JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2008, p. 281–285 Vol. 46, No. 1
0095-1137/08/$08.00+0 doi:10.1128/JCM.01153-07

Evaluation of a *Chlamydophila psittaci* Infection Diagnostic Platform for Zoonotic Risk Assessment▽

Kristel Verminnen,[1]* Barbara Duquenne,[1]† David De Keukeleire,[2,3] Birgitta Duim,[4] Yvonne Pannekoek,[4] Lutgart Braeckman,[2] and Daisy Vanrompay[1]

Department of Molecular Biotechnology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium[1]; Department of Public Health, Faculty of Medicine and Health Sciences, Ghent University, De Pintelaan 185, B-9000 Ghent, Belgium[2]; IDEWE Occupational Health Services, Interleuvenlaan 58, B-3001 Leuven, Belgium[3]; and Department of Medical Microbiology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands[4]

Received 8 June 2007/Returned for modification 29 July 2007/Accepted 31 October 2007

Reports on zoonotic transmission of *Chlamydophila psittaci* originating from poultry are incidentally published. During recent studies in European turkeys we isolated *C. psittaci* genotypes A, B, D, E, F, and E/B, all considered potentially dangerous for humans. This encouraged us to analyze the zoonotic risk on a Belgian turkey farm, from production onset until slaughter, using a *Chlamydophila psittaci* diagnostic platform. Twenty individually marked hens, as well as the farmer and two scientists, were monitored medically. Bioaerosol monitoring, serology, isolation, and nested PCR demonstrated chlamydiosis on the farm leading to symptomatic psittacosis in all 3 persons involved. *ompA* sequencing confirmed the zoonotic transmission of *C. psittaci* genotype A. Strangely, two different antibody microimmunofluorescence (MIF) tests remained negative in all infected persons. The results demonstrate the value of the currently used diagnostic platform in demonstrating *C. psittaci* infections in both birds and humans but raise questions regarding use of the MIF test for diagnosing human psittacosis. In addition, our results suggest the underestimation of psittacosis in the poultry industry, stressing the need for a veterinary vaccine and recommendations for zoonotic risk reduction strategies.

Chlamydophila psittaci (formerly *Chlamydia psittaci*) is found in birds and can be transmitted to humans by inhalation or direct contact causing psittacosis or parrot fever (5). The disease name dates back to 1893 when transmission of *C. psittaci* from parrots (Latin; *psittacus* or parrot) to humans, causing flu-like symptoms, was observed in Paris (8). Until 1932, when the first case of human psittacosis due to *C. psittaci* transmission from diseased chickens was reported, *C. psittaci* infections were thought to occur only in psittacine birds. In the 1950s, after an outbreak in humans due to contact with infected turkeys, the importance of poultry as a source of human infection became more evident (7). Till approximately 20 years ago, American and European turkey farms had to deal with explosive *C. psittaci* outbreaks with high mortality mainly related to outer membrane protein A (*ompA*) genotype D strains (1, 16). These ancient genotype D strains have all been associated with either serious disease in birds or humans or with major epizootics in turkeys, often resulting in human disease.

At present, chlamydial infections are endemic in European turkeys and most likely also in U.S. turkeys, although the latter have not been investigated recently. However, devastating, major outbreaks are currently rare, and respiratory signs with low mortality now characterize *C. psittaci* outbreaks. Recently, we isolated *C. psittaci ompA* genotypes A, B, D, E, F, and E/B in European turkeys. These genotypes are all considered potentially dangerous for humans. Notwithstanding the high prevalence of these genotypes in turkeys, few zoonotic transmissions are reported. This encouraged us to examine the potential zoonotic risk of raising commercial fattening turkeys from production onset until slaughter by using a *C. psittaci* diagnostic platform comprising antigen, gene, and antibody detection, as well as molecular characterization methods in both birds and humans.

The present study clearly demonstrates the usefulness of the diagnostic platform but raises questions as to whether the microimmunofluorescence (MIF) test should be used for demonstrating psittacosis in humans. The results underline the risk of contracting psittacosis from poultry and indicate zoonotic transfer to occur more often than believed, stressing the need for diagnostic monitoring, a veterinary vaccine, and information campaigns with recommendations for psittacosis risk reduction strategies.

