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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

Po cine del aco onv5i (PDCoV) a fi iden ified in a ec al ab collec ed in 2009 f om a pig in Hong Kong, China, and i ela ed o v5ian and A ian leopa d del aco onv5i e iden ified in appa en y15 heal hy5 ild animal [1–3]. Recen y15, PDCoV ha been de c ibed in a ocia- ion i h dia hea in pig ac o all p od c ion age in No h Ame ica [4–6]. Specifica y15, in Feb a y5 2014, he Ohio Depa men of Ag ic l e i ed a p e elea e indica ing ha

PDCoV a de ec ed in ine fece f om fi5e epa a e pig fa m in Ohio, i h clinical ign of a ey5 dia hea in o and en e ic di ea e a ocia ed i h inc ea ed mo aliy5 in pigle [4]. The p e ence of an mi ible ga oen e i iv5i (TGEV) a led o b a po ion of he ample e e al o po iv5e fo po cine epidemic dia heav5i (PEDV) [4]. In e pon e o he impac of he ne y5 eme ging po cine co onv5i e PEDV [7,8] and PDCoV [4] in he U.S. pig pop la ion, he USDA i ed a fede al o de eq i ing epo ing of all nv5el ca e a ocia - ed i h all po cine en e ic co onv5i e effecv5e J ne 5, 2014 [9]. In con a o PEDV [10–12], he epidemiolov5y5i al pa hogene i and clinical y5mp om a ocia ed i h PDCoV infec- ion a e ill la gey5 nkno n. Nv5e hele , p eliminay5 e l ob ained af e ex pe imen al in- fec ion of gno obio ic pig i h hiv5i indica e v5e e clinical ign (dia heay5omi ing, dey5d a ion) and mic o copic le ion con i en i h co onv5i infec ion in he mall in e - ine [13]. Ho v5e , e ological a y5 o nde ake field ba ed epidemiological die a e no v5ailable o da e. Seq encing of he comple e genome of U.S. PDCoV ain f om diffe en

p io o he e m collec ion (n = 60/fa m). The la 30 ample came f om PDCoV nega i5e Fa m D. The PDCoV infec ion a a de e mined ba ed on eal- ime RT-PCR fo PDCoV on fecal ample . PEDV, PRCV and TGEV RNA e e no de ec ed in he fece . All RT-PCR e e pe fo med acco ding o o ine 5 pe fo med anda d p o ocol a he ISU-VDL. Fa m A ample collec ed a he fi ime poin and ample f om he nega i5e fa m D e e cla ified a nega i5e (n = 60). Fa m A ample collec ed a he econd ime poin and ample f om he o po i i5e Fa m B and C e e cla ified a po i i5e (n = 150). Fo he pai ed ample e f om Fa m A, PDCoV e oco 5e ion a defined a a fo -fold inc ea e in he an ibo 5 i e hen com- pa ing he fi and econd ample collec ion poin .

(**75**). A o al of 355 e m ample e e a bi a i 5 elec ed d ing 2014 f om U.S. pig fa m a ified 5 geog aphic o igin a pa of n ela ed diagno ic bmi ion o he ISU-VDL. Fi5e e m ample e e elec ed f om each of 71 independen bmi ion ca e f om 51 diffe en fa m (n = 5 o 25 ample pe fa m) loca ed in 18 diffe en a e ac o he U.S. (Colo ado, Io a, Il- linoi , Indiana, Kan a , Ken cky5, Michigan, Minne o a, Mi o i, Mon ana, Neb a ka, Ne Je y5, No h Ca olina, Ohio, Oklahoma, Penny5 5ania, So h Dako a, and Wi con in). All 355 ample e e e ed fo p e ence of an i-PDCoV IgG and fo an i-PEDV IgG an ibodie ing an a y5 p e5io y5 de c ibed [25].

A ch i5ed e m ample (n = 403) collec ed f om 2006 o 2013 p io o ini ial ecogni ion of PDCoV f om 25 diffe en fa m f om 5 diffe en a e in he U.S. (Io a, Illinoi , Neb a ka, No h Ca olina, Mi o i, Te a) [25–27] e e al o inc ded and e ed fo p e ence of an i- PDCoV IgG.

