Rickettsiales and Chlamydiales

Rickettsiales

- Minute obligate intracellular Gram negative bacteria
- Rods or coccobacilli
- Variable size 0.3-0.6 micron
- Non-motile
- Aerobic , divide by binary fission
- Cell wall similar to Gram negative bacteria but in anaplasmatacea have cells bound by two-layer membrane

General features

- Stain poorly with basic dyes
- Stain well with Giemsa and oyher Romanowsky stains
- Most require living cells for propagation
- Cultured commonly in fertile eggs, or in tissue cultures
- *Haemobartonella felis* not yet propagated in vitro

Key points

- Minute, non-motile Gram-negative bacteria Obligate intracellular pathogens, replicating only in cells
- Demonstrated in blood smears by Romanowsky stains
- Host specificity and tropism for particular cell types evident
- * Extracellular survival brief for most members apart from ***Coxiella burnetii***
- Cause systemic diseases, mainly arthropod borne, in humans and animals
- ***Rickettsiaceae***
- --cell walls often contain peptidoglycan
- --cultured in specific cell lines or in fertile eggs
- -tropism for vascular endothelium or leukocytes
- **m *Anaplasmataceae***
- -lack cell walls, possess cell membranes
- -have not been cultured ***in vitro***
- --tropism for erythrocytes

General features

- They are parasites of arthopods, replicating in cells of gut
- Passed transovarially or transtadially in ticks and mites
- Labile outside the host except *Coxiella brunetti* (resistant due to spores)

Table 111. Genera of the Rickettsiales of veterinary importance.

Family and tribe	Genus	Cells parasitised in host
Rickettsiaceae		
Tribe Rickettsieae	*Rickettsia*	Vascular endothelial cells
	Coxiella	In vacuoles of cells of reticuloendothelial system
Tribe Ehrlichieae	*Ehrlichia*	Circulating leukocytes
	Cowdria	Vascular endothelial cells
	Neorickettsia	Reticular cells of lymphoid tissue
Anaplasmataceae	*Anaplasma*	Within erythrocytes
	Aegyptianella	Within erythrocytes of birds
	Haemobartonella	On, or in, erythrocytes
	Eperythrozoon	On erythrocytes

Various Rickettsiales of veterinary significance

Agent	Main host(s) and distribution	Transmission (vector)	Disease and cells parasitised
Rickettsia rickettsii	Humans, dogs. Western hemisphere, especially Eastern USA	Endemic in ticks (*Dermacentor* spp.). Bites of infected ticks	Rocky Mountain spotted fever in man. Tick fever in dogs. Parasitises vascular endothelial cells
Coxiella burnetii	Humans, cattle and small ruminants. Other animals can be a reservoir. Worldwide	Agent in milk, birth fluids of ruminants and dust. Persists in ticks	Q fever in man: aerosol inhalation, ingestion, tick bites or laboratory accidents. Occasional abortions, weak offspring or infertility in ruminants; usually a tick-borne infection
Ehrlichia canis	Dogs. Americas, Asia, Africa, Caribbean and Mediterranean	Brown dog tick (*Rhipicephalus sanguineus*)	Canine ehrlichiosis (tropical canine pancytopenia). Agent in lymphocytes, monocytes and, rarely, in neutrophils
E. equi	Horses. USA only	Vector unknown, ticks suspected	Equine ehrlichiosis. Granulocytes and vascular endothelial cells affected
E. ondiri	Cattle. East Africa	*Amblyomma* ticks	Bovine petechial fever. Granulocytes and monocytes parasitised
E. (Cytoecetes) phagocytophila	Cattle, sheep and wild ruminants. UK, Ireland and Scandinavia	*Ixodes ricinus*	Tick-borne fever. Pregnant animals may abort. Present in granulocytes and monocytes
E. platys	Dogs. Similar distribution to *E. canis*	Vector not known	Canine infectious cyclic thrombocytopenia. Usually subclinical. Platelets affected
E. risticii	Horses. USA and Europe	Vector unknown. Seasonal, late spring to early autumn	Potomac horse fever (equine monocytic ehrlichiosis). Present in monocytes. Intestinal involvement
Cowdria ruminantium	Domestic and wild ruminants. Africa, Caribbean	*Amblyomma* ticks	Heartwater. Reticular cells, neutrophils, and vascular endothelial cells affected
Neorickettsia helminthoeca	Dogs, coyotes, foxes, bears and ferrets. Pacific coast of USA	Ingestion of salmon containing the agent	Salmon poisoning. Agent present in salmon within the metacercariae of the *Nanophyetus salmincola* fluke. Parasitises reticuloendothelial cells of the lymphoid system, including macrophages
Elokomin fluke fever agent	As above	As above	Salmon fever (milder form). This agent occurs with *N. helminthoeca* or separately. Reticuloendothelial cells of the lymphoid system

