



## FULL PAPER

Bacteriology

# Chlamydial species among wild birds and livestock in the foothills of Mt. Afadjato, Ghana

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**ABSTRACT.** The members of family *Chlamydiaceae* have a broad host range and cause many kinds of diseases in humans and animals. Several cases of *Chlamydiaceae* being detected in atypical hosts have been reported recently. Consequently, cross-species monitoring of *Chlamydia* in wildlife and livestock is pertinent for public health, animal hygiene and wildlife conservation. In this study, we conducted molecular surveillance of *Chlamydia* in wild birds and livestock around a small village in the foothills of Mt. Afadjato, Ghana where direct contact between wildlife and livestock occurs. Among 29 captured wild birds and 63 livestock, 5 sheep, 30 goats and 28 chickens, the positive ratios of *Chlamydia* were 24.1%, 40.0%, 43.3% and 26.9%, respectively. *Chlamydia pecorum* was detected in wild birds, goats, sheep and chickens. On the basis of the variable domain 2 region of *ompA*, several samples from different hosts showed identical sequences and were phylogenetically located to the same clusters. In addition, using *ompA*, *C. psittaci*, *C. abortus* and *C. gallinacea* were also detected in this small habitat. Further genetic and pathogenic analyses of the chlamydial distribution in this area, which represents the interface of wild and domestic animal interactions, may improve our knowledge of their transmission among different hosts.

**KEYWORDS:** biodiversity, *Chlamydia*, Ghana, zoonosis

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*Chlamydia* species are facultative intracellular bacteria widely distributed in various host species, ranging from amphibians to humans. The family *Chlamydiaceae* currently consists of 11 species, including two recently admitted members, *Chlamydia avium* and *C. gallinacea* [26, 27]. From the feral sacred ibis (*Threskiornis aethiopicus*) a new chlamydial species, named as *C. ibidis*, has also been isolated [30]. Several species belonging to *Chlamydiaceae* are recognized as important pathogens in the medical and veterinary fields. *C. trachomatis*, which is highly adapted to humans, is a serious causative agent of blindness and sexually transmitted diseases worldwide. *C. pneumoniae* causes community-acquired pneumonia in humans, and it is sometimes detected in other mammals, such as koala. *C. psittaci* usually infects birds, but occasionally causes psittacosis (ornithosis) in humans. *C. abortus* is a major cause of abortion in ruminants. *C. pecorum* may cause severe diseases, such as encephalitis, polyarthritis and enteritis, in ruminants and pigs, but usually results in asymptomatic infections. In addition, *C. suis* (which mainly infects pigs)

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and *C. felis* (which mainly infects cats) are major pathogens in the veterinary field. The recent addition of three new members of *Chlamydiaceae* in birds, *C. avium*, *C. gallinacea* [26, 27] and *C. ibidis* [30], is indicative of the possibly large diversity of *Chlamydia* spp. associated with avian chlamydiosis [25].

Generally, *Chlamydia* has broad host range, but each *Chlamydia* species tends to be harbored by typical hosts. For example, avian orders *Psittaciformes* and *Columbiformes* are the most typical and prominent hosts for *C. psittaci* [9]. Recently, the detection of *Chlamydia* spp. in atypical or non-typable hosts has been reported [13, 15, 24, 29, 32]. The harboring of *Chlamydia* by atypical hosts may aid in their maintenance and circulation in nature. Most *Chlamydia* species infecting livestock and wild animals can be transmitted to humans as zoonotic agents and *vice versa* [2]. The prevalence of *C. psittaci* in birds correlates with the psittacosis prevalence in humans [12], and the psittacosis in humans has rapidly increased recently in Belgium [23]. Thus, the cross-species monitoring of *Chlamydia* in farm and wild animals is important for public health, animal hygiene and wildlife conservation.

The Republic of Ghana is located along the Gulf of Guinea in the West African sub-region. Ghana is endowed with ecological diversity, ranging from the Sudan Savannah in the north-east to rainforests along the coast, that supports a variety of fauna. Mount Afadjato, at 890 m above sea level, is the highest mountain in Ghana. It lies along the Ghana–Togo border, within the Mount Afadjato and Agumatsa Range Conservation Area (Fig. 1). This area has been designated as an important bird area in Africa [18, 20]. In the foothills of Mt. Afadjato, there are several small villages. In these villages, livestock, like sheep, goats and chickens, are reared under an extensive management system by each family.

In this study, we assessed the prevalence of *Chlamydia* among wild birds and livestock in a small village in the foothills of Mt. Afadjato, Ghana.