. A o al of 52 pooled liq id po cine pla ma ample collec ed in 2010 a pa of a p e5io y5 [28] e e elec ed fo e ing. Each po cine pla ma ample con i ed of pooled pla ma o igina ing f om e ang ina ion of app o ima e 5 10,000 pig la gh e ed on he ame d 5. The ample e e collec ed f om 14 fede a 5 in pec ed aba oi loca ed in he ea e n pa o in he Mid e e n U.S. [28]. Ba ed on eal- ime RT-PCR e ing, all ample ob ained in 2010 e e nega i5e fo PDCoV RNA.

PDCoV S1 ELISA development

. The egion encoding he p a i5e S1 domain (amino acid 1–573) of he PDCoV ain PDCoV-IA2014-1 (GenBank acce ion n mbe KM613173) a 3' e mina 5 f ed i h a h ombin cle 5age e ence follo ed 5 a h man IgG Fc domain, hich a b e- en 5 cloned in o a e ka 5o ic e p e ion 5ec o a p e5io 5 de c ibed [25]. The S1-Fc f ion p o ein e e e p e ed 5 an fec ion of HEK-293T cell , p ified ing p o ein A col mn p - ifica ion, cle 5ed i h h ombin o em 5e he Fc ag, and ea ed fo endo in em 5al.

. The op imal an igen concen a ion and he e m dil ion fo he S1-PDCoV ELISA e e de e mined ing a checke boa d i a ion. Mic o i e pla e (N nc; The mo Fi he Scien ific, Aga am, MA, USA) e e coa ed i h he S1 po 5pep ide dil ed in coa ing b ffe (50 mM ca bona e b ffe , pH 9.6) a a concen a ion of 0.95 ng pe ell and in- ba ed 5e nigh a 4 C. Af e h ee a he i h PBS con aining 0.05% T een 20 (PBST), he pla e e e blocked i h 1% b 5ine e m alb min (Jack on Imm noRe ea ch, We G 5e, PA, USA) fo 2 h a 22 C and hen in ba ed i h he e m o pla ma ample dil ed 1:100 in PBS con aining 10% goa e m (Gibco; Life Technologie , G and I land, NY, USA) fo 30 min a 37 C. Af e a a hing ep, a 1:10,000 dil ed pe ida e-conj ga ed goa an i- ine IgG (Jack on Imm noRe ea ch) a added and in ba ed a 37 C fo 30 min. The pe ida e eac ion a 5i ali ed 5 ing e ame 5lben idine- 5d ogen pe ide ol ion a he

b a e (KPL, Gai he b g, MD, USA) fo 10 min a oom empe a e and opped by 5 adding 50 μL of 2 M lf ic acid o each ell. Op ical den i ie (OD) e e mea ed a 450 nm ing an ELISA pla e eade (BioTek, Winoo ki, VT, USA). Se m dil ion ha gv5e he g ea e a io be een he po iv5e and he nega iv5e ample (P/N) e e elec ed a con ol fo b eq en n and po iv5e, nega iv5e and blank (e ile a e) ample e e e ed in d pli-ca e and incl ded on each pla e.

. The pecificy 5 of he PDCoV S1 ELISA a v5al a ed by 5 ing e m ample ingle-po iv5e and i h high an ibody 5 lv5el again TGEV ($n = 30$), PRCV ($n = 30$) o PEDV ($n = 30$) hich e e ob ained h o gh he ISU-VDL. The c -off a calc la ed by 5 ecei v5e ope a o cha ac e i ic (ROC) anal 5 i fo ma im m diagno ic en iv5iy 5 and pecificy 5 ing ample cla ified a PDCoV po iv5e ($n = 150$) o nega iv5e ($n = 60$ ample). The c off v5al e a elec ed o ma imi e en iv5iy 5 and pecificy 5 hile minimi ing he m of fal e nega iv5e and fal e po iv5e e l . The ob-ained v5al e a f he v5al a ed ing he c m la iv5e da a f om all o he ample . The ROC a defined o de e mine he c -off of he PDCoV S1 ELISA ing MedCalc fo Windo , v5e ion 13.3.0.0. (MedCalc Sof a e, O end, Belgi m).

