

Molecular epidemiology of *Chlamydia psittaci* in psittacine birds and humans

Daisy Vanrompay

Ghent University

Fac. Bioscience Engineering

Department of Molecular Biotechnology

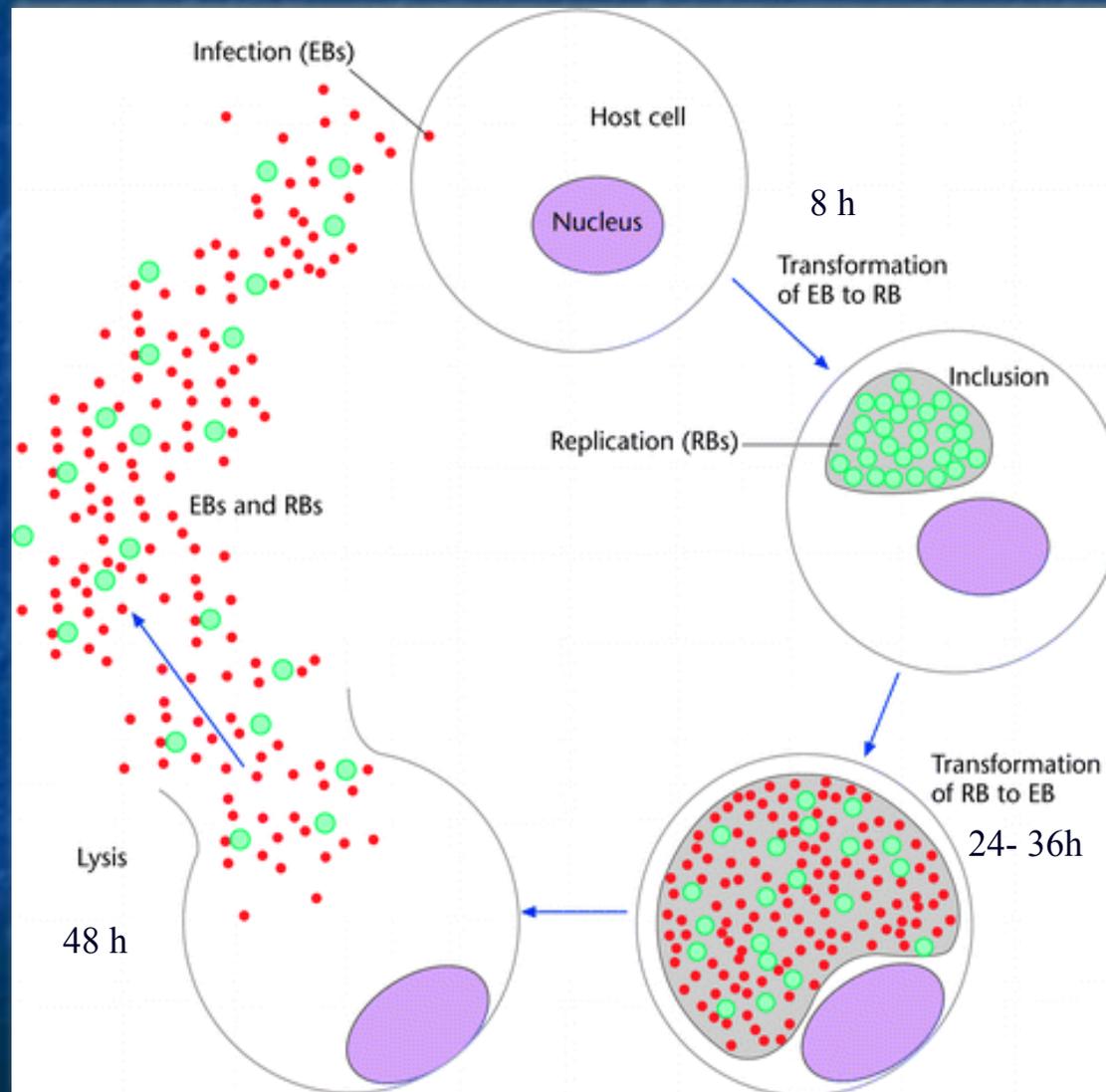


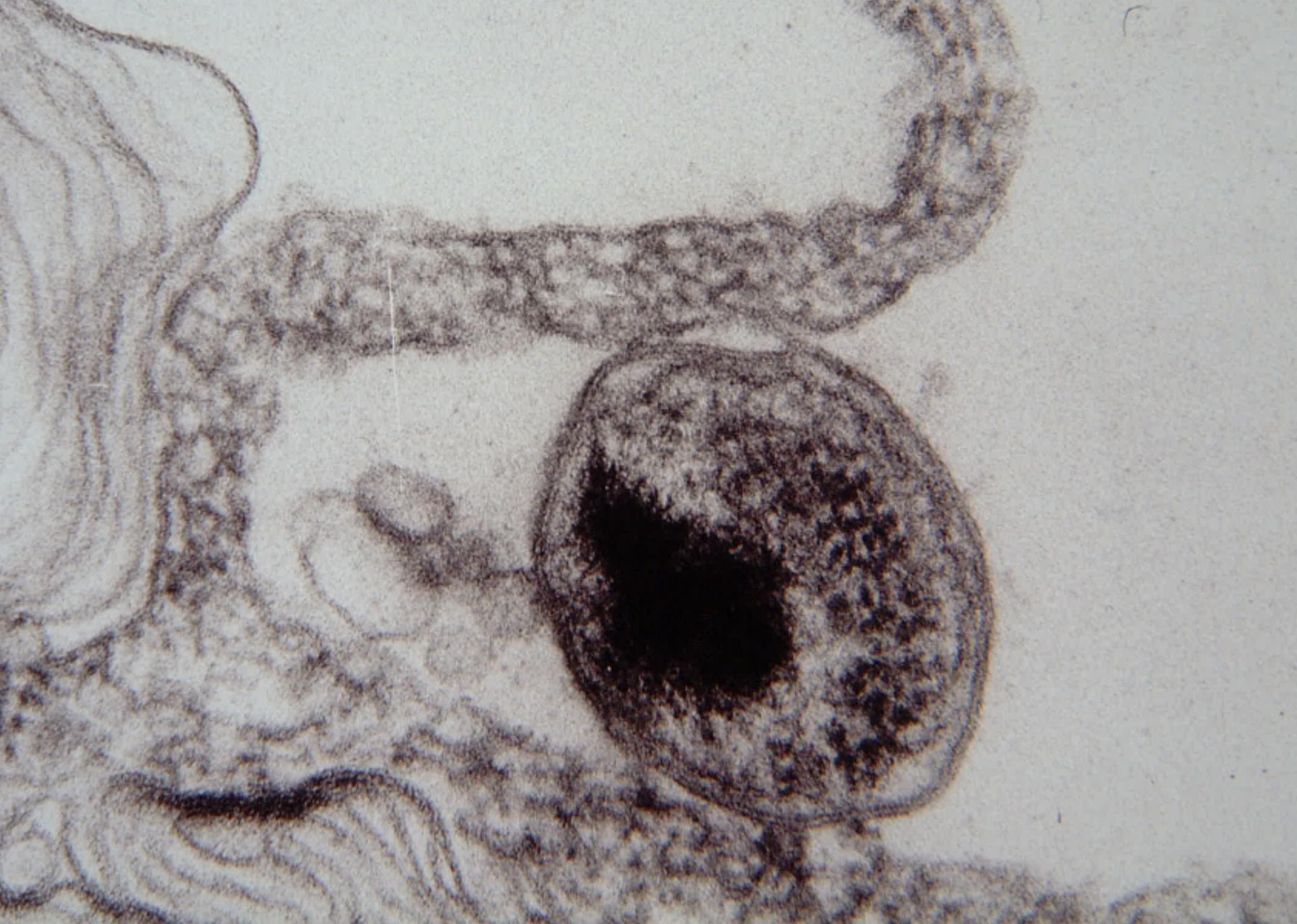
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development

