nd ha 78% of PDCoV RNA po i i:e ample 6e1e ei he1 po i i:e fo1 PEDV RNA, 1o a:i1 g1o p A RNA, 1o a:i1 g1o p B RNA, o1 1o a:i1 g1o p C RNA and 33% 6e1e co-infec ed 6i h PEDV a de e1mined b8 RT-PCR [14]. E pe1imen al 1ial a1e needed o be e1 nde1 and he compa1a i:e :i1 lence of PDCoV o o he1 en e1ic pa hogen .

PDCoV 6a fi1 iden ified in U.S. pig in 2014 [4] and 1e 1o pec i:e die a1ge ing de ec-ion of RNA ha:e been able o iden if8 he :i1 in la e 2013 [30]. Acce o a1chi:ed e1 m ample can be limi ed and o o:e1come hi i e, a1chi:ed po1cine pla ma ample 6e1e al o ed in hi d8. While he PDEV a a8 6a no :alida ed on pla ma, e:idence in he li e1a-1e gge ha he deg1ee of an ibod8 de ec ion in e1 m and pla ma i e en ial8 iden ical [31–34]. O:e1all, he ob ained da a gge ha PDCoV ha been ci1c la ing in he No1 h Ame1ican pig pop la ion p1io1 o 2013 6i ho being 1ecogni ed. Specifical8, an ibodie o PDCoV 6e1e de ec ed in a1chi:ed e1 m and pla ma ample f1om 2010. 6he1ea an i-PDCoV IgG an ibodie 6e1e no de ec ed in e1 m ample collec ed in 2011 o1 2012. Thi co ld be d e o he limi ed n mbe1 of a:ailable 1e 1o pec i:e e1 m ample in combina ion 6i h an o:e1all lo6 PDCoV e1op1e:alence. Al e1na i:el8, he po i i:e ample collec ed in 2010 co ld be fal e po i i:e: ho6e:e1, a lea one ample p1e en ed a high OD :al e. Upon a:ailabili 8 of ano he1 PDCoV e1ological e in he f 1e he e1e l 6ill need o be confi1med.

In concl ion, a al1ead8 indica ed b8 PDCoV RT-PCR 1:eillance 1e l , he ob ained an i-PDCoV IgG p1e:alence da a f 1 he1 confi1m an o:e1all lo6 p1e:alence 1a e of PDCoV in-fec ion in he U.S. pig pop la ion.

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Author Contributions

Conceived and designed the experiments: TO. Performed the experiments: AT CTX. Analyzed the data: PFG. Contributed reagents/materials/analysis tools: YWH. Wrote the paper: AT PFG CTX YWH TO. Carried out the statistical analysis and created the figure: PFG.