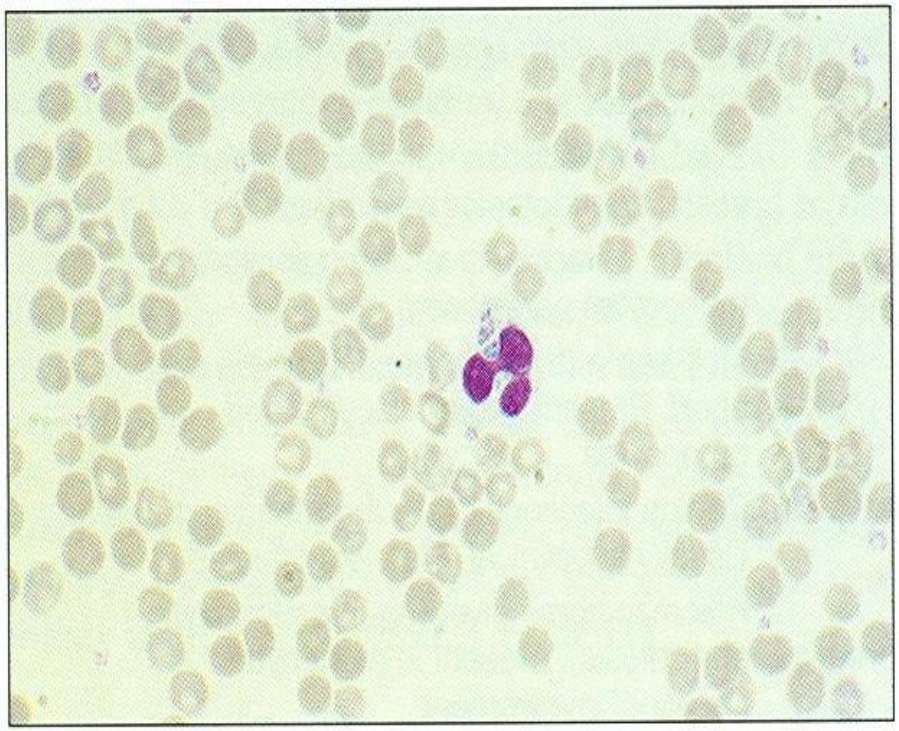
Agent	Main host(s) and distribution	Transmission (vector)	Disease and cells parasitised
Anaplasma marginale	Cattle and other ruminants. Tropical and sub-tropical regions	Ticks and other arthropods and veterinary instruments	Anaplasmosis (gall sickness). Inside erythrocytes with a marginal distribution
A. centrale	As above	As above	Mild or subclinical disease. Can be used to protect animals against the more virulent *A. marginale*. Predominantly central in erythrocytes
A. ovis	Sheep, goats and deer	As above	Infrequent and mild disease. Predominantly marginal in erythrocytes
Aegyptianella pullorum	Poultry. South Africa, South-east Asia and Mediterranean	Ticks such as *Argas persicus*	Aegyptianellosis. Agent in erythrocytes, leukocytes and mononuclear cells. *A. persicus* can also transmit *Borrelia anserina*
Haemobarto-nella felis	Cats. Worldwide	Possibly via cat fights and biting arthropods. Intrauterine infection reported	Feline infectious anaemia. Present on or in erythrocytes
Eperythrozoon ovis	Sheep. Africa, USA, Australia and Europe	Biting arthropods	An infrequent disease in lambs with anaemia and failure to gain weight. Present on red cells
E.suis	Pigs. USA	Biting arthropods and instruments	Porcine eperythrozoonosis. Usually subclinical but occasionally icteroanaemia, embryonic death and abortion. Present on erythrocytes