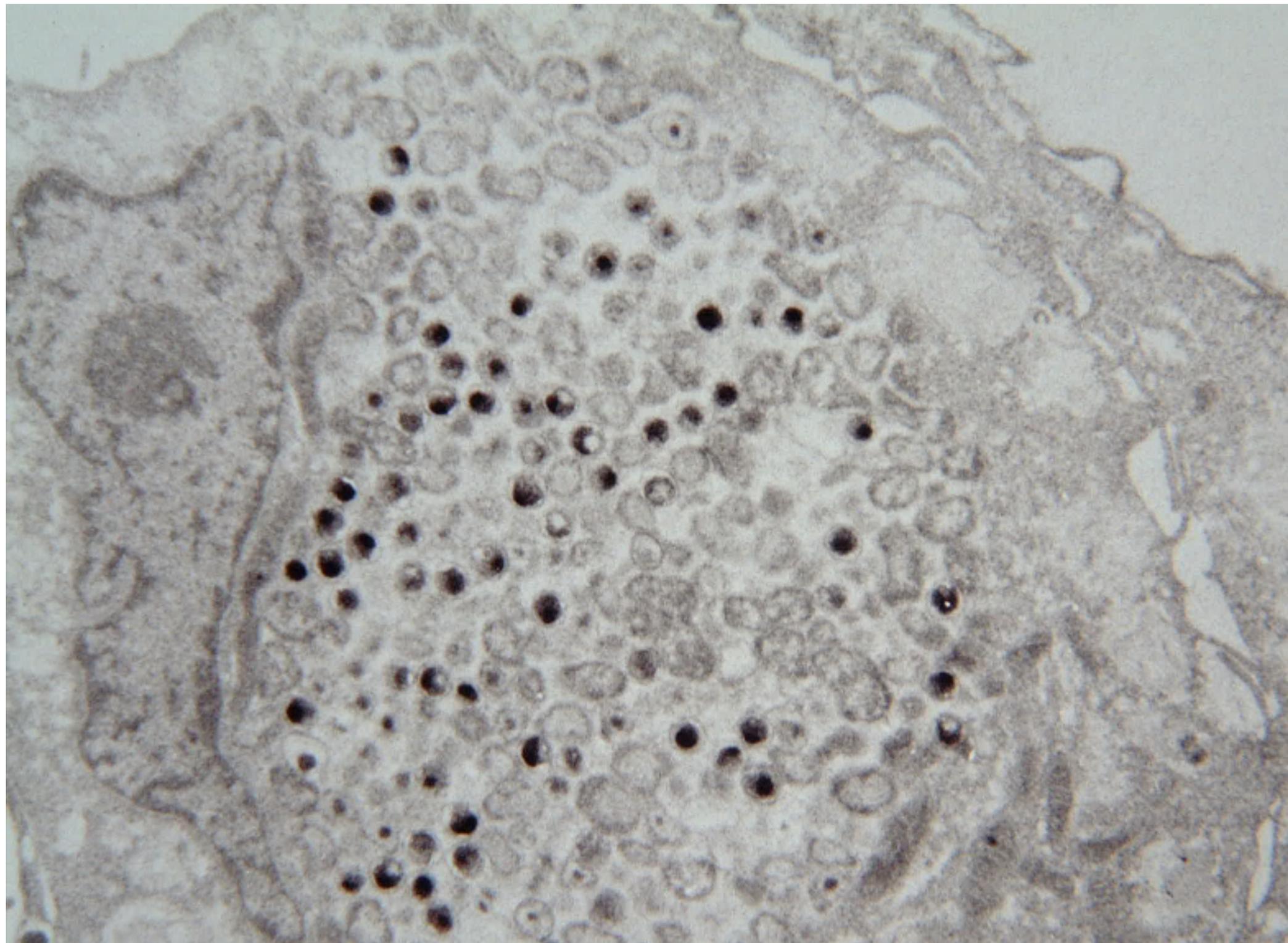
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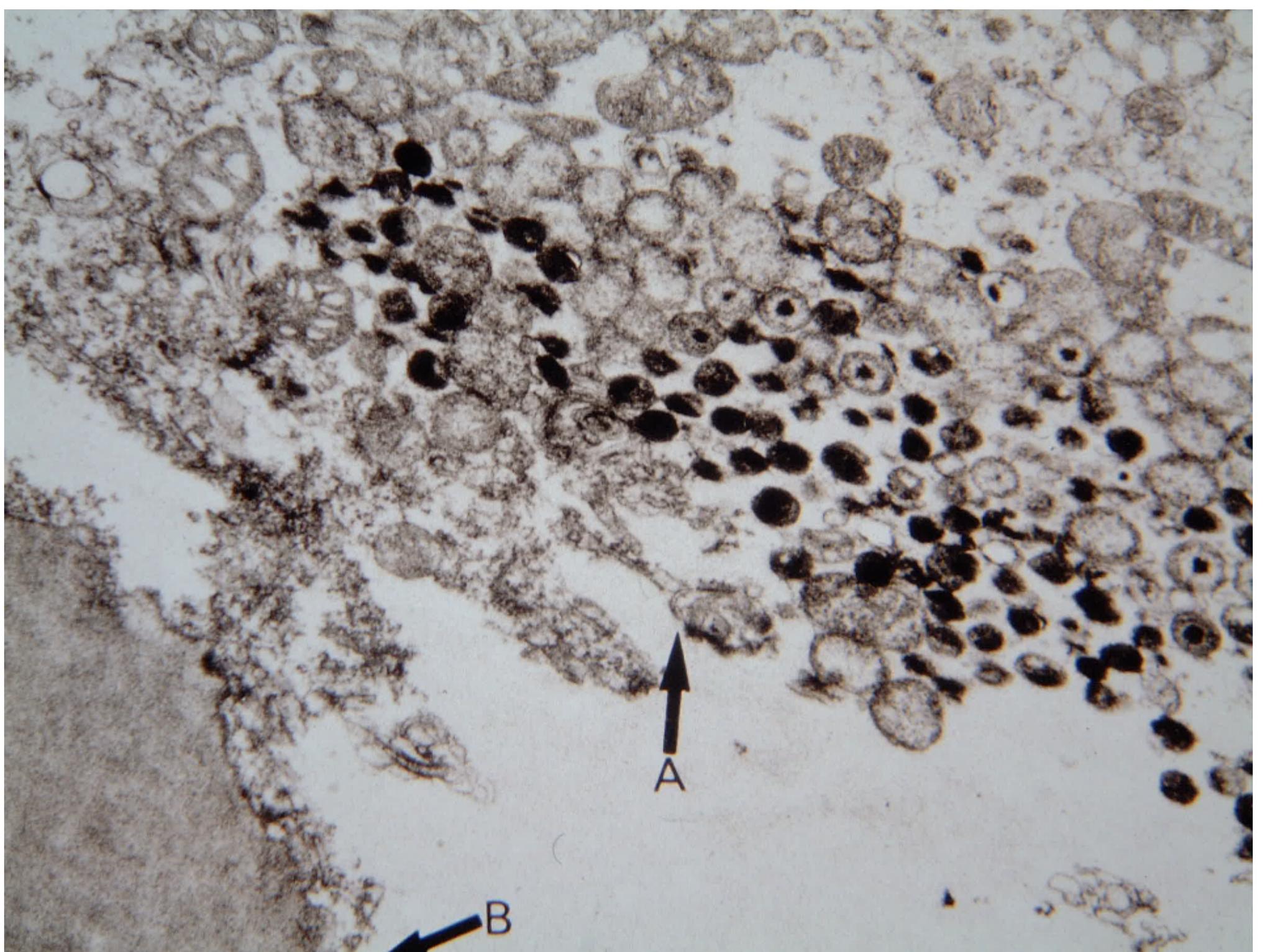
- **Introduction**
- Problem definition
- Prevalence of *C .psittaci* in a human population
- Prevalence of *C . psittaci* in psittacine birds
- General conclusions

