Conditions d'émergences des maladies infectieuses

Moyens d'identifications des maladies infectieuses

- Historique
- Moyens modernes
  - Culture
  - Sérologie
  - Méthodes moléculaires

Culture

- Animal
- Milieu solide
- Culture cellulaire

P3 laboratory

Sérologie

- Immunofluorescence
- ELISA
- WB
- Ab croisée

Outils moléculaires

- Détection amplification PCR
  - Nested
  - Multiplex
  - Suicide
  - Real time QPCR
- Identification
  - PGEF , RFLP
  - Séquençage et BLAST
- Le génome (*Bacillus anthrasis*)

Emergence des Maladies infectieuses

- Rôle de la détection
  - Virus de l'hépatite C
    - infection expérimentale et génétique
  - HHV8
    - Génétique soustractive

Emergence des Maladies infectieuses

- Rôle de la détection (Un concours de circonstance !)
  - La découverte de *Bartonella henselae*
    - Les hypothèses infectieuse de la MGC
    - La découverte de *B henselae* dans l 'angiomatose bacillaire
    - Etude de la séroprevalence de BH chez le VIH / control

Emergence des Maladies infectieuses

- Role de la curiosité médicale
  - *Helicobacter pylori*
  - Fièvre des Iles flinders

Flinder's Islands Spotted Fever

Emerging infectious disease (1991)

- ***Rickettsia honei***
- **Identical to Tick Thai Typhus Rickettsia ?**
- **Transmitted by ?**
- **Located to the Flinder's Islands**
- **Fever, eruption, eschar, enlarged lymph nodes**
- **Described by Dr. Stewart**

Emergence des Maladies infectieuses

- La ville
  - Surpeuplement/paupérisation
    - Péril fécal
      - Choléra
    - Promiscuité
      - Tuberculose, gale
    - Hygiène
      - Typhus murin, peste,
    - Quart Monde
      - SDF et leur pathologie

Emergence des Maladies infectieuses

- Les déplacements
  - Bateau
    - Peste +++
    - *Rickettsia africae* dans les Antilles
    - Légionellose, grippe
  - L'avion
    - Paludisme , Dengue, West Nile ….
    - SARS
    - Ebola, Lassa, Marburg

R. africae invasion of the New World

Dengue

- Dengue virus 1-4
- Vector : *Aedes aegypti, Aedes albopictus*
- Reservoir is vector and infected human
- Control
  - Mosquito control
  - Environmental control
- Breakdown in mosquito control in endemic area
- Development of megapole with shantytown
- Raised of international flight

SARS

- Il s'agit d 'une épidémie d 'infection pulmonaire ou pneumopathie dont l 'origine est un nouveau virus respiratoire de la famille des coronavirus
- Cette épidémie a débutée en Novembre 2002 dans le sud de la Chine et a été reconnue pour la première fois a HongKong et à Hanoi (Vietnam) en Mars 2003.

Emergence des Maladies infectieuses

- L 'alimentation (carnivore et herbivores)
  - Rôle de la religion et des coutumes dans l 'hygiène alimentaire
  - Prions
    - Kuru , Scrapie du mouton (anthropophagie)
    - Greutzfield Jacob
    - Encephalite spongiforme bovine
  - Trichinose
  - Hydatidose
  - Listeria, E coli 0157B7….

Emergence des Maladies infectieuses

- Le sexe
  - HIV ++++
    - Histoire de la découverte de l'infection HIV
  - Syphilis
  - Chlamydia
  - Hépatites
  - Herpes

Emergence des Maladies infectieuses

- Les animaux (Zoonoses)
  - Domestiques
    - Brucellose , fièvre Q, tularémie…
  - De compagnie
    - Chien source d 'ectoparasites
      - Tiques , puces, morsures
    - Chats
      - MCG
  - Sauvages
    - Cervidés (ehrlichioses …)

Human Monocytic Ehrlichiosis

- Epidemiology
  - Vector : *A. americanum*
  - Reservoir : Deer (Odocoïleus virginianus)