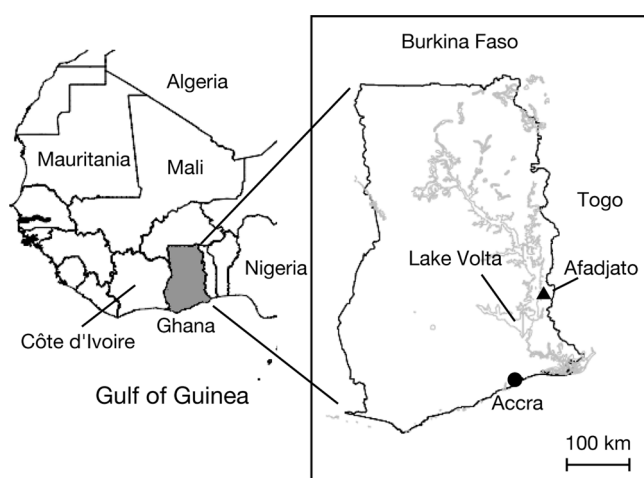
## MATERIALS AND METHODS

### Specimens

All the specimens from wild birds and livestock were collected in the Gbledi Village situated in the foothill of Mt. Afadjato (7°2'6"N, 0°33'42"E) in March 2014 (Fig. 1). Wild birds ( $n=29$ ) were captured using a mist net set approximately 200 m from the village with a license from the Ghana Ringing Scheme. Samples were also taken from domesticated animals reared by villagers, including 5 sheep (*Ovis aries*; the Forest-type breed), 30 goats (*Capra hircus*; the West-African Dwarf breed) and 28 chickens (*Gallus gallus*; the Forest Ecotype). The collection of specimens from wild birds and livestock was approved by the Gifu University Animal Experiment Committee (Approval number: 17070). Cloacal or rectal swabs from wild birds or livestock were collected into sucrose–phosphate–glutamate transportation medium for *Chlamydia* [28]. The specimens were kept at 4°C for approximately 2 days during transportation to the laboratory at the University of Ghana. DNAs from the specimens were extracted using a PureLink Genomic DNA kit (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions.

### Screening for chlamydial DNA in specimens

All the specimens were screened for chlamydial DNA using TaqMan and SYBR Green real-time PCR. The TaqMan real-time PCR amplified 23S rRNA as described elsewhere [5] using the following primers: TQF (5'-GAAAAGAACCCTTGTTAAGGGAG-3') and TQR (5'-CTTAACCTCCCTGGCTCATCATG-3'), with a probe (FAM-CAAAAGGCACGCCGTCAAC-TAMRA). The reaction was performed with the Premix *Ex-Taq* (TaKaRa Bio, Kusatsu, Japan) on a Thermal Cycler Dice TP800 (TaKaRa Bio) in accordance with the manufacturer's instructions. The SYBR Green real-time PCR targeted *envB* as described previously [19] using the following primers: Env-F (5'-AACCTCGGATAGCAAATTAATCTGG-3') and



**Fig. 1.** Map showing the location of the study area, based on shapefiles from GADM database of Global Administrative Areas (<http://gadm.org>) and from DIVA-GIS (<http://www.diva-gis.org>). Ghana is located along the Gulf of Guinea. The Afadjato conservation area lies along the Togolese border.

Env-R (5'-ATTTGGTATAAGAGCGAAGTTCTGG-3'). The reaction was performed with the SYBR Premix *Ex-Taq* (TaKaRa Bio) on a Thermal Cycler TP800 (TaKaRa Bio) in accordance with the manufacturer's instructions. The TaqMan real-time PCR detected a broad range of *Chlamydiaceae*, including *C. psittaci*, *C. abortus*, *C. felis*, *C. caviae*, *C. pneumoniae*, *C. pecorum*, *C. trachomatis*, *C. suis* and *C. muridarum* [5], whereas the SYBR Green real-time PCR detected *C. psittaci*, *C. abortus*, *C. felis*, *C. caviae* and *C. pecorum* with a high sensitivity and a detection limit of 10 inclusion-forming units (IFUs) [19].

#### Phylogenetic analyses of detected species of family *Chlamydiaceae*

Among positive samples for chlamydial DNA, the *ompA* variable domain (VD) 2 region that encompasses portions of outer membrane protein A, 16S rRNA and *enoA*, encoding enolase A, were sequenced for phylogenetic analyses. We first attempted to sequence the *ompA* gene because it has been used to differentiate chlamydial strains and species [11]. However, it was difficult to produce the full-length *ompA* sequence data for each positive sample; consequently, we attempted to amplify and analyze the VD2 region of the *ompA* gene, which contains chlamydial species/strain-specific nucleotide motifs, instead of the full-length *ompA*. The partial VDs of the *ompA* gene, including the VD2 region, were also analyzed to identify chlamydial species and strains [4, 8, 10].

