

# A recombinase polymerase amplification–based assay for rapid detection of *Chlamydia psittaci*

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**ABSTRACT** *Chlamydia psittaci* is a zoonotic agent of systemic wasting disease in birds and atypical pneumonia in mammals including humans, constituting a public health risk. A rapid diagnostic assay would be beneficial in screening *C. psittaci* in the field. In this study, we developed a probe-based recombinase polymerase amplification (RPA) assay for the rapid detection of *C. psittaci*. The specific primer pairs and probe targeting the conserved region of the outer membrane protein A gene were designed and applied to the real-time real-time RPA assay. The test can be performed at 39°C

for 20 min using a portable device, with sensitivities approaching 100 copies of DNA molecules per reaction, with no cross-reaction with other pathogens. The clinical performance of the RPA assay was evaluated in an outbreak of *C. psittaci* and has high accuracy levels in field applications. The epidemic *C. psittaci* strains were classed into 2 genotypes: A and C. Collectively, this study offers a promising approach in screening for *C. psittaci* both in a laboratory setting and in field settings, and RPA can be used as an effective clinical test to monitor outbreaks in domestic fowl populations.

**Key words:** *Chlamydia psittaci*, detection, recombinase polymerase amplification

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## INTRODUCTION

*Chlamydia psittaci* is an obligate intracellular organism with a unique developmental cycle (Wyrick and Richmond, 1989). The infectious agent is considered a zoonotic agent of systemic wasting disease in birds and atypical pneumonia in mammals (Knittler and Sachse, 2015; Bommana and Polkinghorne, 2019). As many as 467 bird species, as well as many nonavian domestic animals, are susceptible to *C. psittaci*, including swine, mice, dogs, horses, cattle, sheep, goats, cats, and humans (Huminer et al., 1992; Hotzel et al., 2004; Szeredi et al., 2005; Kauffold et al., 2006; Kemmerling et al., 2009; Pantchev et al., 2010; Lenzko et al., 2011).

*C. psittaci* infection in birds and the transmission from birds to humans have long been confirmed (Huminer et al., 1992; Hinton et al., 1993; Heddemma et al., 2006; Gaede et al., 2008; Laroucau et al., 2009; Harkinezhad et al., 2009; Vorimore et al., 2015). However, most bird-related cases of psittacosis are usually overlooked owing to latent infections and asymptomatic cases. *C. psittaci* has received little attention owing to the low prevalence in humans (Knittler and Sachse, 2015). Nowadays, pathogenic *C. psittaci* species are distributed globally, inducing significant economic losses and creating a public health risk (Guo et al., 2017). Focus has recently been aimed at *C. psittaci* as a significant zoonosis.

To date, several assays have been developed for screening *C. psittaci* infection in birds. Conventional serological techniques such as pathogen isolation, indirect immunofluorescence, and ELISA are laborious and time-consuming (Souriau and Rodolakis, 1986; Sanderson et al., 1994), whereas PCR-based assays with high sensitivity and specificity are limited by technical difficulties and relatively expensive reagents (Hewinson et al., 1997; Messmer et al., 1997). Thus, it

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is a challenge to quickly identify and monitor *C. psittaci* in the field owing to asymptomatic cases and the lack of rapid detection assay for suspected cases.

To reduce the economic losses in stockbreeding and the potential public health risk for humans, an effective and rapid detection assay is needed to detect *C. psittaci* in the field. In this study, a novel detection assay for rapid and effective detection of *C. psittaci* was developed using recombinant polymerase amplification (RPA). We reported the results of this study, including the development and usage of the RPA assay, and the molecular characterization of the epidemic *C. psittaci* strains in laying ducks. The novel assay with high specificity and sensitivity will benefit by reducing economic loss in the poultry industry.

## MATERIALS AND METHODS

### Ethics Statement

The Committee of the Ethics of Animal Experiments at South China Agricultural University approved the animal experiment protocols (approval ID: SYXK-2014-0136). Permission was issued for the field study and sample collection.

### Viruses

The duck Tembusu virus strain ZJSBL01, adenovirus (AdV) strain CH-GD-12-2014, H9N2 avian influenza viruses (AIV) strain G1, duck hepatitis A virus strain GD-18, and *C. psittaci* strain SZ15 were preserved in our laboratory.