***C. psittaci* is a Gram-negative obligate intracellular bacterium replicating in epithelial cells and macrophages of birds**









C. psittaci outer membrane protein A (ompA) genotypes

- More than 465 bird species
- 7 avian *C. psittaci* genotypes
 - Genotype A: *Psittaciformes*
 - Genotype B: *Columbiformes*
 - Genotype C: *Anseriformes*
 - Genotype D: *Galliformes*
 - Genotype E: *Columbiformes*
 - Genotype F: *Psittaciformes*
 - Genotype E/B: *Anseriformes*
- Certain host specificity
- Respiratory disease



Public Health



Genotype A, B, E



Genotype B



Genotype A, B



Genotype A, B, D, E, F, E/B



Genotype A, B, F, E/B



Genotype A, B, C, E/B



Genotype B

Public health

- **Population at risk**
 - Bird fanciers, pet bird owners
 - Veterinarians
 - Employees: poultry industry, quarantine stations, zoo
-
- **Human infection = psittacosis or parrot fever**
 - Inhaling of aerosolized urine, respiratory secretions or dried faeces of infected birds
 - Human to human transmission is reported

Public health

- **Psittacosis**
- Incubation period 7 to 15 days
- Asymptomatic → clinical disease
 - Flu-like symptoms
 - Pneumonia
 - Fever, headache,
- Several case reports on severe clinical disease

Table. Human cases of psittacosis (available data period 1996-2007)