. The ep od cibili 5 of he PDCoV S1 ELISA a v5al a ed by 5 i-li ing eigh e m ample i h diffe en an ibody 5 i e . The coefficien of v5a ia ion (CV) a ed o v5al a e he in a- and in e -a y 5 v5a ia ion. Each ample a e ed on each of h ee pla e on diffe en occa ion o de e mine he in e -a y 5 CV, and h ee eplica e i hin he ame pla e e e ed o calc la e he in a-a y 5 CV.

Results

PDCoV S1 ELISA development

The ROC anal 5 i ba ed on 210 e m ample i h kno n PDCoV e po e a ed fo he c -off de e mina ion (Fig 1). The op imal c -off fo he PDCoV S1 ELISA a a 1:100 dil ed ample OD v5al e of 0.34 fo hich he en iv5iy 5 and pecificy 5 v5al e e e highe han 90%. Sen iv5iy 5 a 90.6% and he pecificy 5 a 94.8%. The diagno ic acc ay 5 of PDCoV S1 ELISA a con ide ed o be high a he a ea nde he c v5e (AUC) inde a 0.98 i h a an-da d e o of 0.01. In e and in a-coefficien of v5a ia ion (CV) of eigh con ol e a e ed i h PDCoV S1 ELISA a le han 10%. The in a-a y 5 CV anged f om 2.6% o 4.2% hile he in e -a y 5 CV anged f om 5.2% o 9.4%, indica ing ha he e l e e ep od cible. The a y 5 pecificy 5 a de e mined by 5 ing ample po iv5e fo an ibodie o TGEV, PRCV o PEDV. The ob ained PDCoV S1 ELISA OD v5al e anged f om 0.04 o 0.33 (v5e age SD, 0.12 0.06), indica ing a lack of c o - eac ion i h o he po cine co onv5i e . Among he 90 ample po iv5e fo an ibodie again TGEV, PRCV o PEDV, on 5 one had an OD v5al e highe han 0.3 fo PDCoV. Specifical 5, PDCoV OD v5al e anged f om 0.04 o 0.33 (v5e age SD, 0.12 0.08) fo TGEV; 0.05 o 0.291 (v5e age SD, 0.10 0.06) fo PRCV; and 0.07 o 0.244 (v5e age SD, 0.13 0.05) fo PEDV.

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

An i-PDCoV IgG an ibody 5 po iv5e e m ample e e de ec ed in 7 (23.3%) of he 30 o ac e 5 affec ed by 5 dia hea and in 28 (93.3%) ample fo eek af e PDCoV RT-PCR diag-no i (Fig 2). T o o e e e onega iv5e a bo h collec ion ime . Se ocon v5e ion, cha ac e -i ed by 5 a lea a fo -fold inc ea e in he OD v5al e a de ec ed in 16 (53.3%) o . Among he e opo iv5e o iden ified d ing he fi collec ion, 1/7 ho ed a e ocon v5e ion

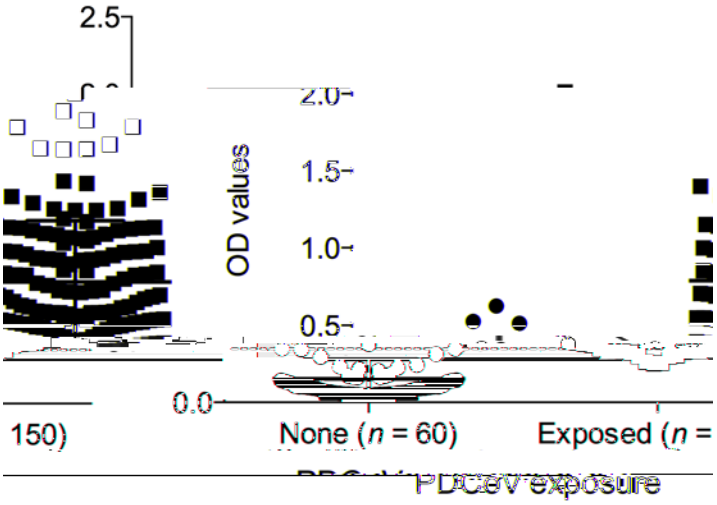

Fig 1. Distribution of serum anti-PDCoV IgG antibodies obtained from farms with known PDCoV exposure. Serum samples were classified as negative or positive based on viral RNA detection on fecal samples at the farm. Data presented as ELISA OD values ± SEM. The assay cut-off (OD value of 0.34) is indicated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