MATERIALS AND METHODS

Farm management. *C. psittaci* zoonotic risk assessment was performed on a Belgian turkey farm located in West-Flanders raising 9,000 broiler turkeys (4,500 hens and 4,500 toms; British United Turkey, strain 6) originating from French layers. Turkeys at the farm had experienced respiratory infections in the past, with an average mortality rate during former broods of 4%. The farm applied an all-in all-out management schedule with a sanitary service period of 2 weeks between slaughter and restocking. During this period, the houses were cleaned and disinfected (formaldehyde fumigation). One-day-old hens and toms were raised together in one farmhouse (12.5 m × 56 m) until the age of 4 weeks. Afterward, they were raised separately in two identical farmhouses (12.5 m × 56 m). Hens and toms were slaughtered at 15 and 18 weeks, respectively. The ambient temperature was 34 to 35°C, and during the second and fourth weeks the temperature was gradually decreased to 20 or 18°C, respectively. Natural and mechanical ventilation was regulated as required. At day 1 and week 5, turkeys

* Corresponding author. Mailing address: Department of Molecular Biotechnology, FBW-Ghent University, Coupure Links 653, B-9000 Ghent, Belgium. Phone: 32 9 264 99 23. Fax: 32 9 264 62 19. E-mail: Kristel.Verminnen@UGent.be.

† Present address: ILVO, Technologie en Voeding, Brusselsestwg 370, B-9090 Melle, Belgium.

▽ Published ahead of print on 14 November 2007.

were vaccinated against the avian metapneumovirus (Nobilis RTV 8544; Intervet). At 3 and 10 weeks of age, turkeys were vaccinated against New Castle Disease (Nobilis ND Clone 30; Intervet).

Experimental design. During the winter and spring, 20 individually marked hens were clinically examined and sampled every 3 weeks, from production onset at day 1 until slaughter at 15 weeks of age. The farmer (male) and two scientists (female), both involved in monitoring chlamydiosis in turkeys, were medically examined and sampled at the same time as the hens. They did not wear a personal face mask. Humans were also sampled 1 day before the turkeys arrived on the farm (day 0). In addition, *C. psittaci* bioaerosol monitoring was performed at day 0 in a cleaned, disinfected empty farmhouse, at day 1 after the arrival of 1 day-old chicks and subsequently every 3 weeks until week 15. The study was approved by the medical (EC UZG 2005/024) and veterinary (EC 2005/20) ethical committees.

Clinical examination and samples. Twenty individually marked hens were clinically examined for conjunctivitis, rhinitis, sinusitis, and dyspnea at the given time points, and chlamydial excretion was monitored by taking two pharyngeal samples, using Dacron-tipped aluminum shafted swabs (Fiers, Belgium). One dry swab, to be examined by the nested PCR was stored at −80°C, while the one for culture was provided with 1 ml of chlamydia transport medium (13) and subsequently stored at −80°C. In addition, blood samples were collected and stored overnight at room temperature. Sera for detecting antibodies against the chlamydial major outer membrane protein (MOMP) were collected after centrifugation (325 × *g*, 10 min, 4°C) and stored at −20°C until tested.

All persons involved had to fill in a medical questionnaire designed to assess information on professional and nonprofessional activities, smoking habits, general health status, use of medication, allergy, and clinical signs specifically related to psittacosis. At each time point, a general medical examination took place, spirography (Microlab type 3500 v5.00; Micromedical, UK) was performed, and blood samples were collected for hematology and *C. psittaci* antibody detection. As for the turkeys, samples for monitoring chlamydial excretion were collected. However, in humans we took pharyngeal and nasal swabs as well as sputum to determine the optimal sampling location. Samples were stored at −80°C until tested. Human specimens were also examined by PCR for the presence of *C. pneumoniae* and influenza virus types A and B.

Upon each visit to the farm, two air samples were collected in the farmhouse by using a conventional AGI-30 all-glass impinger placed at the normal human breathing height (1.60 m), 10 m from the entrance and 10 m from the far end of the farmhouse. The AGI-30 entrapped *C. psittaci* aerosols into 20 ml of sterile double-distilled water at a sampling flow rate of 4 liters/min for 30 min. Samples were stored at −80°C until examined.

***C. psittaci* nested PCR analysis of avian, human, and air samples.** DNA extraction of dry swabs and air samples was performed as previously described (10). Of 200 μl of sputum, DNA was extracted with a QIAamp DNA minikit (blood and body fluid spin protocol) from Qiagen. Samples were eluted with 100 μl of DNase- and RNase-free water.