Emergence des Maladies infectieuses

- Les vecteurs et l 'environnement
  - Les poux
  - Les puces
  - Les tiques
  - Les moustiques
    - West nile

Emergence des Maladies infectieuses

- Nouvelles technologies
  - L 'agriculture
  - la climatisation
    - les legionelloses
  - L 'émission de CO2
  - Le tampon périodique

Emergence des Maladies infectieuses

- Nouvelles technologies
  - l 'hôpital (NOSOCOMIAL)
    - Infection nosocomiale post opératoire
      - lié au patient
      - lié aux opérateurs
      - lié à l'environnement
    - Infection nosocomiale transmises par contact
      - Rôle de l hygiène
      - Rôle de la prescription raisonnée des ATB
    - Infection nosocomiale transmises par voie aérienne

Emergence des Maladies infectieuses

- Nouvelles technologies
  - la transfusion sanguine
    - HIV, Hépatites, paludisme, HHV8, syphilis….
  - La transplantation
    - CMV, légionella, mycobactéries, pneumocystis
  - l 'alimentation artificielle
    - Candidemie, infection sur cathéter
  - la toxicomanie
    - HIV, Hépatites, septicémie, endocardites

Emergence des Maladies infectieuses

- Abandon des mesures de santé publique
  - Vaccination
    - Diphtérie
    - Rubéole
    - Variole

Diphtheria

- Respiratory spread
- Reservoir = human
- Control is vaccination
- Immunity is antitoxinic not antibacterial
- Escape when vaccine cover not sufficient

Diphtheria

- Re-emergence :
  - Northern Africa (Algeria)
  - Eastern Europe (Former USSR)

Rubella

- Respiratory spread
- Reservoir = human
- Control
  - schoolgirl vaccination
  - mass vaccination
- Re-emergence in the non vaccinated male population in the army ++ (British troops in Bosnia, Fort Bragg North Carolina)

Emergence des Maladies infectieuses

- la vulnérabilité
  - niveau économique
    - Choléra péril fécal
  - guerres
    - Choléra
    - Maladies transmises par les poux, et les puces
  - l 'immunossupression
    - Infections opportunistes

Le peril fecal

- **Fecal/ Oral spread**
  - Ex: Cholera
- Control = Public health measure
- Escape = Breakdown of public health measure but also acquisition of novel virulence gene (Ex: V.cholerae)

Cholera

- **First six pandemic (1817-1923) *Vibrio cholerae* 01**
- **Transmission = water (John Snow 1849 London)**
- **Seventh pandemic 150 000 cases in 1996 (85% from Africa)**
- **1992 India and Bangladesh *V cholerae* 0139**

Cholera

- *Vibrio cholerae*
- Fecal-oral transmission
- Reservoir
  - -asymptomatic human
  - aquatic reservoir
- Control
  - potable water (chorine)
  - management of excreta
- Vaccine not efficient yet

Louse

- Make the difference
  - Head louse
  - Body louse
  - Crab (pubic) louse

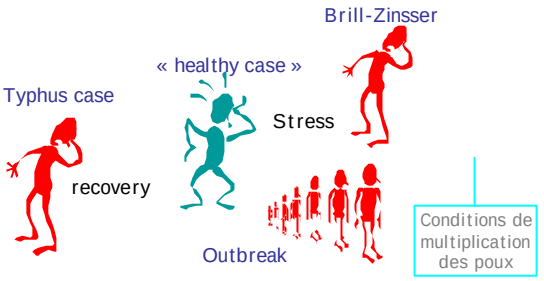
Louse-borne infections

- *Borrelia recurrentis*
  - Relapsing fever
- *Bartonella quintana*
  - Trench fever
  - Bacillary angiomatosis
  - Endocarditis
- *Rickettsia prowazekii*
  - Epidemic typhus

Epidemic typhus

- Jail fever
- Outbreak in refugee camps
  - Burundi
- First imported case in Marseille from Algeria

Epidemic typhus (R prowazekii)



Emergence des Maladies infectieuses

- Bioterrorisme
  - Variole
  - L'anthrax
- Les laboratoires de recherche
  - Les fièvres hémorragiques
  - les virus émergents

Origine

La variole, ou smallpox, était une maladie sévissant à l'état endémique en Afrique. Le réservoir de virus est strictement humain. La transmission s'effectue par voie aérienne et par contact avec les croûtes.