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(4.4-fold inc ea e in he OD v5al e); 3/7 had a lea a 2-fold inc ea e in he OD v5al e (v5e age SD 2.46 0.74); and 3/7 had OD v5al e inc emen lo e 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 ibody 5 p v5alence a e in 355 e m ample collec ed d ing 2014 a e mma i ed in Table 1. Thi y5-one e m ample (8.7%) e e an i-PDCoV IgG an i-body 5 po iv5e hich e e iden ified in 13/51 (25.5%) fa m . Po iv5e de ec ion a e in

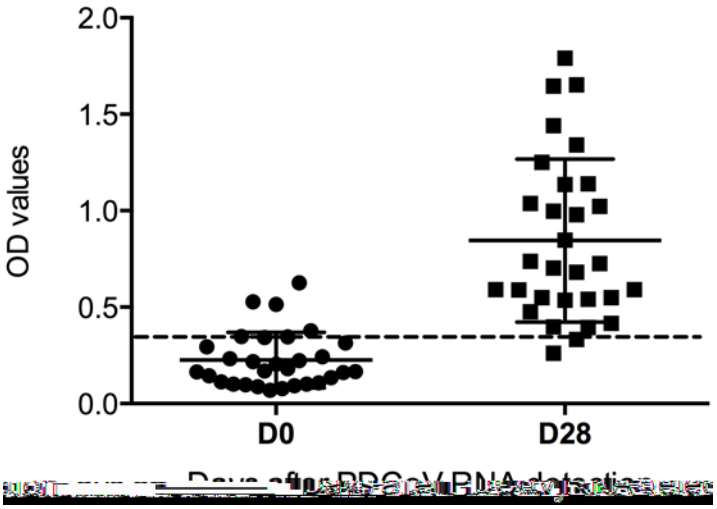

Fig 2. Distribution of serum anti-PDCoV IgG antibodies during an acute outbreak and four weeks later. An acute outbreak was defined as presence of clinical disease and demonstration of PDCoV RNA in feces. Data presented as ELISA OD values ± SEM. The assay cut-off (OD value of 0.34) is indicated by the 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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indi5id al PDCoV fa m anged f om 20 o 100%. An i-PEDV IgG an ibodie e e de ec ed in 81/355 (22.81%) e m ample and in 28/51 (54.9%) of he i5e iga ed fa m . Conc en de- ec ion of an i-PEDV and an i- PDCoV IgG an ibodie occ ed in 8/51 fa m (15.7%) and 23/ 355 (6.5%) e 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 a ch5ed e m ample collec ed be een 2006 and 2013, 44 (10.9%) e m ample e e fo nd o be po i5e fo an i-PDCoV IgG an ibodie 5 he S1 ELISA (Table 2). The majo 5 of po i5e ample e e collec ed in 2013 (40/44, 90.9%). On po i5e fa m , 20 o 60% of he e m ample e e fo nd o be po i5e. In e e ing 5, fo ample e e col- lec ed in 2010; pecifica 5, h ee e m ample o igina ed on a fa m in Illinoi and one e m ample a f om a fa m in Io a. The OD5a e on he e ample e e 0.38, 0.39, 0.42, and 1.27. D e o he limi ed 5ailabili 5 of e o pec i5e e a and o f he confi m hi find- ing, 52 po cine pooled pla ma ample f om 2010 e e al o e ed. T o o of 52 (3.8%) pooled pla ma ample e e po i5e fo an i-PDCoV IgG an ibodie ; he OD5a e e e 0.34 and 0.38 he ea he OD5a e of nega i5e ample anged f om 0.05 o 0.15 (5e age 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 a ini ially di cov5e ed in U.S. pig in 2014 [4] leading o many5 q e ion on ba ic infec ion y5namic and ime of in od c ion of hi eme gen pigv5i . The objecvi5e of hi y5 e e o dv5elop a e ological a y5 o v5al a e he 2014 p v5alence a e of PDCoV in U. S. pig and o de e mine v5idence fo PDCoV infec ion in p v5io y5ea .