All samples were stored at −80°C until use. Pharyngeal DNA extracts of all 20 hens were pooled per time point, whereas human DNA extracts were examined individually. Subsequently, the presence of the *ompA* gene was checked by using a *C. psittaci*-specific nested PCR as described previously (10). Amplification products were visualized by gel electrophoresis (1.2% agarose; Invitrogen, Belgium).

***C. psittaci* isolation from avian, human, and air samples.** Pharyngeal swabs of all 20 hens were pooled per time point, whereas human swabs and air samples were examined individually. Isolation was performed in Buffalo green monkey cells, identifying the organisms by IMAGEN *Chlamydia* immunofluorescence staining (Dakocytomation, Denmark), as previously described (13). The results were presented with scores from 0 to 4 (14).

Molecular characterization of *C. psittaci* isolates from birds and humans. *C. psittaci* isolates were molecularly characterized by sequencing of PCR amplified *ompA* genes generated from genomic DNA by use of CTU/CTL primers (11). All DNA sequence data were double stranded. *ompA* sequences were aligned with related sequences identified by BLAST (http://www.ncbi.nlm.nih.gov). Multiple alignments were done by using CLUSTAL W software (default settings; Vector NTI).

Antibody detection in avian and human sera. Avian MOMP-specific antibody titers were determined by using a recombinant MOMP-based enzyme-linked immunosorbent assay (rELISA) (15). For each time point, the results are presented as mean anti-MOMP antibody titers per 20 hens ± the standard deviation in the sera of individual hens. Sera from specific-pathogen-free turkeys (CNEVA; Ploufragan, France) and experimentally infected specific-pathogen-free turkeys (14) were used as negative and positive controls, respectively.

Human sera were submitted to two Belgian and one foreign medical laboratory. The Belgian laboratories both used the *Chlamydia* MIF immunoglobulin G (IgG), IgM, and IgA detection kit (Focus Diagnostics, Inc.), while the foreign laboratory used a self-made MIF test (2). Afterward, results of the MIF tests were compared to those of the rELISA by using horseradish peroxidase-labeled goat anti-human IgG (H+L; 1/500; Nordic Immunological Laboratories, Tilburg, The Netherlands). For each time point, the results are presented as anti-MOMP antibody titers for each person involved. For the rELISA, human sera negative for *C. trachomatis*, *C. abortus*, *C. pneumoniae*, and *C. psittaci* antibodies were used as a negative control, while serum from a patient infected with *C. psittaci* and negative for *C. trachomatis*, *C. abortus*, and *C. pneumoniae* was used as a positive control. S. Morré (Amsterdam University, Amsterdam, The Netherlands) kindly provided the human control sera.

RESULTS

Chlamydiosis in turkeys. Results are presented in Table 1. Pharyngeal swabs were nested PCR positive from production onset (day 1) until slaughter at 15 weeks of age. At each time point, the PCR results were confirmed by isolation, with the highest scores at week 6 and 9. The rELISA revealed high maternal antibody titers in all 1-day-old-chicks that were almost disappeared at 3 weeks of age. Thereafter antibody titers rapidly increased, especially between 6 and 9 weeks of age, and they kept on rising until the end of the study. Genotyping of chlamydial isolates revealed the presence of genotype A from day 1 until the hens went to the slaughterhouse at 15 weeks of age. Turkeys showed respiratory signs from week 3 onward until the end of the experiment, with most severe clinical signs occurring from 6 to 9 weeks of age. Respiratory signs were characterized by head shaking (rhinitis), serous nasal discharge, and dyspnea. The mortality rate during this brood was 6.6%. During weeks 3 and 9 the turkeys were treated for 5 days with Soludox (Eurovet).

Psittacosis in humans. The results for these evaluations are presented in Table 1. In humans, pharyngeal and nasal sampling yielded comparable results with both nested PCR and culture. However, 9 of 21 times *C. psittaci* was detected by nested PCR in both pharyngeal and nasal swabs, while being absent in the sputum. The farmer and both scientists tested PCR positive from day 0, before the turkeys came into the farmhouse until week 15. Nested PCR results in the farmer and in scientist 1 were confirmed by culture except for week 3, when the culture was negative but the nested PCR was positive. The PCR results for scientist 2 were confirmed by culture except for day 0 and week 3, when the culture was negative but the nested PCR was positive. Genotyping of isolates obtained from days 0 and 1 revealed the presence of genotype E in the farmer, while genotype E/B was present in scientist 1. We were unable to determine the genotype responsible for the positive nested PCR in scientist 2 at day 0, day 1, and week 3. From week 6 to week 15, genotype A was discovered in the farmer and both scientists. All human samples were PCR negative for *C. pneumoniae* and influenza virus types A and B.