Histoire

- Au Moyen âge la variole atteint l'Europe
- Au XVIIème siècle la variole tue 10 % de la population
- Au XVIIème siècle commence la « Variolisation » des aristocrates français, russes et britanniques , entrainant 1 à 2 % de mortalité

La vaccination historique

- Le 14 Mai 1796 Jenner vaccine James Phipps (8 ans) avec le cowpox (de la vache blossom) et le 1er Juillet 1796 il lui inocule la variole

L'éradication de la variole par la vaccination

- 1955 : Dernier cas en France (Bretagne)
- 1967 : Intensification
- 1972 : Dernier cas en Europe
- 1975 : Dernier cas en Inde
- 1977 : Dernier cas en Somalie
- 1980 : Eradication et création de deux conservatoires à Atlanta et Moscou

La variole comme arme biologique

- La variole est utilisée comme arme biologique pour la première fois par Sir Jeffrey Amherst (French and Indian Wars 1754 - 1767) en distribuant des couvertures contaminées aux indiens

La variole comme arme biologique

- Le virus de la variole d'après les informations recueillis après la guerre froide
  - A été militarisé par les russes à Vector
  - Il en a été produit plus de 20 tonnes à Koltsovo, Zagorsk et Pokrov
  - Il existe probablement des souches génétiquement modifiées

Tick transmitted rickettsioses

An updated review

Philippe BROUQUI

Unité des rickettsies, CNRS UMR 60 20

Faculté de médecine, Marseille, France

Ticks

Ixodidae or hard ticks:
(694 species)

Argasidae or soft ticks:
(77 species)

Nuttalliellidae:
1 species

Ixodidae

Argasidae

Ticks ecology

Environnemental factors - Ecosystem

temperature humidity photoperiod vegetation

Interrelation with living life

Each species:

- **Peculiar biotope = specific geographic distribution**
- **Seasonal variation of activity – life cycle**

Hunting the host

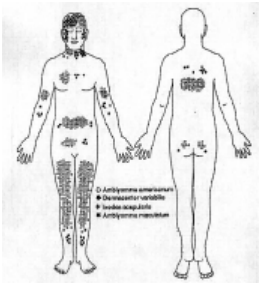
«Ambush»

Ex: *I. ricinus R. sanguineus, D. marginatus*

« Attack »

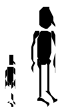
Ex: *Amblyomma variegatum, Amblyomma hebraeum*

Biting sites (1)



Host specificity

High: *R. sanguineus*



Low : *I. ricinus*

The bite

The bite is painless

-Biting

-Anesthesia and attachment

-Tissue lysis with enzymes

-Sticking with glue

-Taking blood meal

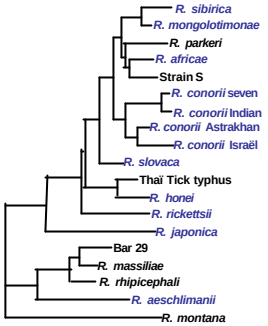
20 hours before transmission

What's a Rickettsia ?

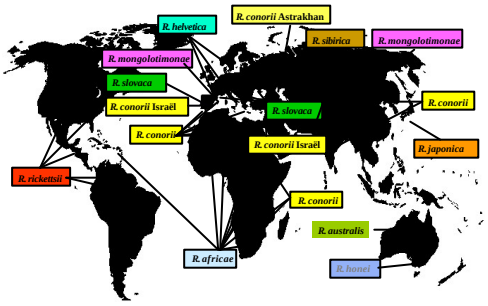
- Gram négatif bacteria
- Strictly intracelluar
- Subgroup α1 of Proteobacteria (16S rRNA)
- SFG rickettsia is motile in the cell
- *R conorii* size : 1,268,755 nucleotides

R belli

Classification of SFG rickettsia based upon (rOmpA)