### Clinical Specimens

In July 2019, an outbreak occurred in laying ducks in Guangdong province of China. Clinical manifestations were characterized by poor laying performance. To identify the pathogens of this disease, a total of 36 clinical samples (including those from the liver, spleen, and ovary) were randomly collected from diseased ducks in 4 laying duck farms (9 ducks per farm) for diagnosis. All the samples were transported on dry ice to the laboratory for sample processing.

### Primer and Probe Design

To design the specific primer and probe set for the RPA assay, we aligned the nucleotide sequences of the *opmA* gene of several *C. psittaci* strains including VS1 (GenBank no. AF269281.1), SZ15 (GenBank no. MK630234), 84-55 (GenBank no. Y16561.1), 6BC (GenBank no. X56980.1), CP3 (GenBank no. AF269265.1), Cal-10 (GenBank no. AB001784), KKCP1 (GenBank no. AB284062.1), 06-859-1 (GenBank no. EU159263.1), and YP84 (GenBank no. AB284058.1) and selected the conserved regions to design the primer and probe sets. The optimal primer was 30–35 bp in length, with 30–70% GC content, and the amplicon

length was 100–200 bp in length. The primer pairs were analyzed carefully using the DNASTAR software (Madison, WI) to avoid any possible primer dimers or self-annealing and screened by BLAST (NCBI) to ensure their specificity. A probe was designed to be compatible with the primer set with the highest amplification efficiency and modified with the tetrahydrofuran residue, a flanking dT-fluorophore, and a corresponding dT-quencher group on the other side of the tetrahydrofuran group. In addition, a suitable 3'-modification group to block the probe from polymerase extension was added in the probe (Table 1).

### Recombinase Polymerase Amplification Assay

The RPA reaction was carried out using the TwistDx RPA kit (TwistDx Inc., Cambridge, UK) as described previously by Zeng et al., 2019. In brief, the RPA was performed in a 25- $\mu$ L volume, containing 19.3  $\mu$ L of rehydration buffer, 2.1  $\mu$ L of each specific primer (10  $\mu$ M), 0.5  $\mu$ L of RPA probe (10  $\mu$ M), and 1  $\mu$ L of nucleic acid template. The reaction was performed at 39°C for 20 min in a Tubescanner (DEAOU Biotechnology, Guangzhou, China). Exponential amplification curves above the threshold of the negative control were considered positive. The amplicons were purified using a DNA clean-up kit (Beyotime, Beijing, China) and subjected to the agarose gel electrophoresis.

To determine the sensitivity of the novel RPA assay for *C. psittaci* detection, 10-fold serial dilutions of DNA standards ranging from  $10^6$  to  $10^1$  copies per microliter were detected using the novel assay. All the runs were performed in triplicate. To analyze the specificity of the novel RPA assay for *C. psittaci* detection, a panel of duck pathogens (including duck Tembusu virus, AdV, H9N2 AIV, and duck hepatitis A virus) was used to evaluate specificity levels. Nucleotide acid extraction and RPA assay were performed as mentioned previously.

### Detection of Clinical Samples

DNA was extracted from the clinical samples and subjected to RPA or quantitative real-time PCR (qRT-PCR) to detect *C. psittaci*. Recombinase polymerase amplification was performed as mentioned previously, and qRT-PCR was performed as previously described by Lin et al., 2019. In brief, a specific primer set targeting the 23S rDNA gene was designed and used for the qRT-PCR reaction. The recipe and thermal cycling parameters of the qRT-PCR reaction were used as described previously by Lin et al., 2019.