The MIF test from Focus Diagnostics, performed in two Belgian medical diagnostic labs, showed no antibodies to *C. pneumoniae* or to *C. trachomatis* but also, strangely enough, no antibodies to *C. psittaci* in all of the infected persons involved. Surprisingly, the self-made MIF test performed in the foreign laboratory detected anti-*C. pneumoniae* IgG (no IgM) in the farmer (1/32 at day 0 to week 15), in scientist 1 (1/16 at day 0 to week 15), and in scientist 2 (1/8 at day 0 and 1/32 at weeks 3 to 15), whereas the *C. pneumoniae* PCR remained negative

TABLE 1. Results for human and turkey samples

Subject	Sample type or parameter[a]	Day 0	Day 1	Wk 3	Wk 6	Wk 9	Wk 12	Wk 15
Farmer	Pharyngeal swab	+	+	+	–	+	+	–
	Nasal swab	+	+	–	–	+	+	+
	Sputum	+	+	+	+	–	+	–
	Isolation	+ (2)	+ (4)	–	+ (2)	+ (1)	+ (4)	+ (3)
	Log_{10} Ab titer	2.7	2.7	2.7	2.7	NT[b]	NT	2.4
	Genotype	E	E	–	A	A	A	A
Scientist 1	Pharyngeal swab	+	+	–	+	+	+	+
	Nasal swab	+	+	+	+	+	+	+
	Sputum	+	–	+	+	–	–	+
	Isolation	+ (4)	+ (3)	–	+ (3)	+ (1)	+ (2)	+ (3)
	Log_{10} Ab titer	3.0	2.7	3.0	2.7	NT	NT	2.7
	Genotype	EB	EB	–	A	A	A	A
Scientist 2	Pharyngeal swab	+	+	–	–	+	+	+
	Nasal swab	+	+	+	+	+	+	–
	Sputum	+	+	–	+	–	–	–
	Isolation	–	+ (3)	–	+ (2)	+ (2)	+ (2)	+ (1)
	Log_{10} Ab titer	2.4	2.4	2.1	2.4	NT	NT	2.1
	Genotype	–	NT	–	NT	A	A	NT
Turkeys	Pharyngeal swab		+	+	+	+	+	+
	Isolation		+ (2)	+ (2)	+ (3)	+ (4)	+ (2)	+ (1)
	Log_{10} Ab titer (mean ± SD)		2.3 ± 1.5	1.7 ± 0.9	2.0 ± 1.3	3.0 ± 1.9	3.3 ± 2.1	3.6 ± 2.5
	Genotype		A	A	A	A	A	A

[a] Values for pharyngeal swabs, nasal swabs, and sputum are nested PCR results. Specific isolation scores are given in parentheses. Ab, antibody.
[b] NT, not tested.

throughout the experiment for all persons involved. Anti-*C. trachomatis* and, unexpectedly, anti-*C. psittaci* antibodies were not detected by this MIF test. The results of all MIF tests, except for those of weeks 9 and 12 since sera were no longer available, were retested using the *C. psittaci* MOMP-based ELISA. The farmer and both scientists tested seropositive at all examined time points.

At day 0, clinical examination of both scientists (S1 and S2) revealed a mild infection of the upper respiratory tract, which disappeared after a few days. The farmer (F) was clinically healthy. During weeks 4, 5, and 6, both scientists, as well as the farmer, experienced a respiratory tract infection characterized by rhinitis (F, S1, and S2), coughing (F, S1, and S2), pharyngitis (S1), and an oppressive feeling in the chest (S1). From week 7 to week 8, the respiratory symptoms were rather mild, but during weeks 9, 10, and 11 these symptoms became more severe, especially in both scientists, with rhinitis (S1 and S2), pharyngitis (S1 and S2), coughing (S1 and S2), abnormal respiratory sounds (F), and painful breathing (S1), as well as fever (S1, 38.5°C; S2, 37,5°C). From week 12 to the end of the experiment respiratory signs diminished again with rhinitis (F, S1, and S2), coughing (S1 and S2), and shortness of breath (F) being the only clinical signs noticed.