References

1. Woo PC, Lau SK, Lam CS, Lai KK, Huang Y, Lee P, et al. Comparative analysis of complete genome sequences of three avian coronaviruses reveals a novel group 3c coronavirus. J Virol. 2009; 83: 908–917. doi: 10.1128/JVI.01977-08 PMID: 18971277
2. Woo PC, Lau SK, Lam CS, Lau CC, Tsang AK, Lau JH, et al. Discovery of seven novel Mammalian and avian coronaviruses in the genus deltacoronavirus supports bat coronaviruses as the gene source of alphacoronavirus and betacoronavirus and avian coronaviruses as the gene source of gammacoronavirus and deltacoronavirus. J Virol. 2012; 86: 3995–4008. doi: 10.1128/JVI.06540-11 PMID: 22278237
3. Dong BQ, Liu W, Fan XH, Vijaykrishna D, Tang XC, Gao F, et al. Detection of a novel and highly divergent coronavirus from asian leopard cats and Chinese ferret badgers in Southern China. J Virol. 2007; 81: 6920–6926. PMID: 17459938
4. Wang L, Byrum B, Zhang Y. Detection and genetic characterization of deltacoronavirus in pigs, Ohio, USA, 2014. Emerg Infect Dis. 2014; 20: 1227–1230. doi: 10.3201/eid2007.140296 PMID: 24964136
5. Marthaler D, Jiang Y, Collins J, Rossow K. Complete genome sequence of strain SDCV/USA/Illinois121/2014, a porcine deltacoronavirus from the United States. Genome Announc. 2014; 2: pii: e00218–14. doi: 10.1128/genomeA.00218-14 PMID: 24723704
6. Li G, Chen Q, Harmon KM, Yoon KJ, Schwartz KJ, Hoogland MJ, et al. Full-length genome sequence of porcine deltacoronavirus strain USA/IA/2014/8734. Genome Announc. 2014; 2: pii: e00278–14. doi: 10.1128/genomeA.00278-14 PMID: 24723718
7. Mole B. Deadly pig virus slips through US borders. Nature. 2013; 499: 388. doi: 10.1038/499388a PMID: 23887408
8. Stevenson GW, Hoang H, Schwartz KJ, Burrough ER, Sun D, Madson D, et al. Emergence of Porcine epidemic diarrhea virus in the United States: clinical signs, lesions, and viral genomic sequences. J Vet Diagn Invest. 2013; 25: 649–654. doi: 10.1177/1040638713501675 PMID: 23963154
9. USDA. Reporting, herd monitoring and management of novel swine enteric coronavirus diseases. Available: http://www.aphis.usda.gov/newsroom/2014/06/pdf/secd_federal_order.pdf. Accessed 17 September 2014.
10. Song D, Park B. Porcine epidemic diarrhoea virus: a comprehensive review of molecular epidemiology, diagnosis, and vaccines. Virus Genes. 2012; 44: 167–175. doi: 10.1007/s11262-012-0713-1 PMID: 22270324
11. Huang YW, Dickerman AW, Pineyro P, Li L, Fang L, Kiehne R, et al. Origin, evolution, and genotyping of emergent porcine epidemic diarrhea virus strains in the United States. MBio. 2013; 4: e00737–13. doi: 10.1128/mBio.00737-13 PMID: 24129257
12. Chen Q, Li G, Stasko J, Thomas JT, Stensland WR, Pillatzki AE, et al. Isolation and characterization of porcine epidemic diarrhea viruses associated with the 2013 disease outbreak among swine in the United States. J Clin Microbiol. 2014; 52: 234–243. doi: 10.1128/JCM.02820-13 PMID: 24197882
13. National Pork Board PEDV Research Updates: Animal-focus 2014. The Ohio State University; Oglesbee. The pathogenesis and characterization of porcine epidemic diarrhea virus (PEDV) and porcine deltacoronavirus (PdCV) in neonatal gnotobiotic swine. American Association of Swine Veterinarians News archive. Available: https://www.aasv.org/news/story.php?id=7712. Accessed 5 December 2014.
14. Marthaler D, Raymond L, Jiang Y, Collins J, Rossow K, Rovira A. Rapid detection, complete genome sequencing, and phylogenetic analysis of porcine deltacoronavirus. Emerg Infect Dis. 2014; 20: 1347–1350. doi: 10.3201/eid2008.140526 PMID: 25075556
15. Wang L, Byrum B, Zhang Y. Porcine coronavirus HKU15 detected in 9 US states, 2014. Emerg Infect Dis. 2014; 20: 1594–1595. doi: 10.3201/eid2009.140756 PMID: 25153521
16. Masters PS. The molecular biology of coronaviruses. Adv Virus Res 2006; 66: 193–292. PMID: 16877062
17. Brian DA, Baric RS. Coronavirus genome structure and replication. Curr Top Microbiol Immunol. 2005; 287: 1–30. PMID: 15609507
18. Woo PCY, Huang Y, Lau SKP, Yuen KY. Coronavirus genomics and bioinformatics analysis. Viruses. 2010; 2: 1804–1820. doi: 10.3390/v2081803 PMID: 21994708

19. Sun DB, Feng L, Shi HY, Chen JF, Liu SW, Chen HY, et al. Spike protein region (aa 636–789) of Porcine epidemic diarrhea virus is essential for induction of neutralizing antibodies. Acta Virol. 2007; 51: 149–156. PMID: 18076304