### Isolation and Identification of *C. psittaci*

The epidemic *C. psittaci* strains were isolated from the positive samples as previously described by Zhang et al., 2008 and Lin et al., 2019. In brief, the homogenate suspension was collected from the homogenized samples, frozen and thawed 3 times, clarified by centrifugation

**Table 1.** Primer pairs used in this study.

Name	Primer pairs (5'-3')	Position	Amplicon (bp)
RPAF1	ACCAAATCAGCTACAATTAATACCATGAA	802–831	177
RPAR1	CCATGTAGTAATGTTAAGAATCTCCGATTT	949–978	
RPAF2	GTTGTGGAAATTTTATACAGACACATCATT	559–588	123
RPAR2	AATCTTAGGATTAGATTGAGCGTATTGGAA	652–681	
RPAF3	GAACCAAGTTTATTAATCGATGGCACTATG	88–117	145
RPAR3	CATCAACTTTAATAACACGATCGAAAACAT	203–232	
Probe	GTAGGTGCACGTGGAGCTTTATGGGAATG [FAM-dT][THF]G[BHQ1-dT]TGTGCAACTTTAG[C3 spacer]	598–643	

and filtrated using a 0.45- $\mu$ m filter, and then inoculated into 6-day-old specific-pathogen-free chicken embryos. The vitelline membranes were harvested from dying embryos during 4 to 10 d after inoculation.

### Sequencing and Genotyping of *C. psittaci*

Specific primer pairs targeting the outer membrane protein A (*ompA*) gene were designed as described previously by Smith et al., 2005. PCR products were sequenced commercially (Songon Biotech, Shanghai, China) using the Sanger method. Multiple sequence alignments were performed using Clustal X (Larkin et al., 2007), and phylogenetic analysis was performed based on the sequences of the *ompA* gene via neighbor-joining method using MEGA version 7.0 (Kumar et al., 2016). All the reference strains used in the present study are listed in Table 2.

## RESULTS

### Development of the RPA Assay

Screening of specific primer pairs and probes was the critical step for RPA assay. In this study, the conservation of the sequences of the *ompA* gene of several *C.*

*psittaci* strains including genotype A, B, EB, E, and Mat116 was evaluated by alignment (Figure 1); then, a total of 3 primer pairs were designed targeting on the highly conserved regions of the *ompA* gene of *C. psittaci* (Table 1). As a result, 2 primer pairs were successfully used to amplify target regions, while the primer set F2/R2 produced the greatest amount of the amplification product. A probe was designed, which was located within the region defined by the primer set F2/R2.

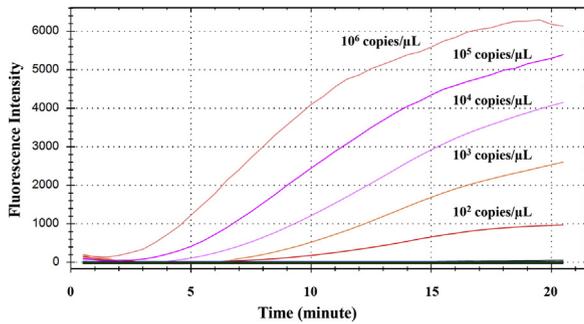
To determine the detection limit of the RPA assay, the sensitivity was assessed using DNA standards as templates. As a result, the RPA reaction produced positive fluorescence signals during 1–10 min when the copy number of DNA template was more than the range from  $10^6$  to  $10^2$  copies per reaction. A typical amplification curve was observed when the copy number ranged from  $10^6$  to  $10^2$  copies per reaction of DNA standard, while no specific signal was detected when the copy number was less than 10 copies per reaction of DNA standard (Figure 2A), indicating a detection limit of  $10^2$  copies per microliter of molecular DNA of the RPA assay. During the sensitivity analysis, 5 consecutive reactions were carried out to evaluate the reproducibility of the novel RPA assay. As expected, all the reactions produced positive signals when DNA input was  $10^6$ – $10^2$  copies per reaction. All these data indicated that the RPA assay possessed high reproducibility. During specificity analysis, the RPA assay was used to detect genome extraction from several duck pathogens. As a result, the specific fluorescence signal was detected only from the corresponding *C. psittaci* positive control, while no cross-reaction with the irrelevant pathogens was found (Figure 2B), indicating high specificity of the novel RPA assay. All these data provided evidence that the novel assay possesses high specificity and sensitivity and scored highly in reproducibility.