Lab diagnosis

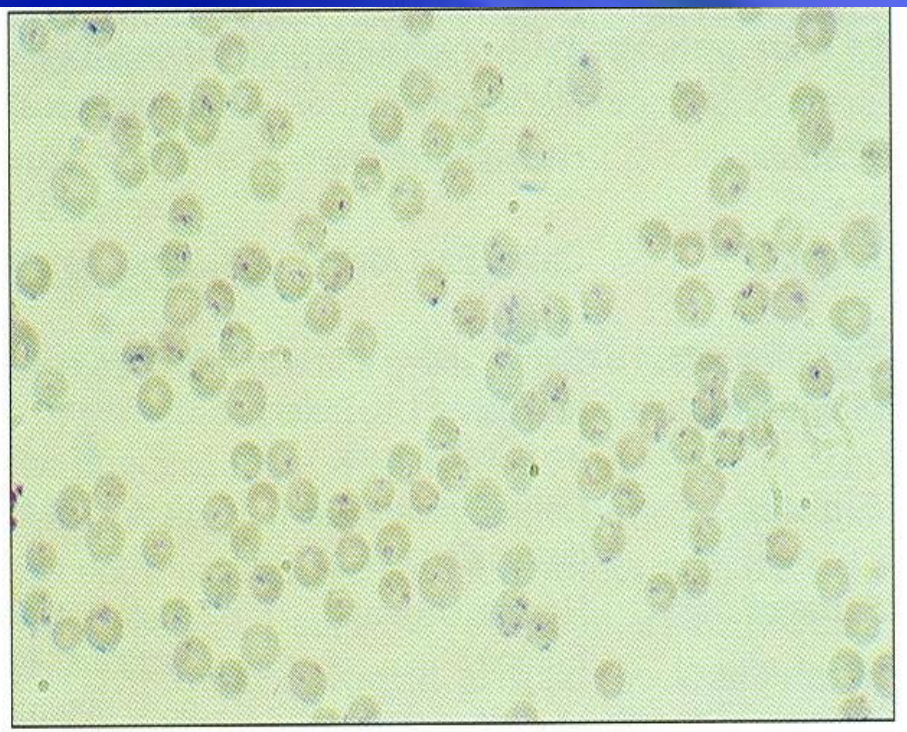
- **Specimen**
  - Vary with disease, may include
  - 1. unclotted blood
  - 2. affected tissue like brain (heart water)
  - 3. paired serum

- **Direct microscopy**

- Poorly stain with Gram stain
- Giemsa and other Romanowsky stains like Giemenez, Machiavello and leishman used
- FAT staining for blood and tissue smears