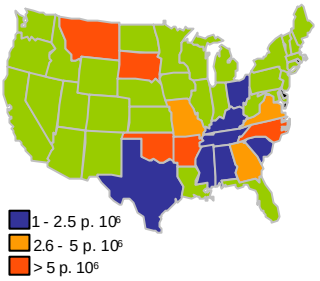


SFG Rickettsioses worldwide



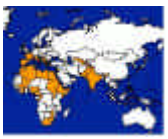
Rocky Mountain Spotted Fever (1899)

- *Rickettsia rickettsii*
- Transmitted by tick bites (*Dermacentor andersoni* and others)
- Summer disease
- USA : 500 cases/year (South-eastern states) Prevalent in Brazil and Central America
- Fever, eruption, 5 - 80 % mortality without treatment



Mediterranean Spotted fever (1910)

- Agent : *Rickettsia conorii*
- Vector : *Rhipicephalus sanguineus*
- Distribution worlwide
- Season; summer
- Mortality : 2 - 5 %



« Tache noire »

Rash

Siberian tick typhus (1935)

- First described in Primorye
- Prevalent in ex-USSR, Pakistan and China
- Vectors: *Dermacentor spp,Hemaphysalis spp and Rhipicephalus spp.*
- Reservoir? mice,rats,and hedgehogs

Siberian tick typhus (1935)

- Incubation period 4-7 days
- Escar and lymphadenopathy
- Fever 38-39°C
- Headaches, myalgia,digestive disturbance
- On day 2- 4 after the onset, rash sometime purpuric
- Mild spotted fever with rare complication

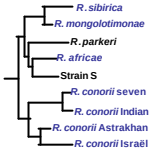
Queensland tick typhus (1946)

- *Rickettsia australis*
- Transmitted by tick bite (Ixodes holocyclus)
- Fever, eruption (vesicular), eschar, enlarged lymph nodes
- Mild to severe

Israeli spotted fever (1974)

- First cases in 1940s but reported as RMSF
- Clinically close to MSF but escar lacking most of time
- Vector : *R sanguineus*
- *Agent*:distinct but closely related to *R conorii*
- Found also in Sicily and Portugal (1999)

Astrakan fever rickettsia (1983)

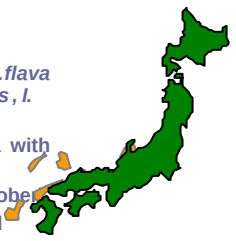


Astrakan fever rickettsia (1983)

- Vector : *R sanguineus* and *R pumilio*
- Agent : astrakan fever rickettsia close to *R conorii* isolated both in tick and humans
- "Tache noire" in 20% of patients only
- Antibodies to *R conorii* present in 50-70% of patients
- Found in human in Chad and in ticks in Kosovo

Japanese or Oriental spotted fever (1984)

- *Rickettsia japonica*
- Vectors : *Dermacentor taïwanensis, Haemaphysalis longicornis,H.flava but also I.ovatus,I. persulcatus , I. monospinosus*
- First described by Dr. Mahara with the use of Weil Felix test
- Described in Japan (april-octobe
- More than 100 cases reported
- Fever, headaches, rash, escar
- Cases of encephalitis reported



Flinder's Island Spotted Fever (1991)

- *Rickettsia honei*
- Identical to Tick Thai Typhus Rickettsia ?
- Transmitted by ?
- Located to the Flinder's Islands
- Fever, eruption, eschar, enlarged lymph nodes
- Described by Dr. Stewart

African Tick bite fever (1992)

- Agent : *Rickettsia africae*
- Vector : *Amblyomma haebraeum*
- South of Africa
- Fever, "tâche noire" (several)
  Rash seldom (vesiculous)
- Mild disease