### Assay Performance on Clinical Samples

In July 2019, an epidemic occurred in duck populations in Guangdong province. All the diseased ducks presented poor laying performance and severe gross and histopathological lesions on many organs. Initial diagnosis as per the clinical manifestations and epidemiological survey suggested the involvement of *C. psittaci* in this outbreak. To confirm the infectious agent, a total of 36 clinical samples were randomly collected from diseased ducks and screened using RPA assay for *C.*

**Table 2.** *C. psittaci* strains used in this study.

Strain	Genotype	Host	GenBank no.
6BC	A	Psittacine	X56980.1
VS1	A	Parrot	AF269281.1
84-55	A	Parakeet	Y16561.1
SZ15	A	Duck	MK630234
CP3	B	Pigeon	AF269265.1
GR9	C	Duck	L25436.1
GD	C	Duck	AF269261
NJ1	D	Turkey	AF269266.1
9N	D	<i>Corvus</i>	EF375557.1
CPMN	E	Human	X12647.1
Cal-10	E	Ferret or human	AB001784
VS225	F	Psittacine	AF269259.1
7778B15	F	<i>Meleagris gallopavo</i>	AY762612
06-859/1	E/B	Duck	EU159263.1
KKCP-1	E/B	Human	AB284062.1
WC	WC	Cow	AF269269.1
M56	M56	Muskrat	AF269268.1
1V	1V	<i>Corvus</i>	EF028916.1
6N	6N	<i>Corvus</i>	EF197820.1
YP84	Mat116	Psittacine	AB284058.1
R54	R54	Skua	AJ243525.1
YP84	Daruma-1981	Psittacine	AB284065.1
CPX0308	CPX0308	Stork	AB284064.1

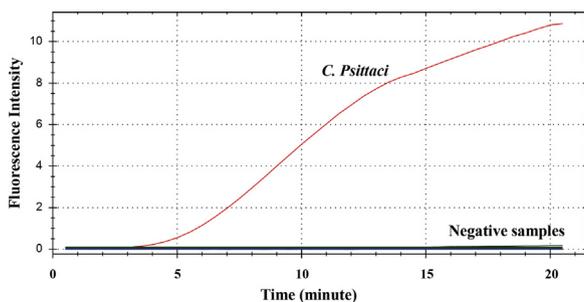


**Figure 1.** Alignment of the sequences of the primer and probe set used for the real-time RPA assay. Nucleotide sequences of the primers (F2/R2) and probe are shown at the top of the frames, whereas the corresponding nucleotide sequences of 9 *C. psittaci* strains (including genotype A, B, EB, E, and Mat116) are shown at the bottom. Dots represent that nucleotides are identical to those of the *C. psittaci* strain VS1. Abbreviation: RPA, recombinant polymerase amplification.

*psittaci*. As a result, all these samples were positive for *C. psittaci*, indicating that the major pathogen *C. psittaci* was responsible for this disease outbreak. To confirm the detection results, qRT-PCR assay was used in this study. As a result, all 36 samples proved positive for *C. psittaci* by qRT-PCR. All negative controls tested negative, and positive controls were tested positive for qRT-PCR detection. These findings provided evidence for the 100% level of agreement of *C. psittaci* detection between RPA assay and qRT-PCR assay (Figure 3). These data indicated that the RPA assay developed in this study possesses good practicability and high accuracy for rapid diagnosis in field settings.

### Molecular Characteristics of the *C. psittaci* Strains

To analyze the molecular characteristics of the *C. psittaci* strains, *C. psittaci* isolation was performed and the nucleotide sequences of the *ompA* gene were sequenced commercially. As a result, a total of 36 *C. psittaci* strains were successfully isolated. However, only 4 distinct



**Figure 2.** Sensitivity and specificity of the real-time RPA assay. The sensitivity of the real-time RPA assay: The sensitivity of the RPA assay was determined using the DNA standard. The DNA was diluted from  $10^6$  to 10 copies per microliter, and the detection limit of the assay was  $10^2$  copies per reaction. The specificity of the real-time RPA assay: Duck Tembusu virus (DTMUV), adenovirus (AdV), H9N2 avian influenza viruses (AIV), duck hepatitis A virus type 1 (DHAV-1), and distilled water were used as the negative samples. Abbreviation: RPA, recombinant polymerase amplification.