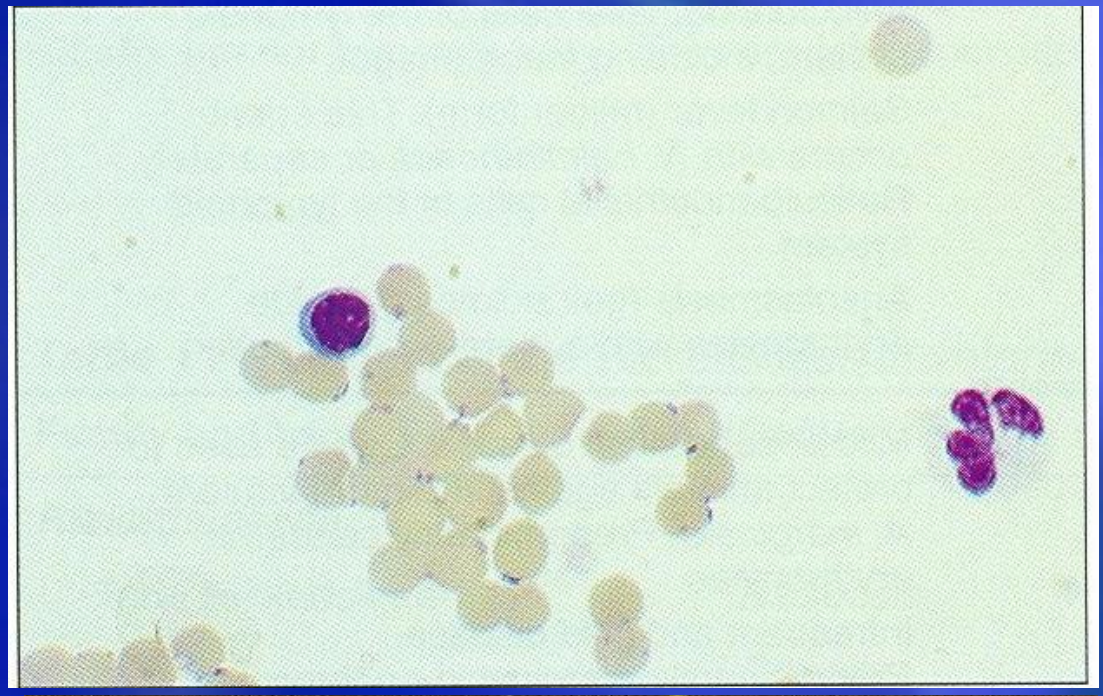


371 Leishman-stained blood smear from a sheep showing a neutrophil containing *Ehrlichia (Cytoecetes) phagocytophila*. (×1000)

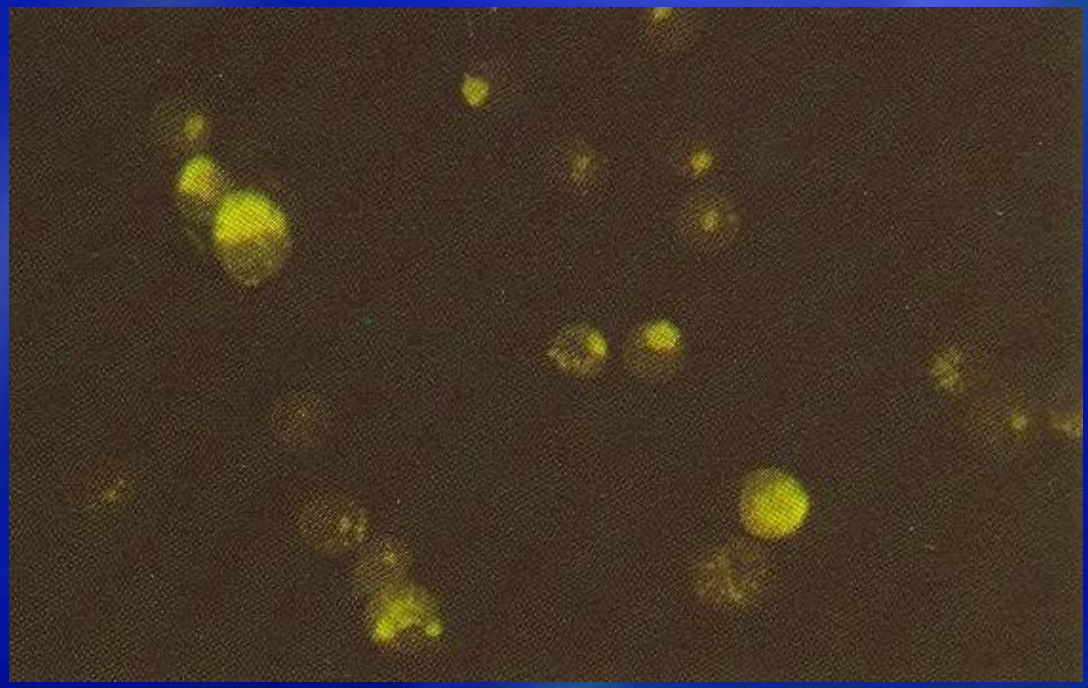


372 Leishman-stained blood smear from a sheep with *Eperythrozoon ovis* parasitising the red cells. (×1000)

Leishman-stained blood smear from a cat with *Haemobatonella felis*



***Ehrlichia risticii* in mouse macrophages (FAT, X1000)**



Isolation and cultivation

- Often difficult
- *Rochalimaea* will grow on inert media but other Rickettsiales require living cells for replication