Country	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	per million of habitants
Austria	1			1	2	3	0	3	6				0.36
Argentina					38	1							0.50
Australia	86	35	55	81	99	137	213	200	239	164	171	62	5.54
Belgium	0	0	8	12	13	10	23	39	12	7	2	3	0.95
Croatia	7	4	0	5	8	3		5	4				1.10
Denmark	0				31	8	13	14	8				2.37
Germany	134	124	155	109	86	53	40	42	15	33	25	10	0.51
Hungary	0	0	0	1	5	1	6	85	7				0.30
Japan				23	18	35	54	44	40	34			0.27
Poland	2	2	0	2	0	5		2	2				0.05
Slovakia	2	0	1	3	10	0	0	1	0				0.18
Spain						5	4	0	1				0.06
Sweden	25	66	30	29	24	12	13	12	7	5	2	9	1.3
The Netherlands	0	28		25	36	24	17	27	33	49	59	27	1.70
U.K.	353	322	293	207	204	106	68	100	62	59			1.75
U.S.A.	45	38	54	15	13		19	13	11				0.05

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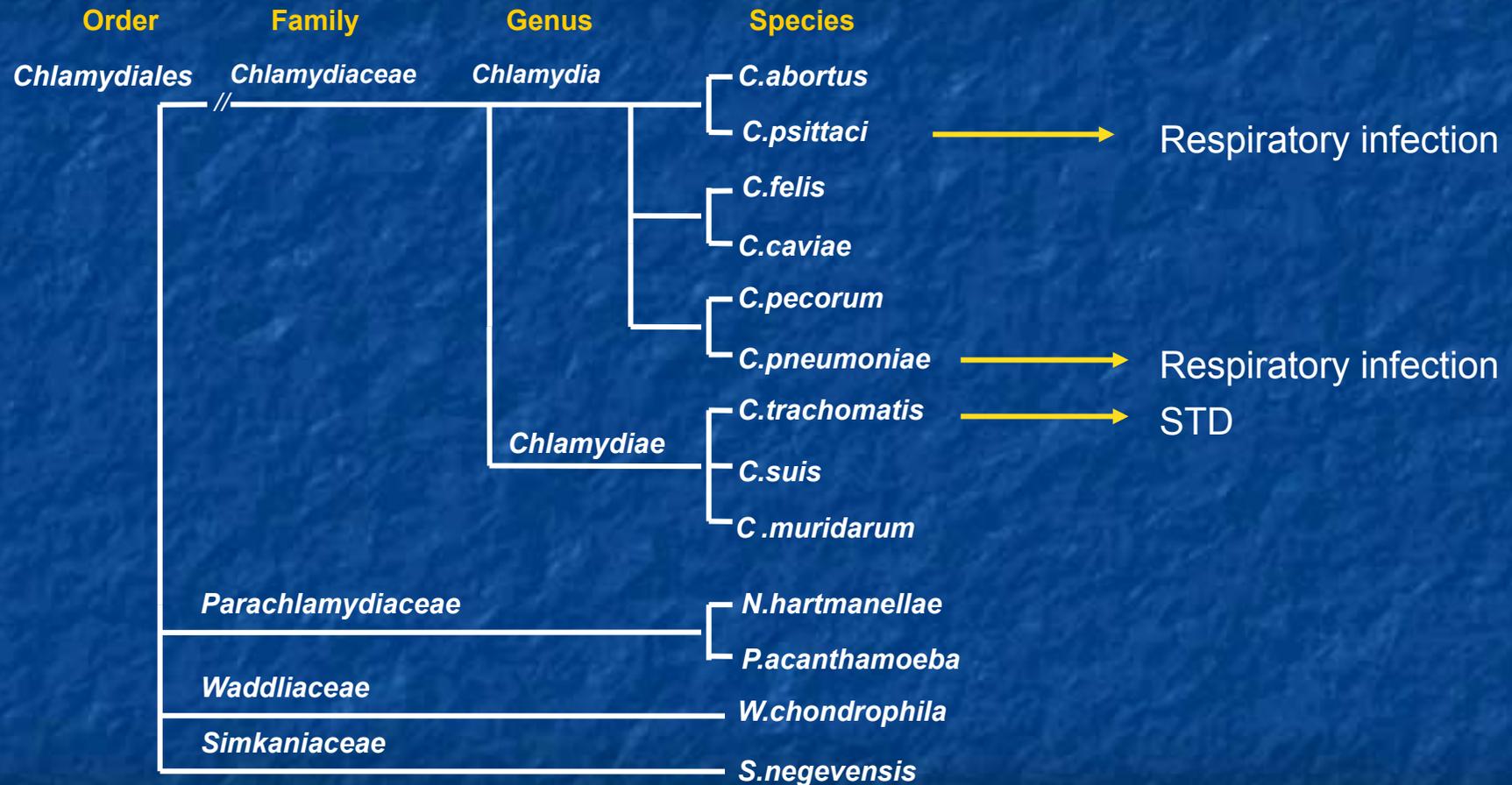
Problem definition