For scientist 2, spirography revealed no significant changes for forced expiratory volume (FEV), forced vital capacity (FVC), or peak expiratory flow (PEF) values. However, based on age, gender, and body length, all values for scientist 2 were generally lower than predicted. For scientist 1 at week 6, the PEF had declined from a normal value of 359 liters/min (day 0) to 258 liters/min, and at 12 weeks the FEV was 2.56 liters/s instead of a normal value of 2.86 (day 0). At the same time, the FVC was 2.99 liters instead of the normal value of 3.31 (day 0). For the farmer, PEF declined from 484 liters/min (day 0) to 395 and 409 liters/min at weeks 12 and 15, respectively. The FEV remained steady, but from week 12 to week 15 the FVC declined from 5.07 to 4.50 liters.

For all humans, leukocyte values over time stayed within the normal range. However, for both scientists, leukocytes values varied identically over time, with peak values at 3 and 9 weeks. The eosinophil and basophil percentages never rose above the normal reference values. Neutrophil percentages rose above the normal reference value (males, 64%; females, 68%) during week 3 (73%), week 9 (73%), and week 12 (74%) for scientist 1; week 9 (79%) for scientist 2; and day 0 (65%) and week 6 (65%) for the farmer. At week 12, the monocyte percentages of scientist 2 (11%) rose above the normal reference value (8%). The lymphocyte percentages rose above the normal reference value (males, 37%; females, 32%) at day 0 (33%) and week 6 (33%) for scientist 2 and at week 15 (42% for scientist 2 and 39% for the farmer). Actually, for the farmer, the lymphocyte percentages increased continuously for the last 6 weeks of the study. For all persons, no significant changes were found in normal C-reactive protein, aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyl transpeptidase blood parameters.

Bioaerosol monitoring. Two air samples were collected in the farmhouse each time the farm was visited. Chlamydial organisms were detected by nested PCR in all air samples taken at two different locations from day 0, before the turkeys came into the farmhouse, until week 9. Afterward, during weeks 12 and 15, only one of two locations tested positive in the nested PCR. We were unable to isolate *C. psittaci* from air samples.

DISCUSSION

We evaluated a diagnostic platform for the detection and characterization of *C. psittaci* in birds and in the mean time for tracing zoonotic transmission to humans. The platform comprises the old and new "gold standard" chlamydial diagnostic methods for detection such as culture and nucleic acid amplification testing and for strain characterization such as MOMP serotyping and *ompA* genotyping. The ultimate goal will be to introduce such a diagnostic platform in veterinary and medical reference clinical microbiology laboratories to improve diagnosis and notification since chlamydiosis in birds and psittacosis in humans is highly underestimated.

Using the MOMP-based rELISA, high maternal antibody titers were discovered in day-old turkeys. *C. psittaci* vaccines are unavailable; thus, French layers must have been infected. Notwithstanding high maternal antibody titers, nested PCR and culture showed the presence of *C. psittaci* in day-old hens. The cultured strain was characterized as *ompA* genotype A. *C. psittaci* infections in the presence of high maternal antibody titers have been reported previously (9, 12) and are thought to occur due to vertical transmission (6, 17), contact with wild birds or infected rodents or failure in cleaning and disinfecting farmhouses between broods. The importance of the latter was demonstrated by the discovery of chlamydial DNA in air samples taken 1 day before starting the new brood. Thus, the former brood most probably had to deal with a *C. psittaci* infection, although live organisms could not be isolated using the present bioaerosol sampling technique, which needs to be improved.

During the first 2 weeks, high maternal antibody titers apparently protected the birds against clinical disease. Thus, antibodies contribute to the protection against *C. psittaci* outbreaks in commercial fattening turkeys. In the future, layers must be vaccinated to avoid vertical transmission of *C. psittaci* and to create high maternal antibody titers in all day-old turkeys. However, as demonstrated in former studies (9, 12) as well as in the present research, *C. psittaci* outbreaks may still occur at 3 to 4 weeks of age when maternal antibodies titers declined. Thus, future vaccines must be able to induce protection in young fattening turkeys with high maternal antibody titers.