The whole-genome amplification of positive samples was conducted using an illustra GenomiPhi DNA Amplification kit (GE healthcare, Chicago, IL, USA) to enhance the PCR efficiency. The *ompA* VD2 region was amplified using nested PCR with an outer pair of primers (CMGP-1F: 5'-CCTTGTGATCCTTGCGCTACTTG-3'/CMGP-1R: 5'-GTGAGCAGCTCTTCGTTGAT-3') and an inner pair of primers (CMGP-2F: 5'-GCCTTAAACATCTGGGATCG-3'/CMGP-2R: 5'-GCACAACCACATTCATCCATAAAG-3') and previously described conditions [3] on a PCR Thermal Cycler Dice (TaKaRa Bio). The nested PCR reaction was performed with BIOTAQ HS DNA Polymerase (Bioline Reagents, London, UK) in Ampdirect Plus buffer (Shimadzu Corporation, Kyoto, Japan) instead of *Taq* polymerase as reported previously [3, 4] because of better recorded amplification results. The 16S rRNA was amplified with the pan-*Chlamydiales* primers panCh 16F2: 5'-CCGCCAACACTGGGACT-3' and panCh16R2: 5'-GGAGTTAGCCGGTGCTTCTTAC-3' that could amplify a broad range of *Chlamydiales*, including *parachlamydia*, with high sensitivity and a detection limit of 5 copies [1, 16]. The *enoA* gene was amplified with the primers YPenA5: 5'-CCWATGATGAAYCTYATTAACGG-3' and YPenA6: 5'-TCTTCYTCAGGRAGGCCATCT-3', (W=A or T; Y=C or T), following a method described elsewhere [22]. The 57-, 169- and 381-nt sequences were determined for *ompA*, 16S rRNA and *enoA*, respectively. Amino acid sequences deduced from the nucleotide sequences were aligned using ClustalW (<https://www.genome.jp/tools-bin/clustalw>) and phylogenetically analyzed using Mega 7 [14]. The independent dendrograms of *ompA* VD2 and 16S rRNA were constructed using the Maximum-likelihood method based on the Le\_Gascuel\_2008 model. A dendrogram of the *enoA* gene was constructed by the Maximum-likelihood method based on the Kimura 2-parameter model.

#### Nucleotide sequence accession numbers

Nucleotide sequences obtained in this study have been deposited at the DNA Data Bank of Japan (DDBJ) under accession numbers LC271681 to LC271692 for the VD2 region of *ompA*, LC484863 to LC484866 for partial 16S rRNA and LC271693 for the partial *enoA* gene.

## RESULTS

The wild birds ( $n=29$ ) captured and screened in the present study belonged to two orders (*Passeriformes* and *Columbiformes*) and comprised 10 species (Table 1). Livestock samples ( $n=63$ ) were from sheep (the Forest-type breed), goats (the West-African Dwarf breed) and chicken (the Forest Ecotype). Using real-time PCR, three out of nine species of *Passeriformes* tested positive for chlamydial DNA, namely *Phyllastrephus icterinus* (33.3%), *Cyanomitra olivacea* (33.3%) and *Alethe castanea* (100%). In all, 24.1% of wild birds were positive for *Chlamydia* spp. (Table 1). For livestock, DNA samples from 43.3% of goats, 40.0% of sheep

**Table 1.** Prevalence of *Chlamydia* spp. in the wild birds captured at the foothill of Mt. Afadjato, Ghana (Nomenclature follows Borrow and Demey, 2010)

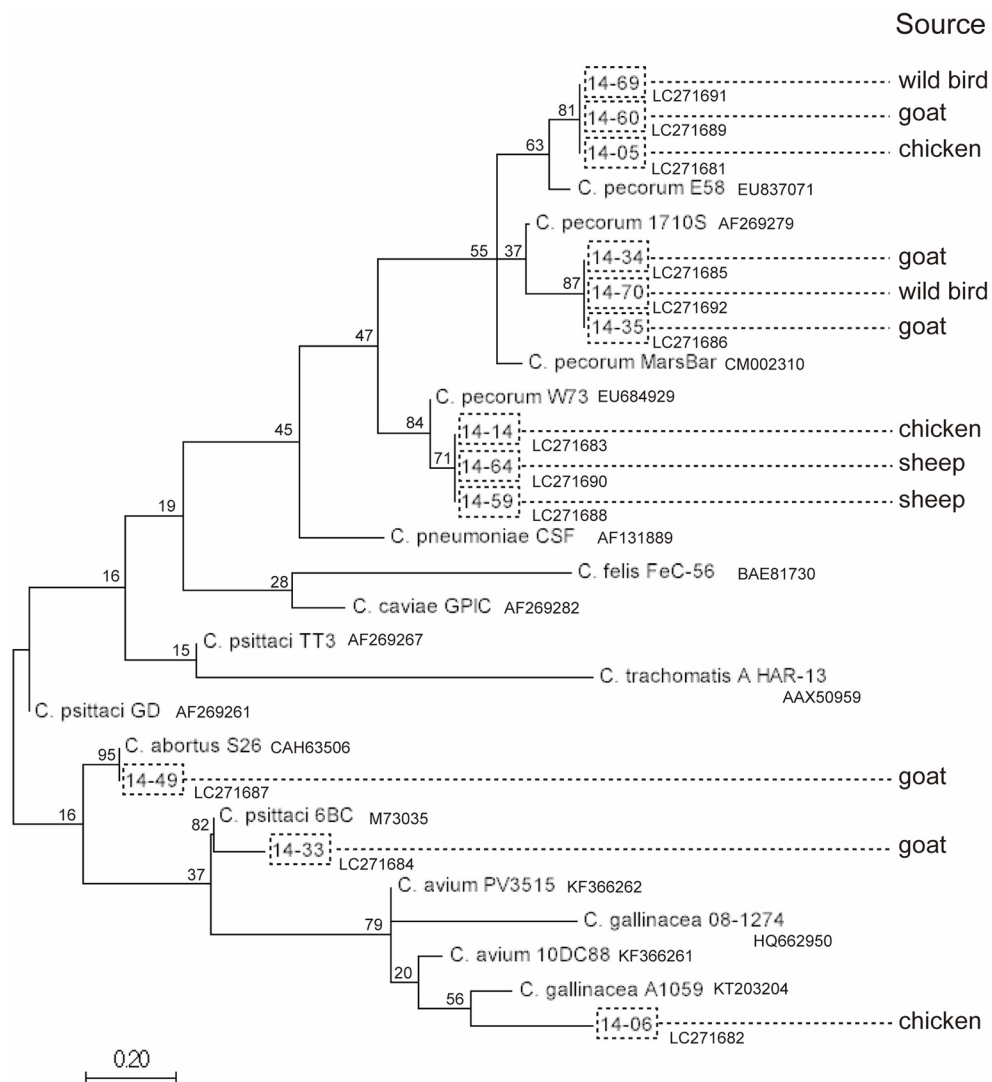
Order	Species		Number of specimens	Number of positive specimens	Percentage of positive specimens
	Latin name	English name			
Passeriformes	<i>Stiphornis erythrorhax</i>	Forest Robin	2	0	0
	<i>Terpsiphone rufiventer</i>	Red-bellied Paradise Flycatcher	1	0	0
	<i>Bleda canicapillus</i>	Grey-headed Bristlebill	5	0	0
	<i>Bleda eximius</i>	Green-tailed Bristlebill	2	0	0
	<i>Eurillas latirostris</i>	Yellow-whiskered Greenbul	2	0	0
	<i>Phyllastrephus icterinus</i>	Icterine Greenbul	6	2	33.3
	<i>Cyanomitra olivacea</i>	Olive sunbird	6	2	33.3
	<i>Alethe diademata</i>	White-tailed Alethe	3	3	100
	<i>Hylia prasina</i>	Green Hylia	1	0	0
	Columbiformes	<i>Tutur tympanistria</i>	Tambourine Dove	1	0
Total			29	7	24.1