- Yolk sac of chick embryos, cell cultures or lab animals

- **Identification**
- Based on animal species, clinical signs and demonstration of organism in stained blood or tissue smears and by specific serological tests

- Serological tests
- Q fever
- Weil felix reaction

Chlamydiales

- Order Chlamydiales consist of one family Chlamydiaceae with one genus Chlamydia
- Posses cell wall similar to Gram negative but lack muramic acid
- Obligate intracellular parasites with requirement of high energy compounds like ATP
- Also called ***energy parasites***
- Developmental cycle consist alternating, morphologically distinct, infectious and reproductive forms

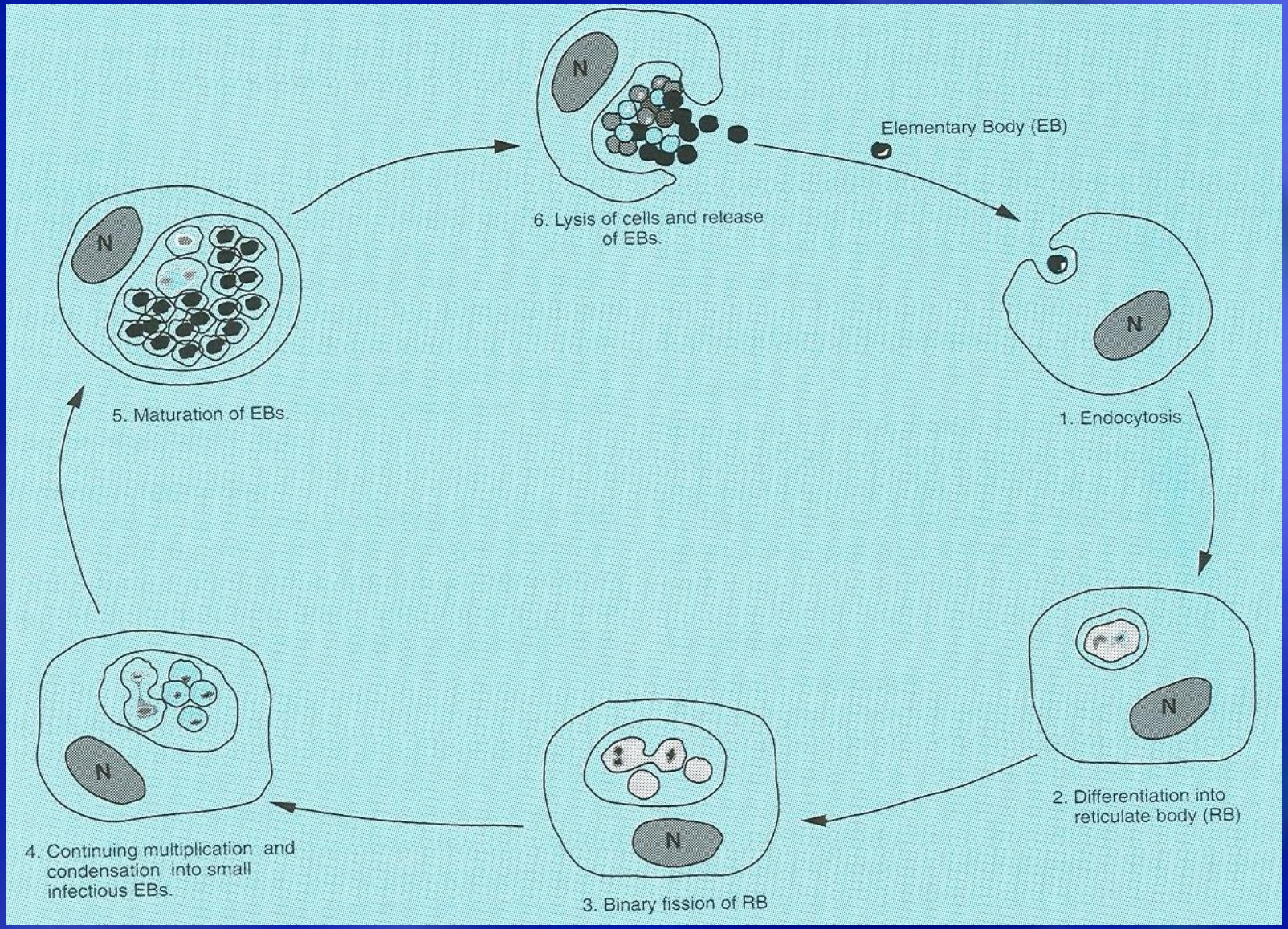
- *Elementary body is small (200-300 nm), infectious and represents extracellular form of organism*
- EB enters cell by endocytosis and differentiates into larger non-infectious but metabolically active reticulate body inside an expanding vacoule