*C. psittaci* strains (designated GD19A1, GD19A7, GD19A11, and GD19 C) were identified owing to the 100% identity of many *C. psittaci* isolates from the same duck flock, indicating the same strain circulating in the laying duck farm during this outbreak. The *ompA* genes of GD19A1, GD19A2, and GD19A3 are 1,209 nt in length, whereas the *ompA* gene of GD19 C is only 1,176 nt in length. The sequences of the *ompA* gene obtained in this study have been deposited in the GenBank database under the accession numbers MT386537–MT386540.

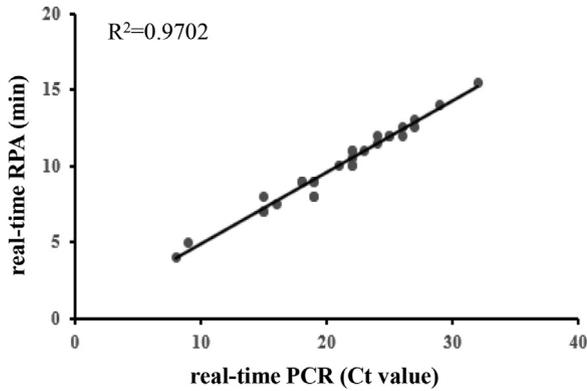
Sequence alignment based on the *ompA* gene was performed using the Clustal X program. As a result, the nucleotide sequence identities of the *ompA* gene between the isolates ranged from 80.68 to 99.76%. The GD19A1 isolate was 99.83 and 99.34% identical to that of strain SZ15 and 6BC, respectively. The GD19A7 isolate was 99.92 and 99.42% identical to that of strain SZ15 and 6BC, respectively. The GD19A11 isolate was 99.59 and 99.09% identical to that of strain SZ15 and 6BC, respectively. All these data indicated that GD19A1, GD19A7, and GD19A11 possess high similarity with strains SZ15 and 6BC. Interestingly, the GD19 C isolate was 98.98% identical to the GD strain, indicating high similarity of the isolate with GD strain.

### Genotyping of the *C. psittaci* Strains

It has been reported that strains of *C. psittaci* are usually divided into 15 genotypes (Sachse et al., 2008). Thus, we analyzed the genotypes of the *C. psittaci* isolates using several reference strains (Table 2). Interestingly, genotype A, B, C, D, E, F, EB, Mat116, M56, CPX 0308, WC, 6N, 1V, Daruma-1981, and R54 were clearly separated from each other (Figure 4). The isolates GD19A1, GD19A2, and GD19A3 are closely related to strains SZ15, 6BC, and VS1, indicating that these epidemic strains belong to the genotype A, which is usually found in psittacine birds. Interestingly, the isolate GD19 C is closely related to the strain GR9 and GD, which belongs to the genotype C. These data indicated that the *C. psittaci* strains collected in laying ducks belong to 2 genotypes.

## DISCUSSION

*C. psittaci* is an economically significant avian pathogen associated with a systemic wasting disease in birds and also a mammalian pathogen causing atypical pneumonia in a variety of species (Knittler and Sachse, 2015). Transmission of *C. psittaci* from birds to humans has already been described with chickens, ducks, parrots, and turkeys, which serve as reservoirs or sources (Hummer et al., 1992; Hinton et al., 1993; Heddema et al., 2006; Gaede et al., 2008; Harkinezhad et al., 2009; Laroucau et al., 2009; Vorimore et al., 2015). In our previous study, we found that parrot-type *C. psittaci* is responsible for a disease outbreak in laying ducks in 5 provinces of China (Lin et al., 2019). During that outbreak, 3 duck breeders in a farm who showed severe



**Figure 3.** Performance of RPA in comparison with real-time RT-PCR. Comparison of clinical performance between the threshold time of RPA assay (y-axis) and Ct value of real-time PCR (x-axis) on *C. psittaci*-positive clinical samples (n = 30). Abbreviation: RPA, recombinant polymerase amplification.