- **The actual number of *C. psittaci* infections is probably underestimated** because psittacosis is difficult to diagnose, is covered by antimicrobials and often is not reported.
 - **Currently: molecular epidemiology in domestic/companion birds & humans and development of a diagnostic platform**
- **Reduction of *C. psittaci* zoonotic risk**
 - **Future: developing a vaccine for the bird species being most dangerous to public health**

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Prevalence of *Chlamydia psittaci* in a Flemish human population in contact with domestic/companion birds



New Classification: *Chlamyphila* is no longer used!!! – only one genus *Chlamydia*

Prevalence of *Chlamydia psittaci* in a Flemish human population in contact with domestic/companion birds

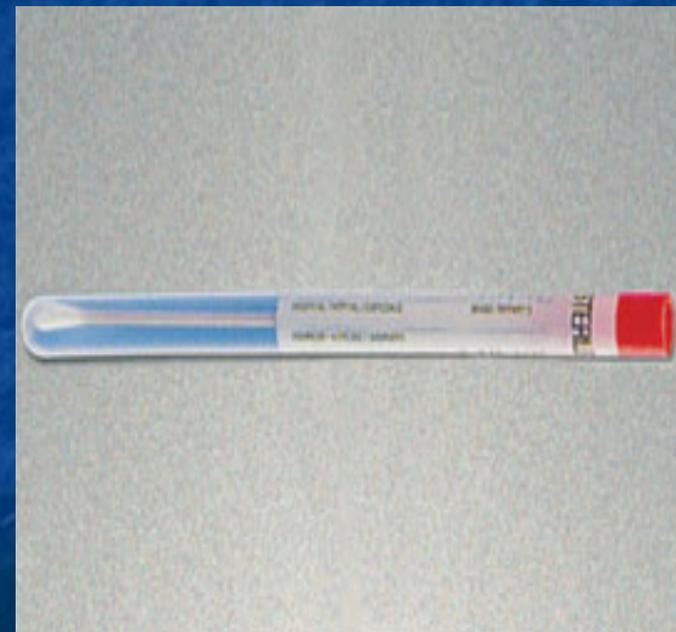
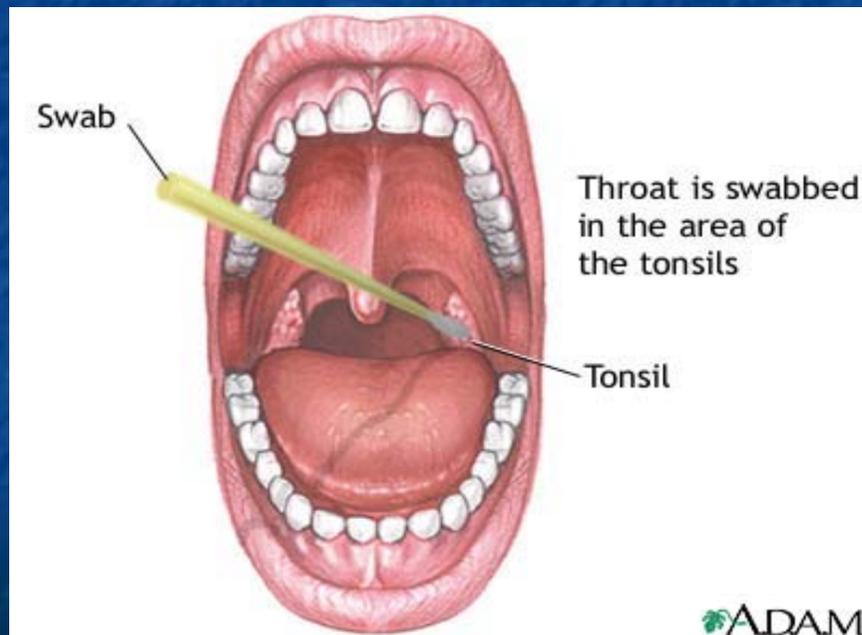
- **Population (n = 987)**
- 420 participants had contact with domestic/companion birds
- 567 had no-contact with birds
 - 120 (randomly) of them were included in the study
- 420 + 120 = 540 participants; 264 males and 276 females, 35-55 year