The current genotype A infection caused severe respiratory signs, especially from weeks 6 to 9, and was characterized by rapidly augmenting antibody titers, as demonstrated by the rELISA. The infection was accompanied by *Escherichia coli* and *Ornithobacterium rhinotracheale* infections. *C. psittaci* genotype A was still present when the hens went to the slaughterhouse, as demonstrated by both nested PCR and culture. The latter underlines the zoonotic risk for slaughterhouse personnel. In the past, similar *C. psittaci* infection kinetics have been observed on European turkey farms (9, 12).

The present diagnostic platform demonstrated the zoonotic transmission of a *C. psittaci* genotype A infection to all three persons who came in contact with infected turkeys. Interestingly, nasal and pharyngeal swabs were more suitable than sputum for diagnosing a chlamydial infection. Perhaps some people had difficulty providing representative sputum samples. Surprisingly, the farmer was *C. psittaci* positive in both nested PCR and culture even before the turkeys arrived on the farm. The strain involved was characterized as genotype E and most likely originated from the former brood. Remarkably, the genotype E infection did not protect the farmer against a subsequent genotype A infection, since live *C. psittaci* genotype A bacteria could be isolated at week 6. Nevertheless, the farmer was the one with less severe respiratory signs, and he showed, in contrast to both scientists, no fever during the infection. Natural immunity after multiple *C. psittaci* infections during subsequent broods probably protected the farmer against a severe symptomatic course of infection since both scientists also experienced a *C. psittaci* infection before going to the farm, but they both became seriously ill and febrile.

At day 0, scientist 1 was positive in both nested PCR and culture, and the strain involved was characterized as genotype E/B. At the same time, scientist 2 was only positive in nested PCR, but we were unable to type the strain as the CTL/CTU PCR performed directly on clinical specimens remained negative. This is not unusual since the nested PCR is more sensitive than the latter nucleic acid amplification test (3, 10). The infection source was traced using the present diagnostic platform and was found to be a parrot relief and breeding center (4). Both scientists visited this center 7 weeks before starting the present zoonotic risk assessment study, but they were unaware of being infected since they experienced no clinical signs. Genotype E and E/B strains detected in the beginning of the study, in the farmer and in scientist 1, were apparently not transmitted to turkeys. Perhaps the excretion levels in humans were not high, and/or maternal antibody titers protected the turkeys.

Psittacosis in humans was correlated with severe respiratory signs, especially in both scientists, although all persons involved were formerly infected. During the present study, none of the three persons was treated for a *C. psittaci* infection. Spirography showed FEV, FVC, and PEF levels to decline for two on three persons at risk during the present study. However, we cannot exclude the influence of rising ammonia concentrations in the air during the brooding period. Thus, the impact of the present *C. psittaci* infection on respiratory functions is difficult to determine. No significant changes were found in C-reactive protein, aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyl transpeptidase blood parameters. Little information is known about changes in these parameters in patients with psittacosis. In the case of systemic psittacosis, changes in liver enzyme levels can be observed, but in fact this is all dependent on the seriousness of the infection (J. M. Ossewaarde, unpublished data). Leukocyte values can be normal, but leukopenia can also be observed. For the farmer, leukocyte values in time showed no significant variations although lymphocyte percentages almost doubled from the beginning to the end of the study. This might be due to an immunological memory created by recurrent *C. psittaci* zoonotic transfer. Various leukocyte values in both scientists tend to correspond to changes in neutrophil percentages, especially at week 9, when severe respiratory signs occurred.

The present study is to our knowledge the first to examine a *C. psittaci* zoonotic transmission in "real time" in a clinical setting. *C. psittaci* infections are common in turkeys, and everyone in contact with fattening turkeys is at risk of contracting psittacosis with either a symptomatic or an asymptomatic course of infection. Psittacosis probably occurs more often

than previously thought, and nothing is known of natural protection, the consequences of recurrent infections, and the possible pathogenic interactions between *C. psittaci* and other human respiratory pathogens. The present diagnostic platform is a step toward the improvement of *C. psittaci* diagnosis in both birds and humans and therefore might contribute to our understanding of the course of infection.

ACKNOWLEDGMENTS

P. Zwaenepoel (NV Versele-Laga, Deinze, Belgium) is acknowledged for industrial support. We also thank F. Daenick and T. Harkinezhad for technical assistance on the farm.

This study was supported by COST855 (Animal Chlamydiosis and the Zoonotic Implications) and FOD Volksgezondheid grant RT-05/08-Minspec-7.