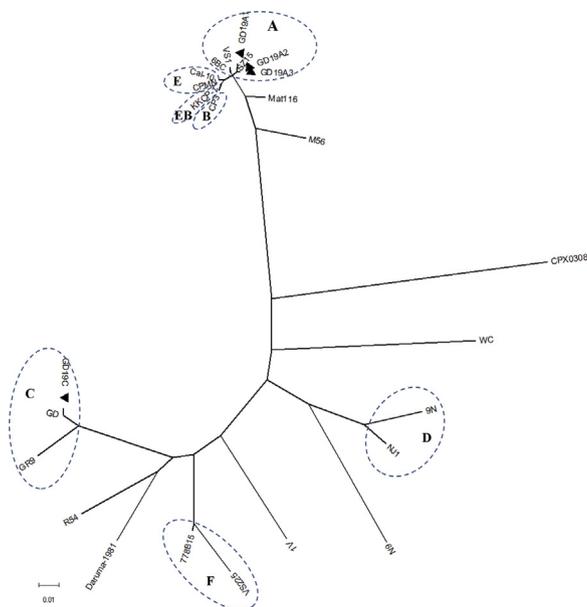
clinical signs of respiratory disease were diagnosed with atypical pneumonia from this organism by PCR, and they subsequently recovered from the disease after doxycycline treatment. Thus, a rapid and effective screened system to monitor *C. psittaci* in fields would benefit monitoring *C. psittaci* in laying ducks and preventing the transmission of *C. psittaci* from birds to humans.

Nowadays, PCR-based assays, such as PCR, nested PCR, and qRT-PCR, are routinely used for *C. psittaci* detection of suspected cases. However, these assays usually required more time (approximately 90–120 min) to perform the test and cannot be used for point-of-care detection outside of a well-equipped laboratory owing

to the limit of expensive and complex equipment. Recombinase polymerase amplification represents one of the fastest isothermal amplification methods (Piepenburg et al., 2006), which can be performed in 20–30 min at a temperature of around 37°C–42°C. Recombinase polymerase amplification uses the recombinase and single-stranded DNA-binding protein to match primers to their target on the template DNA; then, DNA amplification is carried out by DNA polymerase in the next 20–30 min (Harris and Griffith, 1987; Piepenburg et al., 2006). Importantly, RPA amplicons can be visualized by lateral flow dipstick or analyzed by gel electrophoresis. Compared with PCR-based assays for the detection of *C. psittaci*, the RPA assay needs lower temperatures and shorter time cycle increments. Moreover, the RPA assay can be performed using a portable device containing the essential reagents and equipment, which can be used in the field or resource-limited settings, enabling the novel assay to be used for rapid diagnosis of *C. psittaci* in the field. Although RPA assay was developed comparatively late, it may play a critical role and occupy a large market for rapid detection of animal pathogens in future owing to its simplified equipment requirements and fast reaction times (Li et al., 2018).

To date, owing to isothermal properties, fast reaction time, and high sensitivity and specificity, RPA has been widely used for rapid detection of pathogens in fields, such as AIV, AdV, Newcastle disease virus, infectious bronchitis virus, Marek’s disease virus, *Escherichia coli*, causative agent of equine piroplasmiasis, and respiratory syncytial virus (El-Tholoth et al., 2019; Rames and Macdonald, 2019; Zeng et al., 2019; Hu et al., 2020; Lei et al., 2020; Wang et al., 2020; Xu et al., 2020). However, no reports about rapid detection of *C. psittaci* using RPA assay are available. In this study, a probe-based RPA assay for rapid detection of *C. psittaci* was successfully developed. The novel assay can perform the test in a timely manner (approximately 20 min), with a detection limit of 100 copies per molecule and high specificity with other pathogens.

RPA assay could potentiate highly accessible and sensitive amplification of nucleic acids outside of the laboratory (Li et al., 2018). Previous studies reported that a mobile laboratory is established for RPA detection of dengue virus in the field (Abd et al., 2015). Considering the lack of a well-equipped laboratory in the field or in remote areas, point-of-care diagnosis is essential for disease treatment and preventing pathogen transmission. In this study, we tried to construct a mobile laboratory containing the DNA/RNA extraction kit, TwistDx RPA kit, Tubescanner, pipettor, and a miniature centrifuge. Using the portable mobile laboratory, we performed RPA detection of *C. psittaci* in an outbreak. A total of 36 clinical samples were collected during this outbreak and proved positive for *C. psittaci* using RPA detection. Then, all the clinical samples were taken back to a laboratory and confirmed positive for *C. psittaci* by qRT-PCR and *C. psittaci* isolation. These data provide evidence that the RPA assay performed using