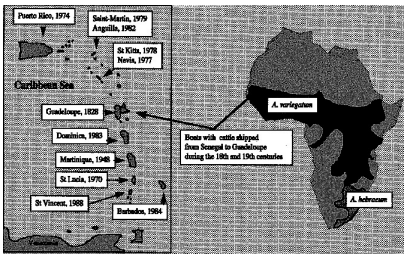
African Tick bite fever

Features	African Tick-bite fever	Mediterranean spotted fever	Rocky Mountain spotted fever
"Tache noire"	98/100 (98 %)	72 %	0 %
Multiple	51/97 (53 %)	0 %	0%
Location			NA
- Lower limbs	61/100 (61 %)	Limbs 46 %	
- Upper limbs	11/100 (11 %)		
- Chest, abdomen, groin	19/100 (19 %)	Trunk 46 %	
- Back, buttock	4/100 (4 %)		
- Neck and head	6/100 (6 %)	8 %	
Regional lymph nodes	40/70 (57 %)	NA	NA
Grouped cases	74/100 (74 %)	0 %	0 %
Doxycycline therapy	55/74 (74 %)	86 %	NA
No treatment	12/74 (16 %)	8 %	NA
Death	0/100 (0 %)	2.5 %	4 %

African Tick bite fever

	R. conorii	*R. africae*
RASH	Frequent : Maculo-papular	Seldom : Vesicular
ESCARRE		
Lymph nodes		

R. africae invasion of the New World



Indian tick typhus (1995)

- **Prevalent in India**
- ***R conorii* (ITT strain)**
- ***R Sanguineus* ?**

R. mongolotimonae (1996)

- 1993 : Isolated from ticks *Hyalomma asiaticum* China
- 1996 : 1st case in Marseilles
- 1998 : 2nd cases in Marseilles
- 2000 Niger in *Hyalomma sp.*
- Migrating bird ?
- Since then more than 15 cases documented

R. mongolotimonae (1996)

- Fever , Multiple Escarre, Lymphnodes, Lymphangitis

R heilongjiangensis (1996)

- First isolated from *Dermacentor salivarum* in 1982 in China
- 1992, serological diagnostic in 12 patients with fever, headaches, rash, eschar lymphadenopathy and conjunctivitis
- 1996 isolation of the bacteria from 7 patients

Rickettsia slovaca (1997)

- *Dermacentor marginatus*
- Escarre of the scalp, lymphadenopathy , fever
- Neurological involvment ? (méningo-encéphalitis) ?
- 25 confirmed cases PCR and culture ***(Raoult et al., Lancet, 1997)***

Rickettsia slovaca (1997)

Features	Number / 25	(%)
Male/Female	10/15	NA
Incubation time (median in days	6	NA
Tick bite*	25	100 %
Inoculation lesion *	25	100 %
Fever	6	24 %
Headaches	4	16 %
Cervical lymph node *	25	100 %
Painful lymph node	11	44 %
Rash	2	8 %
Chronic fatigue	3	12 %
Localized alopecia	4	16 %

R. helvetica (1999)

- 2 patients deseased with perimyocardite (Sweden) diagnostic with PCR in cardiac tissues
- 9.2 % seroprevalence in forest workers in Northern France
- 1 case published of unexplained fever (France)
- *Ixodes ricinus*

Aneruptive Fever Associated with Antibodies to *Rickettsia helvetica* in Europe and Thailand†

Pierre-Edouard Fournier,[1] Caroline Allombert,[1] Yupin Supputamongkol,[2] Giuseppe Caruso,[3] Philippe Brouqui,[1] and Didier Raoult[1]*

R. helvetica (1999)

- 1 case with antibodies to *R helvetiva* in a traveler been biten in Australia and returning to Japan ? (Dr Inokuma et al.)
- Detected by PCR in *Ixodes ovatus, persulcatus,* and *monospinosus* (Dr Takada)

R aeschlimannii (2000)

- Isolation from *Hyalomma marginatum* in 1997 in Morocco and then from sub-Saharan Africa
- Fist human case in 2000 in a patient returning from Morocco with fever, an eschar and a generalized maculopapular rash. Another case was reported from South Africa

Rickettsia of unknown pathogenicity (1)