and 26.9% of chicken were positive for *Chlamydia* spp. (Table 2).

We succeeded in sequencing the VD2 region of the *ompA* gene from 12 of 29 positive samples (7 wild bird and 22 livestock). Among the 12 samples, 9 were located on clusters near *C. pecorum*, which strongly indicates that these samples belonged to *C. pecorum*. Additionally, *C. pecorum* positive samples were obtained from two wild birds (*P. icterinus*), three goats, two sheep and two chickens (Fig. 2). *C. pecorum* strains can be classified into two major clusters based on the *ompA* genotype [17]. In the present study, at least three clusters of *C. pecorum* were identified. The first cluster consisted of samples 14–69, 14–60 and 14–05

**Table 2.** Prevalence of *Chlamydia* spp. in livestock reared in a village at the foothill of Mt. Afadjato, Ghana

Livestock (breed)	Number of specimens	Number of positive specimens	Percentage of positive samples
Goat (- Forest type)	30	13	43.3
Sheep (- West-African Dwarf)	5	2	40.0
Chicken (- Forest Ecotype)	28	7	26.9
Total	63	22	34.9

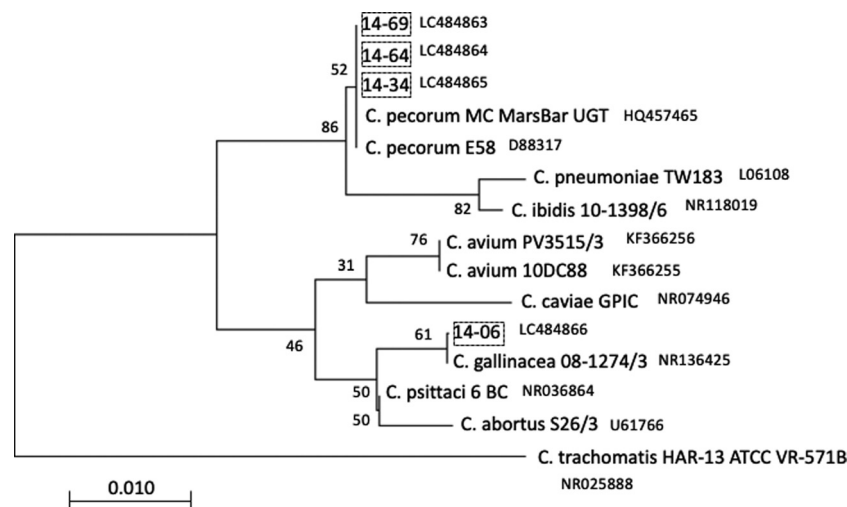


**Fig. 2.** Phylogenetic analysis of the *ompA* variable domain 2 (VD2) region using the by Maximum-likelihood method. Some of the *Chlamydiaceae* positive samples in this study from which the *ompA* VD2 region (dotted squares) was sequenced successfully were compared with those of other reported *Chlamydiaceae* strains. The tree with the greatest log likelihood is shown. Branch lengths are measured in amino acid substitutions. Numbers indicate percentages of the bootstrap replicates ( $n=1,000$ ). The scale bar represents the percentage of sequence diversity. The sources of the collected samples are shown, and the ID number of each nucleotide or protein in GenBank is provided.