- RB multiply by Binary fission producing further RBs

- At present only two species are recognised
  - *C. trachomatis*
  - *C. psittaci*

Key points

- Spherical intracellular bacteria with unique developmental cycle
- Appropriate staining procedures include the modified Ziehl-Neelsen and Giemsa methods
- Unable to synthesize ATP and replicate only in living cells
- Cell walls lack peptidoglycan but contain genus-specific lipopolysaccharide
- Species vary in virulence for particular hosts; some strains are associated with specific diseases in domestic animals
- Produce respiratory, enteric, plural and reproductive tract diseases in animals and humans



Representation of the life cycle of chlamydiae (N = Nucleus).

Table 110. Clinical conditions associated with chlamydial infections.

Chlamydia species	Host species	Clinical condition
C. trachomatis	Human	• Ocular disease a) Trachoma b) Inclusion conjunctivitis of neonates • Genital tract infections a) Non-gonococcal urethritis b) Salpingitis c) Cervicitis d) Epididymitis • Respiratory disease of infants • Proctitis • Lymphogranuloma venereum • Arthritis
***C. psittaci* (TWAR strains) (*C. pneumoniae*)**	Human	• Pneumonia
C. psittaci	Human (zoonosis)	• Psittacosis (ornithosis) • Abortion • Conjunctivitis
C. psittaci	Mammalian	• Intestinal infection and diarrhoea • Gastritis • Pneumonia • Abortion, e.g. enzootic abortion of ewes, chlamydial abortion of cows • Genital infections • Mastitis • Polyarthritis/polyserositis • Encephalomyelitis, e.g. sporadic bovine encephalomyelitis • Hepatitis • Conjunctivitis, e.g. feline pneumonitis
C. psittaci	Avian (psittacosis/ornithosis)	• Pneumonia and air sacculitis • Pericarditis • Encephalitis • Conjunctivitis • Intestinal infection and diarrhoea