Rickettsial name	*Vector*	Year first discovery	Geographic repartition	type of detection
R rhipicephali	*R sanguineus* *D occidentalis* *D andersonii* *D variabilis*		France Portugal USA	PCR and Culture
JC 880	*?*	1970 1973	Pakistan	Culture
R belli	*D variabilis* *D occidentalis* *D alpopictus*	1966	USA	Culture/PCR
	Ornithodoros spp			
R montanensis	*D variabilis* *D andersoni*	1953	USA	Culture
Strain S R massiliae Bar 29	*R sanguineus* *R sanguineus* *R turanicus*		Armenia France, Portugal, Greece,Spain	Culture Culture/PCR
	R mushsamae		Mali	

Rickettsia of unknown pathogenicity (2)

Rickettsial name	Vector	Year first discovery	Geographic repartition	type of detection
R peacockii	*D andersonii*		USA	PCR and Culture
Strain DbS14	*?*		Eastern siberica , Kasakhstan	PCR
R PA4	*D reticulatus* *D niveus*		Kasakhstan	PCR
R parkerii	*A maculatum*	1937	USA	Culture
R texicana (bullis fever)	*A americanum*	WWII	Texas	Culture /PCR
R canadensis	*H leptorispalustris*		Canada	Culture/PCR
Rav1,Rav3,Rav9	*A variegatum*	2000	Niger and Mali	PCR only
R amblyommii	*A americanum*		USA	Culture /PCR

Rickettsia of unknown pathogenicity (3)

Rickettsial name	Vector	Geographic repartition	type of detection
R hulinii	*H concinna*	China	PCR and Culture
Irs3,IrS4	*I ricinus*	Slovakia	PCR/culture (Irs4)
R monacensis	*I ricinus*	Germany	PCR/culture
AT-1	*A testudinarium*	Japan	PCR
Candidatus Rickettsia tarasevicheae	*I persulcatus*	Russia	Culture/PCR

Diagnostic

- Serology
  - Microimmunofluorescence
    - Not specifique (cross reaction)
  - Western-blot
    - rOmpA
    - rOmpB
    - LPS
- Cross adsorbsion
- Monoclonal antibodies

Diagnostic

- Culture
  - Animal
  - Embryonated eggs
  - Cells (shell-vials)
- Identification
  - Serology
  - PCR

Treatment

- *In vitro* Suseptible :
  - Doxycycline
  - Quinolones
  - Josamycine
  - Roxithromycine
  - Clarithromycine
  - Telithromycine
  - Rifampin ?
- Treatment
  - Mild
    - Doxycycline 200mg qd once (A,C)
    - Josamycine 25 mg/kg/ bd 10 days
  - Malignant
    - Doxycycline 200 mg /qd for 10 days

Rifampicin resistant isolates

Human Anaplasmosis :

a zoonotic emerging disease

Pr Philippe BROUQUI
Unité des rickettsies, CNRS UMR 60 20
Marseilles, France

History of a discovery

- **Emerging infection**
  - 1991 Dumler et al.
    - A clinical human case
  - 1995 Chen et al.
    - Molecular detection
  - 1996 Goodman et al.
    - Cell culture isolation

Blood smear

Spleen smear

Taxonomy

Reorganization of genera in the families *Rickettsiaceae* and *Anaplasmataceae* in the order *Rickettsiales*: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*

J. Stephen Dumler,[1] Anthony F. Barbet,[2] Cornelis P. J. Bekker,[3] Gregory A. Dasch,[4] Guy H. Palmer,[5] Stuart C. Ray,[6] Yasuko Rikihisa[7] and Fred R. Rurangirwa[8]

A.phagocytophilum

Genomic

- p44 ou msp2 paralog
  (multi-gene family)

20-50 P 44 paralogs encoding a Msp2 multimeric protein which is suggested to play a role in adhesion to granulocytes. Expression of this Msp2 vary depending upon bacterial conditions (ticks , mice, in vitro culture, human acute infection) and may represent an adaptative capability of the bacterium

Epidemiology

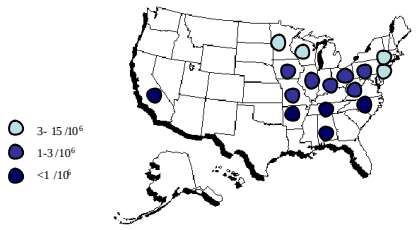
- Vectors In Europe
  - *Ixodes ricinus*
  - *Ixodes trianguliceps*
  - *Ixodes persucatus*