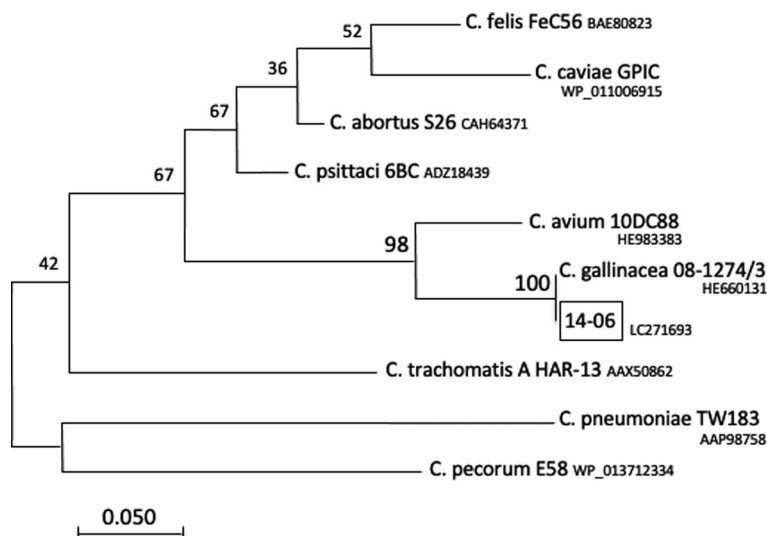
from wild bird, goat and chicken, respectively. The second cluster contained samples 14–70 (wild bird), 14–34 (goat) and 14–35 (goat), whereas the third cluster contained samples 14–14 (chicken), 14–59 (sheep) and 14–64 (sheep). The partial VD2 *ompA* sequences were identical in all the samples in the first and second clusters, and identical for 14–14 and 14–64 in the third cluster. Representative samples of each cluster, 14–69, 14–34 and 14–64, respectively, were subjected to a phylogenetic analysis of their 16S rRNAs and classified within the cluster of *C. pecorum*, which confirmed that these samples belonged to *C. pecorum* (Fig. 3).

We also detected strains as *C. abortus* (sample 14–49) and as *C. psittaci* (sample 14–33) in goats (Fig. 2). *C. psittaci* can be divided into 10 clusters (I–X) of the VD2 variant, which is correlated with the *C. psittaci* genotype (equivalent to *C. psittaci* serotype determined with anti-major outer membrane protein antibodies) as described previously [3]. The sample 14–33 was located on the same cluster as *C. psittaci* 6BC. *C. psittaci* 6BC belongs to genotype A, which is the most prevalent genotype, and is usually harbored by psittacine birds [7]. *C. psittaci* 6BC also belongs to cluster I as determined by analyzing the VD2 region [3], suggesting that the 14–33 sample from goat belongs to genotype A.

In addition to *C. pecorum*, *C. abortus* and *C. psittaci*, we detected another *Chlamydia* sp. in this area from a chicken sample, 14–06, and identified it as *C. gallinaceae* on the basis of the *enoA* gene (Fig. 4), as well as *ompA* VD2 (Fig. 2) and 16S rRNA (Fig. 3) analyses.



**Fig. 3.** Phylogenetic analysis of the 16S rRNA gene using the Maximum-likelihood method. Some representative *Chlamydia pecorum* and *C. gallinaceae* positive samples from which the *ompA* variable domain 2 region was sequenced successfully were subjected to 16S rRNA (dotted squares) sequencing and compared with those of other reported *Chlamydiaceae* strains. The tree with the greatest log likelihood is shown. Branch lengths are measured in nucleotide substitutions. Numbers indicate percentages of the bootstrap replicates ( $n=1,000$ ). The scale bar represents the percentage of sequence diversity. The ID number of each nucleotide or protein in GenBank is provided.



**Fig. 4.** Phylogenetic analysis of the *enoA* gene using the Maximum-likelihood method. The *enoA* gene of sample 14-06 (square) from a chicken that tested positive for *Chlamydiaceae* was compared with those of other reported *Chlamydiaceae* strains. The tree with the greatest log likelihood is shown. Branch lengths are measured in nucleotide substitutions. Numbers indicate percentages of the bootstrap replicates ( $n=1,000$ ). The scale bar represents the percentage of sequence diversity. The ID number of each nucleotide or protein in GenBank is provided.