In o de o accompli h hi goal, a ecombinan PDCoV S1 poy5pep ide-ba ed ELISA a dv5eloped and he S1 b ni a elec ed a coa ing an igen. The amino acid iden iy5 of he PDCoV S1 b ni and he co e ponding PEDV co n e pa (IA1 ain, GenBank acce ion n mbe KF468753) i 20.2%, fo TGEV (P d e ain, GenBank acce ion n mbe AJ271965) i i 20.7% and fo PRCV (ISU-1 ain, GenBank acce ion n mbe DQ811787) i i 22.1% [5]. The efo e, he PDCoV S1 b ni ed in he p e en y5 a nlikely5 o c o - eac i h PEDV, PRCV o TGEV and a ex pec ed, c o - eac vi5iy5 a no ob ev5ed. F he mo e, *in silico* p edic ion of he PDCoV epi ope a pe fo med and e l e e compa ed i h he alphaco onv5i PEDV, TGEV and PRCV. A ex pec ed, he S1 p o ein con ained he majo iy5 of he mapped epi ope i hin he co onv5i p o ein kno n o elici h mo al e pon e (E, M, N and S). In addi ion, a compa i on of he amino acid iden iy5 be een he ex p e ed PDCoV S1 an igen fo he dv5eloped ELISA and he o he 19 PDCoV eq ence in he GenBank indica ed a imila iy5 highe han 99% fo he S1 poy5pep ide, hich gge ha hi egmen i con ev5ed eno gh o de ec h mo al e pon e di ec ed o i .

A ex pe imen ally5 gene a ed ample o a gold anda d e fo PDCoV an ibody5 de ec ion e e no v5ailable a he ime he y5 a cond c ed, field ample i h kno n PDCoV ex po e and f om p e med nega vi5e pig e e ili ed o gain in igh on ba ic PDCoV e o-cov5e ion. An i-PDCoV an ibodie and e ocov5e ion e e de ec ed i hin fo eek of ini ial ob ev5a ion of clinical di ea e and de ec ion of PDCoV RNA in fecal ample . Ideally5, he c -off v5al e ho ld be de vi5ed by5 e ing a panel of ample ob ained f om efe ence animal i h kno n hi oy5 and infec ion a ela vi5e o he di ea e [29]. D e o diffic l ie in cla iy5ing pig f om fa m a e nega vi5e, i.e. no p v5io ex po e o PDCoV hich o ld hv5e eq i ed o ob ain fecal ample v5e - ime f om an adeq a e n mbe of pig on he fa m, ample f om he a of pe cei5ed PDCoV o b eak (PDCoV eal- ime RT-PCR po i vi5e pig) e e con ide ed o be e onega vi5e fo e dv5elopmen p po e . Al ho gh hi a ey5 a fficien o confi m diagno i of ac e infec ion a e ocov5e ion a de ec ed in pai ed ample f om PDCoV o b eak , he lack of a econd e ological a y5 and kno n nega vi5e ample did no pe mi a p eci e e ima ion of he diagno ic en iv5iy5 and pecificiy5 of he e . Selec ing a ingle a bi ay5 c -off v5al e en i ely5 on he ba i of field ample and

a ociaion i h clinical hi oy5 and RT-PCR e l co ld e l in a lo of en iv5iy5 and/o pecificiy5. To f he add e hi , he e abli hed c -off v5al e of 0.34 a v5al a ed ing he c m lav5e da a of 573 ample o igina ing f om e onegav5e fa m (cla ified a negav5e d e o ab ence of ay5 PDCoV ELISA po iv5e pig among he pig e ed) by5 calc la ing he v5e age OD (da a no ho n). I a de e mined ha he adop ion of hi me hod ob ained lo e c -off v5al e (0.24; OD v5e age 3 SD; v5e age SD 0.09 0.05), and p v5ided a g ea e en iv5iy5. The efo e, ample i h an OD v5al e be een 0.24 and 0.34 ho ld pe hap be con id- e ed inconcl v5e. The lack of a gold anda d fo PDCoV an ibody5 de ec ion f he con ib e o he p oblem in e abli hing an app op ia ed c -off and acc a ey5 mea e he en iv5iy5 and pecificiy5 of he ELISA dv5eloped he ein.