REFERENCES

1. **Andersen, A. A.** 1991. Serotyping of *Chlamydia psittaci* isolates using serovar-specific monoclonal antibodies with the microimmunofluorescence test. J. Clin. Microbiol. **29:**707–711.
2. **Comanducci, M., R. Manetti, L. Bini, A. Santucci, V. Pallini, R. Cevenini, J. M. Sueur, J. Orfila, and G. Ratti.** 1994. Humoral immune response to plasmid protein pgp3 in patients with *Chlamydia trachomatis* infection. Infect. Immun. **62:**5491–5497.
3. **Geens, T., A. Dewitte, N. Boon, and D. Vanrompay.** 2005. Development of a *Chlamydophila psittaci* species-specific and genotype-specific real-time PCR. Vet. Res. **36:**787–797.
4. **Harkinezhad, T., K. Verminnen, C. Van Droogenbroeck, and D. Vanrompay.** 2007. *Chlamydophila psittaci* genotype E/B transmission from African grey parrots to humans. J. Med. Microbiol. **56:**1097–1100.
5. **Longbottom, D., and L. J. Coulter.** 2003. Animal chlamydioses and zoonotic implications. J. Comp. Pathol. **128:**217–244.
6. **Lublin, A., G. Shudari, S. Mechani, and Y. Weisman.** 1996. Egg transmission of *Chlamydia psittaci* in turkeys. Vet. Rec. **139:**300.
7. **Meyer, K. F.** 1965. Ornithosis, p. 675–770. *In* H. E. Biester and L. H. Schwarte (ed.), Diseases of poultry. Iowa State University Press, Ames, IA.
8. **Morange, A.** 1895. De la psittacose, ou infection speciale determinee par des perruches. Academie de Paris, Paris, France.
9. **Van Loock, M., T. Geens, L. De Smit, H. Nauwynck, P. Van Empel, C. Naylor, H. M. Hafez, B. M. Goddeeris, and D. Vanrompay.** 2005. Key role of *Chlamydophila psittaci* on Belgian turkey farms in association with other respiratory pathogens. Vet. Microbiol. **107:**91–101.
10. **Van Loock, M., K. Verminnen, T. O. Messmer, G. Volckaert, B. M. Goddeeris, and D. Vanrompay.** 2005. Use of a nested PCR-enzyme immunoassay with an internal control to detect *Chlamydophila psittaci* in turkeys. BMC Infect. Dis. **5:**76.
11. **Vanrompay, D., P. Butaye, C. Sayada, R. Ducatelle, and F. Haesebrouck.** 1997. Characterization of avian *Chlamydia psittaci* strains using *omp1* restriction mapping and serovar-specific monoclonal antibodies. Res. Microbiol. **148:**327–333.
12. **Vanrompay, D., P. Butaye, A. Van Nerom, R. Ducatelle, and F. Haesebrouck.** 1997. The prevalence of *Chlamydia psittaci* infections in Belgian commercial turkey poultry. Vet. Microbiol. **54:**85–93.
13. **Vanrompay, D., R. Ducatelle, and F. Haesebrouck.** 1992. Diagnosis of avian chlamydiosis: specificity of the modified Gimenez staining on smears and comparison of the sensitivity of isolation in eggs and three different cell cultures. Zentbl. Veterinarmed. B **39:**105–112.
14. **Verminnen, K., M. Van Loock, E. Cox, B. M. Goddeeris, and D. Vanrompay.** 2005. Protection of turkeys against *Chlamydophila psittaci* challenge by DNA and rMOMP vaccination and evaluation of the immunomodulating effect of 1α,25-dihydroxyvitamin D_3. Vaccine **23:**4509–4516.
15. **Verminnen, K., M. Van Loock, H. M. Hafez, R. Ducatelle, F. Haesebrouck, and D. Vanrompay.** 2006. Evaluation of a recombinant enzyme-linked immunosorbent assay for detecting *Chlamydophila psittaci* antibodies in turkey sera. Vet. Res. **37:**623–632.
16. **Winsor, D. K., Jr., and J. E. Grimes.** 1988. Relationship between infectivity and cytopathology for L-929 cells, membrane proteins, and antigenicity of avian isolates of *Chlamydia psittaci*. Avian Dis. **32:**421–431.
17. **Wittenbrink, M. M., M. Mrozek, and W. Bisping.** 1993. Isolation of *Chlamydia psittaci* from a chicken egg: evidence of egg transmission. Zentbl. Veterinarmed. B **40:**451–452.