Table 1. Contact frequency of humans with domestic and/or companion birds

Birds	Daily n=254	Weekly n=58	Sporadic n=108	Total n=420
Canaries	166 (65.3%)	35 (60.3%)	31 (28.7%)	232 (55.2%)
<i>Psittaciformes</i>	118 (46.4%)	24 (41.3%)	32 (29.63%)	174 (41.4%)
Racing pigeons	58 (22.8%)	37 (63.7%)	24 (22.2%)	119 (28.3%)
Ducks	53 (20.8%)	14 (24%)	21 (19.4%)	90 (21.4%)
Geese	48 (19%)	21 (36.2%)	21 (19.4%)	88 (21%)
Turkeys	24 (9.4%)	7 (12%)	15 (13.8%)	46 (11%)

Prevalence of *Chlamydia psittaci* in a Flemish human population in contact with domestic/companion birds

- **Pharyngeal swabs**
- *ompA* nested PCR enzyme immuno assay \Rightarrow *C. psittaci* specific
- 16S-rRNA nested PCR \Rightarrow *C. pneumoniae* specific



Prevalence of *Chlamydia psittaci* in a Flemish human population in contact with domestic/companion birds

- **Sera**
- MOMP ELISA ⇒ *C. psittaci* genotype A recombinant MOMP
- MIF (Focus Diag) ⇒ *C. psittaci* genotype A strain (LPS removed)
- ELISA (Serion) ⇒ *C. psittaci* genotype D strain
- ELISA (Medac) ⇒ MOMP peptides = *C. trachomatis* specific
- ELISA (Anilabsystems) ⇒ *C. pneumonia* (LPS removed)

Prevalence of *Chlamydia psittaci* in a Flemish human population in contact with domestic/companion birds

Table. *C. psittaci* zoonotic risk related to contact with one certain bird species

Contact with:	Number (%) of persons on 540 examined individuals, in contact with only one bird species	PCR positives *(%)	PCR and/or rMOMP ELISA positives *(%)	OR (95% CI)* **	P value
Psittacine birds	31 (5.7)	7 (22.5)	8 (25.8)	6.3 (2.6-15.2)	0.00001
Parakeet	23 (4.2)	6 (26.0)	6 (26)	5.9 (2.2-15.8)	0.00001
Parrot	8 (1.5)	1 (12.5)	2 (25)	4.9 (1-24.3)	0.050
Canary	69 (12.8)	9 (13.0)	12 (17.4)	4.8 (2.2-10.5)	0.00001
Pigeon	32 (5.9)	7 (22)	8 (25)	6.0 (2.5-14.7)	0.00001
Duck	2 (0.3)	0 (0)	0 (0)	-	-
Goose	5 (0.9)	0 (0)	0 (0)	-	-
Turkey	5 (0.9)	1 (20)	1 (20)	3.8 (0.43-33.8)	0.23
Chicken	1 (0.18)	0 (0)	0 (0)	-	-
Total	145	24	29	-	-

*Each individual had only contact with one bird species

**Odd ratio for PCR and/or ELISA positives with 95% confidence intervals

Prevalence of *Chlamydia psittaci* in a Flemish human population in contact with domestic/companion birds

Table. *C. psittaci* zoonotic risk related to daily contact with a single bird species.

Total number of persons daily in contact with						
Parakeet	Parrot	Canary	Pigeon	Duck	Goose	Turkey
16	5	55	17	1	3	4
Number of persons (%) positive by PCR and/or rMOMP ELISA						
5	1	10	7	0	0	1
(31.2%)	(20%)	(18.2%)	(41.2%)	0	0	(20%)

Prevalence of *Chlamydia psittaci* in a Flemish human population in contact with domestic/companion birds

Table. *C. psittaci* zoonotic risk related to weekly contact with a single bird species

Total number of persons weekly in contact with						
Parakeet	Parrot	Canary	Pigeon	Duck	Goose	Turkey
3	2	8	9	0	1	1
Number of persons (%) positive by PCR and/or rMOMP ELISA						
1	1	2	1	0	0	0
(33.3%)	(50%)	(25%)	(11%)	0	0	0