## DISCUSSION

Generally, the members of family *Chlamydiaceae* have a broad host range and cause many kinds of diseases in humans and other animals. In this study, we conducted molecular surveillance of *Chlamydia* among livestock and wild birds around a small village, where direct contact between wildlife and livestock occurs, in the foothills of Mt. Afadjato, Ghana. We detected a variety of chlamydial species in different hosts in this area. Our major results were as follows: (i) at least, four kinds of *Chlamydia* (*C. pecorum*, *C. psittaci*, *C. abortus* and *C. gallinacea*) were harbored asymptotically in livestock and wild birds near the village and (ii) according to the phylogenetic analysis, *C. pecorum* detected in wild bird, goat and chicken located to the same cluster.

In this study, we were unable to obtain full-length *ompA* sequences and other sequences, such as 16S/23S rRNA, owing to limitations of the target DNA available and perhaps the presence of PCR inhibitors [31] in field fecal samples. This inherent limitation in molecular epidemiological studies prevented us from carrying out further genetic analyses, such as multi-locus sequence typing, which is an appropriate way to analyze the genetic diversity of *Chlamydia* [21]. For the purpose of achieving a high sensitivity of *Chlamydia* detection by PCR for nucleic acid sequencing, we amplified total DNA using several DNA amplification kits, as well as PCR enzymes and buffers, that suppressive inhibitors in biological samples. Finally, we succeeded in obtaining the partial VD2 *ompA* sequences in 12 samples and constructed a phylogenetic tree. In this phylogenetic tree, three clusters of *C. pecorum* were identified in this narrow sampling area (Fig. 2). Each cluster included at least three samples from the different animal species sampled, goat, sheep, chicken and wild birds. *C. pecorum* is common in ruminants but has been rarely identified in wild birds [15, 17]. Although previous studies reported the presence of *C. pecorum* in birds, most reports are from captive birds [6, 24]. Therefore, to confirm the presence of *C. pecorum* in the wild birds of the Afadjato area, we attempted to analyze the 16S rRNA region using pan-*Chlamydiales* PCR that could detect a broad range of *Chlamydiales*, including *parachlamydia*, with a high sensitivity and a detection limit of 5 copies as described previously [1, 16]. In this pan-*Chlamydiales* PCR, we used locked nucleic acids in the PCR primers to improve PCR performance [1, 16]. With this improved PCR method, we successfully constructed a phylogenetic tree of partial 16S rRNA sequences. The 14–69, 14–64 and 14–34 samples were within the *C. pecorum* cluster in the resulting phylogenetic tree, which corroborated the results of the tree constructed using partial VD2 *ompA* sequences.

Furthermore, *C. pecorum* samples belonging to the same clusters as determined by the *ompA* VD2 region analysis were not necessarily from the same hosts. For example, the cluster containing *C. pecorum* E58 included the 14–69 sample from wild bird, the 14–60 sample from goat and the 14–05 sample from chicken. In each cluster, the partial VD2 *ompA* sequences from different host were identical for 14–69 (wild bird), 14–60 (goat) and 14–05 (chicken); identical for 14–34 (goat), 14–70 (wild bird) and 14–35 (goat); and identical for 14–14 (chicken), 14–64 (sheep) and 14–59 (sheep). This revealed the existence of *C. pecorum* among wild birds, goats and chickens in this small area.

The detection of *Chlamydia* in atypical hosts has been reported. In these reports, samples were collected from different countries over a long period [32] or from different provinces/regions for several months in each country [15, 29]. In contrast, we collected samples in a small village (consisting of approximately 10 families), where direct contact between farm and wild animals occurs, over 2 days. All the wild birds examined in this study were native birds. Because they were captured near the village, contact between wild birds and livestock could be frequently occurring. In fact, livestock reared in the village was always free range and grazed and fed in the foothills, which ensured that they were in contact with wild birds. In addition, all the *Chlamydia* were detected in healthy wild birds and livestock. Therefore, *Chlamydiaceae* bacteria may infect asymptotically, resulting in its circulation and maintenance among the wild birds and livestock in this area. Here, the existence of *C. pecorum* among different hosts, such as wild birds, livestock and chickens, in the small area might imply the circulation of *Chlamydia*. Certainly, multi-locus sequence typing or whole-genome sequencing analyses of detected strains and a fixed-point sampling are necessary for discussing the circulation of pathogens in a specific environment, like those conducted in urban pigeons in one German town [24].

This is the first report that examined the prevalence and diversity of *Chlamydiaceae* in several kinds of mammals and birds in the Afadjato and Agumatsa area of Ghana, West Africa. It is imperative to further investigate the genetic diversity of harbored by wild birds and livestock in this area to explore their evolutionary relationships, host specificity and cross-species transmission mechanisms. These results will help to evaluate the impact of *Chlamydiaceae* on public health, livestock hygiene and wildlife conservation.