**Figure 4.** Phylogenetic analysis of *C. psittaci* isolates based on the nucleotide sequence of the ompA gene. The phylogenetic tree was constructed using the neighbor-joining method from phylogenetic distances calculated using MEGA 7.0 software. Clades representing an established genotype of *C. psittaci* are encircled by a dashed line and designated accordingly in bold print. Basic data of other reference strains are listed in Table 2. The *C. psittaci* strains isolated in this study are indicated by solid triangles.

a portable mobile laboratory possesses good practicability and high accuracy, which will be beneficial to detect *C. psittaci* in the field and reduce economic loss in ducks. More effort will be required to further assess the practicability of the novel assay in the field.

Currently, *C. psittaci* infection in ducks has been reported globally (Arzey et al., 1990; Huminer et al., 1992; Hinton et al., 1993; Vorimore et al., 2015; Lin et al., 2019). A recent seroepidemiological survey revealed that the seroprevalence of *C. psittaci* was 26.13 and 45.29% in caged and free-range ducks, respectively (Laroucau et al., 2009). In our previous study, we reported a parrot-type *C. psittaci* strain associated with egg production drop in laying ducks is responsible for an outbreak (Lin et al., 2019). Soon after identifying the outbreak cause in that break, doxycycline and aureomycin were widely used in many laying duck farms, which protected laying ducks from *C. psittaci* infection and reduced the economic loss caused by this disease. However, antibiotic application usually results in the accumulation of drug concentrations in the plasma of *C. psittaci*, increasing the possibility of the development of drug-resistant strains (Tell et al., 2003; Bommana and Polkinghorne, 2019). In July 2019, the infectious disease re-emerged in laying ducks in Guangdong province of China, and the antibiotics were also used in this outbreak to control this disease. Surprisingly, aureomycin resistance was observed on one duck farm, where isolated the GD19A1 strain. Our findings directly indicated that drug-resistant *C. psittaci* strains have emerged in ducks and may circulate more widely in future. Further studies continuously surveilling laying ducks for *C. psittaci* infection, along with vaccine development and antibiotic prohibition, would help control this disease and reduce the risk of the drug-resistant bacteria and human infection.

The major outer membrane protein encoded by the *ompA* gene was the major predominant protein, containing a series of serotype-specific and antigenic epitopes. Based on the *ompA* gene, *C. psittaci* can be divided into 15 genotypes: genotype A, B, C, D, E, F, EB, MatI16, M56, CPX 0308, WC, 6N, 1V, Daruma-1981, and R54 (Sachse et al., 2008). Interestingly, genotypes of *C. psittaci* tend to be associated with species of birds. Generally, genotype A was found in psittacine birds, genotype B was identified in pigeons, genotype C was screened in ducks and geese, genotype D was usually isolated in turkeys, genotype E was detected in pigeons and turkeys, genotype F was found in turkeys and psittacine birds, and genotype E/B was identified in ducks, turkeys, and pigeons (Geens et al., 2005). In this study, 4 strains were isolated in the outbreaks. Of these, GD19A1, GD19A2, and GD19A3 strains were closely related to the parrot-type strain SZ15, which was responsible for a previous outbreak in laying ducks (Lin et al., 2019). Another isolate GD19 C was closely related to the GD strain, which belongs to genotype C. These findings provided clues that the epidemic of *C. psittaci* in Chinese duck populations is complicated. More effort will be required to systematically monitor

*C. psittaci* in the field and analyze the association of genotypes with host preference.

In summary, this is the first demonstration of a point-of-care diagnostic assay for *C. psittaci* detection using the RPA method. The probe-based RPA assay can be performed in 20 min using a portable device, with a detection limit of 100 copies of DNA molecules per reaction and no cross-reaction with other pathogens. Moreover, the assay was applied to screen *C. psittaci* in an outbreak and was presented as a practical and highly accurate test in field application. Collectively, this study offers a promising approach to detect *C. psittaci* both in the laboratory and in fields, which would benefit identifying the agent in ducks.

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## DISCLOSURES

The authors have no potential conflict of interests with this manuscript.

## SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at <https://doi.org/10.1016/j.psj.2020.11.031>.

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