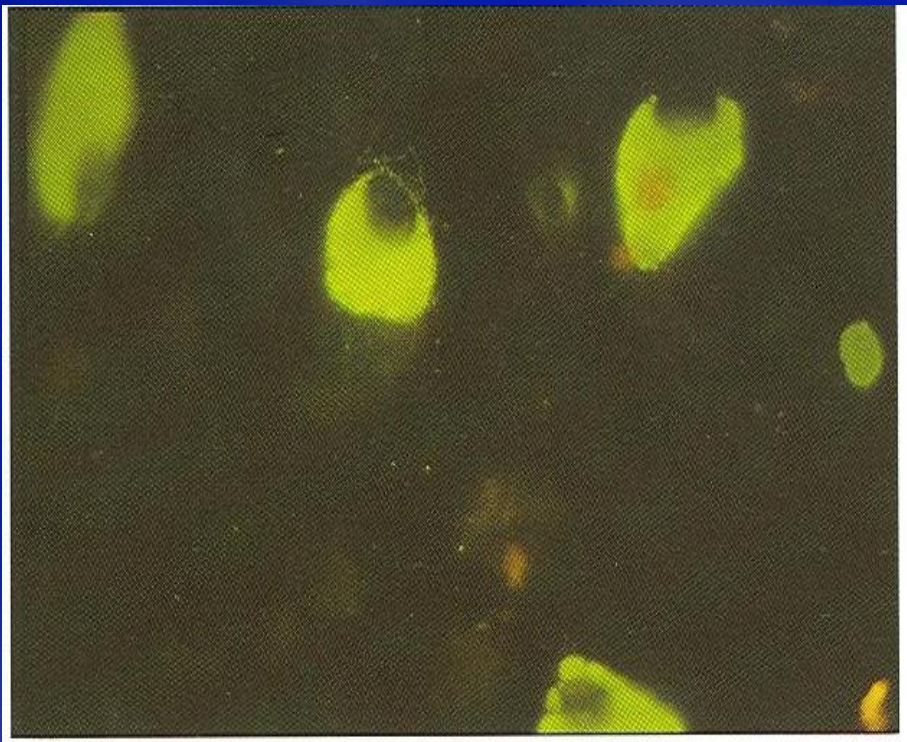
Lab diagnosis

- **SPECIMEN**

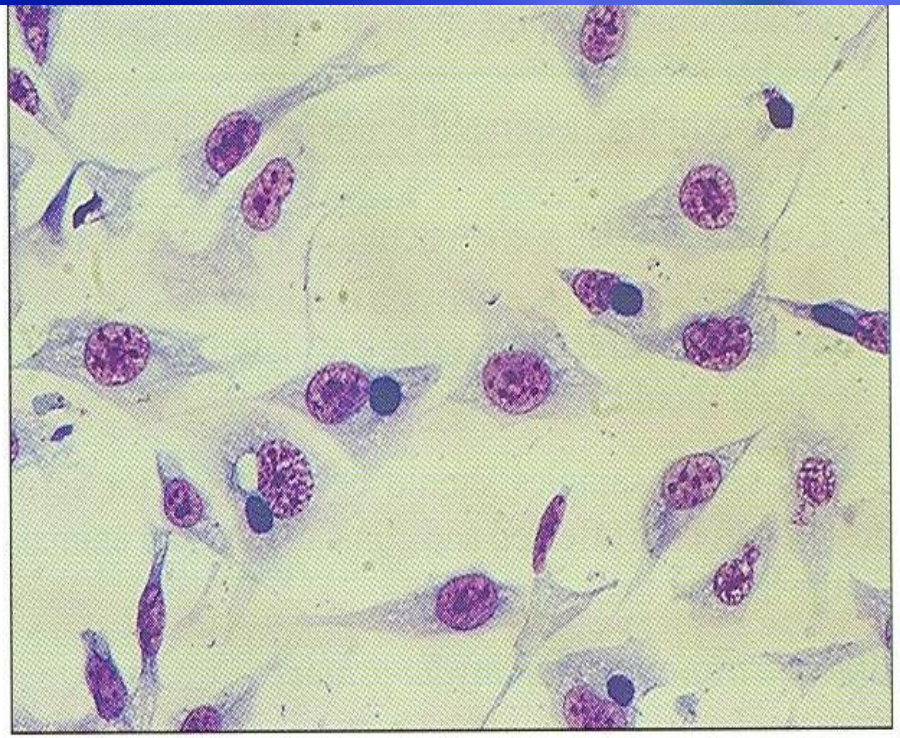
- Abortion-
  - stained smear from affected cotyledons/vaginal swabs/wet surface of aborted fetus
  - Pieces of cotyledon/ foetal liver/ lung
- Polyarthitis-
  - aspirated synovial fluid
- Conjuctivitis-
  - conjuctival swab
- Systemic infection-
  - samples of lung, liver and spleen

Isolation and cultivation

- Mouse inoculation
- Inoculation of embryonated eggs(6-7days) via yolk sac route
- Cell culture-McCoy, baby hamster kidney-21, L929 and Vero
- **Serology**
  - CFT
  - ElISA
  - Indirect FAT



368 *C. psittaci* (EAE isolate) inclusions in McCoy cells. (Direct FA technique, ×400)



369 *C psittaci* (EAE) inclusions in McCoy cells. (Giemsa stain, ×400)

THE END