**POTENTIAL CONFLICTS OF INTEREST.** No author has a potential conflict of interest relevant to this article.

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REFERENCES

1. Adenyo, C., Ohya, K., Qiu, Y., Takashima, Y., Ogawa, H., Matsumoto, T., Thu, M. J., Sato, K., Kawabata, H., Katayama, Y., Omatsu, T., Mizutani, T., Fukushi, H., Katakura, K., Nonaka, N., Inoue-Murayama, M., Kayang, B. and Nakao, R. 2020. Bacterial and protozoan pathogens/symbionts in ticks infecting wild grasscutters (*Thryonomys swinderianus*) in Ghana. *Acta Trop.* **205**: 105388. [Medline] [CrossRef]
2. Burnard, D. and Polkinghorne, A. 2016. Chlamydial infections in wildlife-conservation threats and/or reservoirs of 'spill-over' infections? *Vet. Microbiol.* **196**: 78–84. [Medline] [CrossRef]
3. Chahota, R., Ogawa, H., Mitsuhashi, Y., Ohya, K., Yamaguchi, T. and Fukushi, H. 2006. Genetic diversity and epizootiology of *Chlamydomphila psittaci* prevalent among the captive and feral avian species based on VD2 region of *ompA* gene. *Microbiol. Immunol.* **50**: 663–678. [Medline] [CrossRef]
4. Chahota, R., Ogawa, H., Ohya, K., Yamaguchi, T., Everett, K. D. E. and Fukushi, H. 2018. Involvement of multiple *Chlamydia suis* genotypes in porcine conjunctivitis. *Transbound. Emerg. Dis.* **65**: 272–277. [Medline] [CrossRef]
5. Everett, K. D. E., Hornung, L. J. and Andersen, A. A. 1999. Rapid detection of the *Chlamydiaceae* and other families in the order *Chlamydiales*: three PCR tests. *J. Clin. Microbiol.* **37**: 575–580. [Medline] [CrossRef]
6. Frutos, M. C., Monetti, M. S., Vaulet, L. G., Cadario, M. E., Fermepin, M. R., Ré, V. E. and Cuffini, C. G. 2015. Genetic diversity of *Chlamydia* among captive birds from central Argentina. *Avian Pathol.* **44**: 50–56. [Medline] [CrossRef]
7. Geens, T., Desplanques, A., Van Looock, M., Bönner, B. M., Kaleta, E. F., Magnino, S., Andersen, A. A., Everett, K. D. E. and Vanrompay, D. 2005. Sequencing of the *Chlamydomphila psittaci ompA* gene reveals a new genotype, E/B, and the need for a rapid discriminatory genotyping method. *J. Clin. Microbiol.* **43**: 2456–2461. [Medline] [CrossRef]
8. Guo, W., Li, J., Kaltenboeck, B., Gong, J., Fan, W. and Wang, C. 2016. *Chlamydia gallinacea*, not *C. psittaci*, is the endemic chlamydial species in chicken (*Gallus gallus*). *Sci. Rep.* **6**: 19638. [Medline] [CrossRef]
9. Harkinezhad, T., Geens, T. and Vanrompay, D. 2009. *Chlamydomphila psittaci* infections in birds: a review with emphasis on zoonotic consequences. *Vet. Microbiol.* **135**: 68–77. [Medline] [CrossRef]
10. Kabeya, H., Sato, S. and Maruyama, S. 2015. Prevalence and characterization of *Chlamydia* DNA in zoo animals in Japan. *Microbiol. Immunol.* **59**: 507–515. [Medline] [CrossRef]
11. Knittler, M. R. and Sachse, K. 2015. *Chlamydia psittaci*: update on an underestimated zoonotic agent. *Pathog. Dis.* **73**: 1–15. [Medline] [CrossRef]
12. Kozuki, E., Arima, Y., Matsui, T., Sanada, Y., Ando, S., Sunagawa, T. and Oishi, K. 2020. Human psittacosis in Japan: notification trends and differences in infection source and age distribution by gender, 2007 to 2016. *Ann. Epidemiol.* **44**: 60–63. [Medline] [CrossRef]
13. Krawiec, M., Piasecki, T. and Wieliczko, A. 2015. Prevalence of *Chlamydia psittaci* and other *Chlamydia* species in wild birds in Poland. *Vector Borne Zoonotic Dis.* **15**: 652–655. [Medline] [CrossRef]
14. Kumar, S., Stecher, G. and Tamura, K. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **33**: 1870–1874. [Medline] [CrossRef]
15. Li, J., Guo, W., Kaltenboeck, B., Sachse, K., Yang, Y., Lu, G., Zhang, J., Luan, L., You, J., Huang, K., Qiu, H., Wang, Y., Li, M., Yang, Z. and Wang, C. 2016. *Chlamydia pecorum* is the endemic intestinal species in cattle while *C. gallinacea*, *C. psittaci* and *C. pneumoniae* associate with sporadic systemic infection. *Vet. Microbiol.* **193**: 93–99. [Medline] [CrossRef]
16. Lienard, J., Croxatto, A., Aeby, S., Jatou, K., Posfay-Barbe, K., Gervais, A. and Greub, G. 2011. Development of a new chlamydiales-specific real-time PCR and its application to respiratory clinical samples. *J. Clin. Microbiol.* **49**: 2637–2642. [Medline] [CrossRef]