I.ricinus

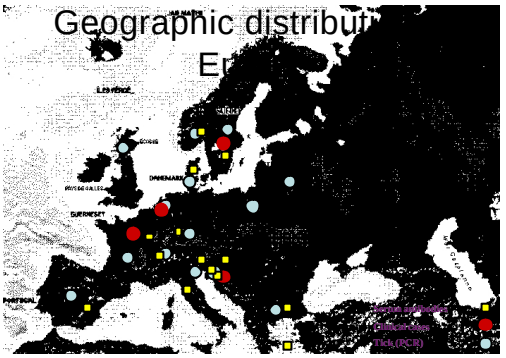
Epidemiology

- Reservoirs
  - *Small mammals*
    - *Peromyscus leucopus, Apodemus sylvaticus* and Aflavicollis, *Sorex araneus* and *Clethrionomys glareolus*
  - Rabbits (USA)
  - Deer, Moose Calf
  - Dogs, Cow, Goat, Sheep Horse ?

Geographic distribution USA



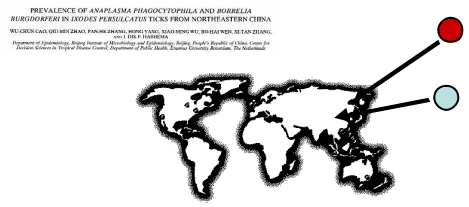
Geographic distribution Europe



Human Granulocytic Ehrlichiosis – A Clinical Case in Scandinavia

Human Granulocytic Ehrlichiosis in Western Europe

Geographic distribution world



PREVALENCE OF *ANAPLASMA PHAGOCYTOPHILA* AND *BORRELIA BURGDORFERI* IN *IXODES PERSULCATUS* TICKS FROM NORTHEASTERN CHINA

Serologic and Molecular Detection of *Ehrlichia chaffeensis* and *Anaplasma phagocytophila* (Human Granulocytic Ehrlichiosis Agent) in Korean Patients

Seroprevalence in Europe

- France 0-17 % , Germany 12-14%, Swizerland 7.4-17%, Slovenia 15% , Norway 10%...

Risk factor /Tick exposure

- Prospective study in Slovenia 1995-1996
  - Diagnosis in 64/130 (49,2%)
  - *A phagocytophilum* in 4/130 (3,1%)
  - Lyme borelioses in 10/130 (7,7%)
  - TBE in 36/130 (27,7%)
- Prospective study in Sweeden in 2002
  - *A phagocytophilum* : 4/27 (14.8 %)
  - Borreliose de Lyme : 12/23 (52%)
- In Belluno Northern Italy
  - *A. phagocytophilum* 21/242 (8.6%), forester 6/109, (5.5%) hunters, and 3/193 (1.5%) resident
- Prospective study in Belluno Northern Italy (355 patients , 353 ticks and 1088 sera)
  - 1088 sera from 355 asymptomatic patients bitten by Ixodes ticks no seroconversion to *A phagocytophilum*
  - Prevalence of *A phagocytophilum* in these tick 1/353

Incubation Time

- Tick bite 7 to 30 days before the onset of symptoms
  - 24 hours attachment are needed for *A.phagocytophilum* transmission
  - Incubation period goes unnoticed

The disease

- Asymptomatic infections frequent +++
- Flu-like syndrome
  - Frequent > 50%
    - Fever
    - Headaches
    - Nausea
    - Myalgia

Human granulocytic ehrlichiosis in Europe
J. R. Blanco and J. A. Oteo

The disease

- Clinical presentation
  - Less frequent 20%-50%
    - Arthralgia
    - Caught
    - Conjonctivitis
    - Interstitial pneumonitis