20. Liu DQ, Ge JW, Qiao XY, Jiang YP, Liu SM, Li YJ. High-level mucosal and systemic immune responses induced by oral administration with *Lac obacill s*-expressed porcine epidemic diarrhea virus (PEDV) S1 region combined with *Lac obacill s*-expressed N protein. Appl Microbiol Biotechnol. 2012; 93: 2437–2446. doi: 10.1007/s00253-011-3734-0 PMID: 22134641

21. Knuchel M, Ackermann M, Muller HK, Kihm U. An ELISA for detection of antibodies against porcine epidemic diarrhoea virus (PEDV) based on the specific solubility of the viral surface glycoprotein. Vet Microbiol. 1992; 32: 117–134. PMID: 1441196

22. Simkins RA, Weilnau PA, Van CJ, Brim TA, Saif LJ. Competition ELISA, using monoclonal antibodies to the transmissible gastroenteritis virus (TGEV) S protein, for serologic differentiation of pigs infected with TGEV or porcine respiratory coronavirus. Am J Vet Res. 1993; 54: 254–259. PMID: 8381626

23. Sestak K, Zhou Z, Shoup DI, Saif LJ. Evaluation of the baculovirus-expressed S glycoprotein of transmissible gastroenteritis virus (TGEV) as antigen in a competition ELISA to differentiate porcine respiratory coronavirus from TGEV antibodies in pigs. J Vet Diagn Invest. 1999; 11: 205–214. PMID: 10353350

24. Elia G, Decaro N, Martella V, Lorusso E, Mari V, Maria SL, et al. An ELISA based on recombinant spike protein S for the detection of antibodies to transmissible gastroenteritis virus of swine-like canine coronaviruses. J Virol Methods. 2010; 163: 309–312. doi: 10.1016/j.jviromet.2009.10.015 PMID: 19878695

25. Gerber PF, Gong Q, Huang YW, Wang C, Holtkamp D, Opriessnig T. Detection of antibodies against porcine epidemic diarrhea virus in serum and colostrum by indirect ELISA. Vet J. 2014; 202: 33–36. doi: 10.1016/j.tvjl.2014.07.018 PMID: 25135339

26. Opriessnig T, Xiao CT, Gerber PF, Halbur PG. Identification of recently described porcine parvoviruses in archived North American samples from 1996 and association with porcine circovirus associated disease. Vet Microbiol. 2014; 173: 9–16. doi: 10.1016/j.vetmic.2014.06.024 PMID: 25081955

27. Shen HG, Halbur PG, Opriessnig T. Prevalence and phylogenetic analysis of the current porcine circovirus 2 genotypes after implementation of widespread vaccination programmes in the USA. J Gen Virol. 2012; 93: 1345–1355. doi: 10.1099/vir.0.039552-0 PMID: 22398315

28. Shen HG, Schalk S, Halbur PG, Campbell JM, Russell LE, Opriessnig T. Commercially produced spray-dried porcine plasma contains increased concentrations of porcine circovirus type 2 DNA but does not transmit porcine circovirus type 2 when fed to naive pigs. J Anim Sci. 2011; 89: 1930–1938. doi: 10.2527/jas.2010-3502 PMID: 21278103

29. OIE. Principles and methods of validation of diagnostic assays for infectious disease. In: World Organisation for Animal Health, editor. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 2013. Paris. PMID: 24547648

30. USDA. Swine enteric coronavirus disease testing summary report. Available: http://www.aphis.usda.gov/animal_health/animal_dis_spec/swine/downloads/secd_wkly_lab_%20rpt_09_11_14.pdf. Accessed 19 September 2014.

31. Dyckman JD, Wende RD. Comparison of serum and plasma specimens for syphilis serology using the reagin screen test. J Clin Microbiol. 1980; 11: 16–18. PMID: 7354126

32. Scott Y, Parker P, McArdle B, Wallis JP. Comparison of plasma and serum for antibody detection using DiaMed microtubes. Transfus Med. 1996; 6: 65–67. PMID: 8696450

33. Siev M, Yu X, Prados-Rosales R, Martiniuk FT, Casadevall A, Ach(0/eoprl6M.,)-215.1Coerrltatio09.8(n)-220.5betv
a754plasma antibody toial ant(ges.n)-212.8(Clin)-214.5(Vaccinl)-208.9Iimmuiol. 2013;
10.128/Ca