17. Mohamad, K. Y., Kaltenboeck, B., Rahman, K. S., Magnino, S., Sachse, K. and Rodolakis, A. 2014. Host adaptation of *Chlamydia pecorum* towards low virulence evident in co-evolution of the *ompA*, *incA*, and ORF663 Loci. *PLoS One* **9**: e103615. [Medline] [CrossRef]
18. Ntiamao-Baidu, Y., Owusu, E. H., Daramani, D. T. and Nuoh, A. A. 2001. Ghana. pp. 367–389. In: Important Bird Areas in Africa and Associated Islands: Priority Sites for Conservation (Fishpool, L. D. C. and Evans, M. I. eds.), Pisces Publications, Berkshire.
19. Okuda, H., Ohya, K., Shiota, Y., Kato, H. and Fukushi, H. 2011. Detection of *Chlamydomphila psittaci* by using SYBR green real-time PCR. *J. Vet. Med. Sci.* **73**: 249–254. [Medline] [CrossRef]
20. Owusu, E. H., Ekpe, E. K. and Asamoah, A. 2005. Large mammal fauna of the Afadjato and Agumatsa range in Ghana: An important bird area. *West Afr. J. Appl. Ecol.* **8**: 1.
21. Pannekoek, Y., Morelli, G., Kusecek, B., Morré, S. A., Ossewaarde, J. M., Langerak, A. A. and van der Ende, A. 2008. Multi locus sequence typing of *Chlamydiales*: clonal groupings within the obligate intracellular bacteria *Chlamydia trachomatis*. *BMC Microbiol.* **8**: 42. [Medline] [CrossRef]
22. Pannekoek, Y., Dickx, V., Beeckman, D. S. A., Jolley, K. A., Keijzers, W. C., Vretou, E., Maiden, M. C. J., Vanrompay, D. and van der Ende, A. 2010. Multi locus sequence typing of *Chlamydia* reveals an association between *Chlamydia psittaci* genotypes and host species. *PLoS One* **5**: e14179. [Medline] [CrossRef]
23. Rybarczyk, J., Versteede, C., Lernout, T. and Vanrompay, D. 2020. Human psittacosis: a review with emphasis on surveillance in Belgium. *Acta Clin. Belg.* **75**: 42–48. [Medline] [CrossRef]
24. Sachse, K., Kuehlewind, S., Ruettger, A., Schubert, E. and Rohde, G. 2012. More than classical *Chlamydia psittaci* in urban pigeons. *Vet. Microbiol.* **157**: 476–480. [Medline] [CrossRef]
25. Sachse, K. and Laroucau, K. 2014. Avian chlamydiosis: two more bacterial players discovered. *Vet. J.* **200**: 347–348. [Medline] [CrossRef]
26. Sachse, K., Laroucau, K., Riege, K., Wehner, S., Dilcher, M., Creasy, H. H., Weidmann, M., Myers, G., Vorimore, F., Vicari, N., Magnino, S., Liebler-Tenorio, E., Ruettger, A., Bavoil, P. M., Hufert, F. T., Rosselló-Móra, R. and Marz, M. 2014. Evidence for the existence of two new members of the family *Chlamydiaceae* and proposal of *Chlamydia avium* sp. nov. and *Chlamydia gallinacea* sp. nov. *Syst. Appl. Microbiol.* **37**: 79–88. [Medline] [CrossRef]
27. Sachse, K. and Laroucau, K. 2015. Two more species of *Chlamydia*-does it make a difference? *Pathog. Dis.* **73**: 1–3. [Medline] [CrossRef]
28. Spencer, W. N. and Johnson, F. W. A. 1983. Simple transport medium for the isolation of *Chlamydia psittaci* from clinical material. *Vet. Rec.* **113**: 535–536. [Medline]
29. Szymańska-Czerwińska, M., Mitura, A., Niemczuk, K., Zaręba, K., Jodelko, A., Pluta, A., Scharf, S., Vitek, B., Aaziz, R., Vorimore, F., Laroucau, K. and Schnee, C. 2017. Dissemination and genetic diversity of chlamydial agents in Polish wildfowl: Isolation and molecular characterisation of avian *Chlamydia abortus* strains. *PLoS One* **12**: e0174599. [Medline] [CrossRef]
30. Vorimore, F., Hsia, R. C., Huot-Creasy, H., Bastian, S., Deruyter, L., Passet, A., Sachse, K., Bavoil, P., Myers, G. and Laroucau, K. 2013. Isolation of a new *chlamydia* species from the feral Sacred Ibis (*Threskiornis aethiopicus*): *Chlamydia ibidis*. *PLoS One* **8**: e74823. [Medline] [CrossRef]
31. Wilson, I. G. 1997. Inhibition and facilitation of nucleic acid amplification. *Appl. Environ. Microbiol.* **63**: 3741–3751. [Medline] [CrossRef]
32. Zocevic, A., Vorimore, F., Marhold, C., Horvatek, D., Wang, D., Slavec, B., Prentza, Z., Stavianis, G., Prukner-Radovic, E., Dovc, A., Siarkou, V. I. and Laroucau, K. 2012. Molecular characterization of atypical *Chlamydia* and evidence of their dissemination in different European and Asian chicken flocks by specific real-time PCR. *Environ. Microbiol.* **14**: 2212–2222. [Medline] [CrossRef]