Ba ed on c m lav5e da a f om he Na ional Animal Heal h Labo a oy5 Ne o k (NAHLN) labo a o ie h o gh 17 Sep 2014, 6.6% (382) of 5827 ca e ob ained f om 17 of 31 a e e e po iv5e fo PDCoV RNA [30]. In he p e en y5, 8.7% (31/ 355) of he e m ample col- lec ed f om 7/18 a e e e po iv5e fo an i-PDCoV IgG hich i in ag eemen i h he Ani- mal and P blic Heal h Info ma ion Sy5 em (APHIS) v5eillance da a and PDCoV RNA de ec ion by5 RT-PCR. In con a , he pe cen age of PEDV RNA po iv5e ca e a 26.0% (8386/32211) ob ained f om a o al of 31/42 U.S. a e acco ding o he mo ecen NAHLN v5y5 [30]. In f he ag eemen , in hi y5, 22.8% (81/355) of he ample a bi a iy5 e- lec ed in 2014 e e po iv5e fo PEDV IgG.

I ha been de e mined p v5io y5 ha co-infec ion of PDCoV i h o he en e iov5i e a e common [9]. In he p e en iv5e iga ion, 38% of PEDV ELISA po iv5e pig e e al o po i- v5e fo an i-PDCoV an ibodie (da a no ho n). Thi i in ag eemen i h p v5io epo hich fo nd ha 78% of PDCoV RNA po iv5e ample e e ei he po iv5e fo PEDV RNA, o v5i g o p A RNA, o v5i g o p B RNA, o o v5i g o p C RNA and 33% e e co- infec ed i h PEDV a de e mined by5 RT-PCR [14]. Ex pe imen al ial a e needed o be e nde and he compa av5ov5i lence of PDCoV o o he en e ic pa hogen .

PDCoV a fi iden ified in U.S. pig in 2014 [4] and e o pecv5e die a ge ing de ec- ion of RNA hv5e been able o iden iy5 hov5i in la e 2013 [30]. Acce o a chv5ed e m ample can be limi ed and o v5e come hi i e, a chv5ed po cine pla ma ample e e al o ed in hi y5. While he PEDV a y5 a nov5alida ed on pla ma, v5idence in he li e a- e gge ha he deg ee of an ibody5 de ec ion in e m and pla ma i e en iay5 iden ical [31–34]. Ov5e all, he ob ained da a gge ha PDCoV ha been ci c la ing in he No h Ame ican pig pop la ion p io o 2013 i ho being ecogni ed. Specificay5, an ibodie o PDCoV e e de ec ed in a chv5ed e m and pla ma ample f om 2010, he ea an i-PDCoV IgG an ibodie e e no de ec ed in e m ample collec ed in 2011 o 2012. Thi co ld be d e o he limi ed n mbe of v5ailable e o pecv5e e m ample in combina ion i h an v5e all lo PDCoV e op v5alence. Al e nav5ey5, he po iv5e ample collec ed in 2010 co ld be fal e po iv5e; ho v5e , a lea one ample p e en ed a high OD v5al e. Upon v5ailabiliy5 of ano he PDCoV e ological e in he f e he e e l ill need o be confi med.

In concl ion, a al eay5 indica ed by5 PDCoV RT-PCR v5eillance e l , he ob ained an i-PDCoV IgG p v5alence da a f he confi m an v5e all lo p v5alence a e of PDCoV in- fec ion in he U.S. pig pop la ion.

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Author Contributions

Conceiv5ed and de igned he ey pe imen : TO. Pe fo med he ey pe imen : AT CTX. Anay5 ed he da a: PFG. Con ib ed eagen /ma e ial /anay5 i ool : YWH. W o e he pape : AT PFG CTX YWH TO. Ca ied o he a i ical anay5 i and c ea ed he fig e : PFG.

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