- We demonstrated the usefulness of a nested PCR/EIA for detecting *C. psittaci* in humans
- Clinicians should be recommended to use nucleic acid amplification tests as:
 - Molecular diagnosis is more sensitive
 - Direct evidence for the presence of *C. psittaci* (while serology is retrospective)
 - Current serological tests are not *C. psittaci*- specific
 - Difficult to use in a human population where *C. pneumoniae* is highly prevalent
- Psittacosis occurs more often than reported
- The occurrence of less severe, clinically ‘unnoticed’ infections
- Racing pigeons, and especially *Psittaciformes* are a main risk for attracting psittacosis
- Need for:
 - More accurate diagnostic monitoring (birds) and reporting
 - Information campaigns, recommendations for risk reduction
 - Veterinary vaccine
 - *C. psittaci*- specific serological assay for testing human sera (epidemiology)

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C. psittaci infections in psittacine birds and transmission to humans

- 50 psittacine breeding facilities



- Fresh faeces from birds for culture
- Fresh faeces from birds for PCR
- Human pharyngeal swab for culture
- Human pharyngeal swab for PCR
- Questionnaire



OmpA nested PCR/EIA

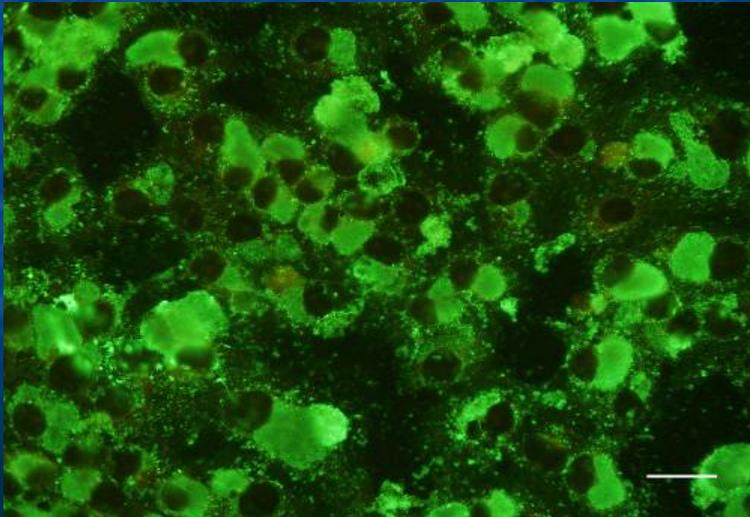
OmpA genotyping real-time PCR

Culture in BGM cells + IF identification

C. psittaci infections in psittacine birds and transmission to humans

Response in 39 of 50 contacted breeders: 308 birds and 46 humans

Birds	PCR positives (%)	Culture positives (%)	Humans	PCR positives (%)	Culture Positives (%)
308	59 (17.5%)	25/59 (42.3%)	46	6 (13%)	4/6 (66.6%)



IF staining of BGM monolayer

- **Genotype specific real-time PCR:**
 - Genotype A in 5/6
 - Rhinitis and coughing
 - Genotype E/B in 1/6
 - Shortness of breath

***C. psittaci* infections in psittacine birds and transmission to humans**

▪ Conclusions

- Proven zoonotic transmission of genotype A and E/B
 - First time genotype E/B has been identified in humans
- Psittacosis without severe clinical symptoms
- No vaccine:
 - 46.2% treated their birds with antibiotics over the past year
 - 10.2% were using tetracyclines prophylactically

➡ Risk of developing antibiotic resistant zoonotic *C. psittaci* strains?

➡ Urgent need for a vaccine and information campaigns on sensible antibiotic use in *Psittaciformes*

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General conclusions

- **Nested PCR/EIA** can be used to specifically detect *C. psittaci* in humans.
- **Serological tests** for humans are not *C. psittaci* - specific: **cross reaction** with antibodies against *C. trachomatis*, *C. pneumonia* and other *Chlamydiales* occurs.
- Human *C. psittaci* infections are **underestimated**.
- Zoonotic transmissions from ***Psittaciformes* and racing pigeons** often occurs.
- The course of infection in humans can be **symptomatic or asymptomatic**.
- Information campaigns on sensible use of antimicrobial drugs are needed in order to prevent the creation of **antibiotic resistant zoonotic *C. psittaci*** strains.
- **Legislation, prevention and control** measurements must be reconsidered.
- In the future, **vaccination** could be used to reduce zoonotic risk.