The disease

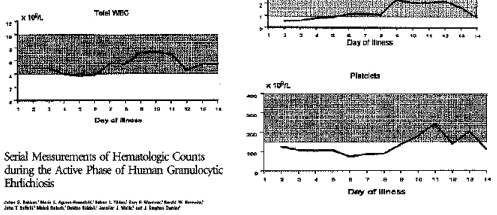
- Clinical presentation
  - Seldom < 10%
    - Rash
    - Lymph nodes
- Hospitalization required in 60% of patients
- No chronic form describe at this time

Laboratory data

- Haematology
  - Thrombopenia 12/13
  - Leucopenia 9/13
  - Lymphopenia 7/13
- Hepatitis (ALAT) 13/13

Laboratory data

- Laboratory features in the course of the disease in 144 patients (USA)



Serial Measurements of Hematologic Counts during the Active Phase of Human Granulocytic Ehrlichiosis

Atypical presentation

- Co-infection ++
  - Frequent
    - Lyme borelioses (5%)
      - There is no fever in EC ++++
    - TBE (7%)
    - Babesioses ?
    - Rickettsioses (*R.helvetica*) ?

Other diagnosis

- Other infectious agent transmitted by *Ixodes spp*
  - Borreliose de Lyme

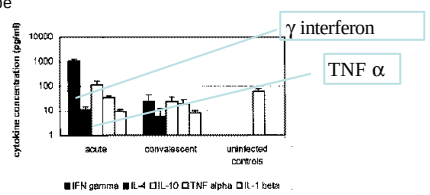
  - Tick-Borne encephalitis
  - Babesioses
    - *B divergens* , *B microtii* ?

Prognosis

- Mean duration of fever # 7.5 days
- No death among the 20 patients reported in Europe BUT
  - In the USA
    - Lethality is 3%
    - Opportunistic infections described
    - Perinatal transmission reported

Physiopathology

- Immune response
  - TH1 type



In Immunocompromised (FIV) cats *A phagocytophilum* induced a raised in IL10 but not of γ interferon

Physiopathology

- Pathology in Mice
  - Pathological modification vary with time and INF γ secretion
    - 2 à 4 days PI :
      - Moderate modifications (A)
    - 7 à 10 days PI :
      - Detection of Anaplasma (C)
    - at day 14 :
      - numerous liver lesions with frequent hepatocyte apoptosis (E)

Physiopathology

- Pathology in humans
  - multi-organ distribution of lesions
    - Spleen (E,F), liver, lymph nodes, bone marrow (G), lung (H)

Physiopathology

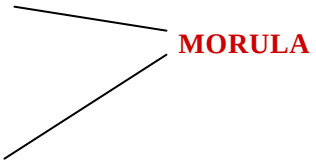
- In a patient with chronic myelogenous leukemia (CML) *A.phagocytophilum* better grows in CML cells than in the immature HL60 cells suggesting that the target cell is rather a mature than an immature granulocyte

Non specific diagnosis

- Flu-like syndrome after exposure to Ixodes ticks
  - The PPV is better if there is a thrombopenia, elevated ALAT and leucopenia .
  - Be careful to atypical Lyme borrelioses (febrile EC) or TBE +++

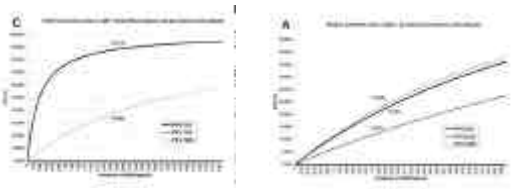
Specific diagnosis

- Blood smear



Specific diagnosis

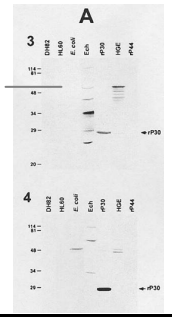
- Serology
  - IFA (In house / MRL ® test)
    - IgG > 32 (S > 80%, Sp > 90 %)
    - IgM > 32 (S > 30% , Sp > 98 %)



Specific diagnosis

- Western immunoblot

Cross reaction within the *Anaplasmacetae*



Specific diagnosis

- Serological response
  - 99 % of patient have antibodies in the first month AOS
  - Mean detection of antibodies occurs on day J11 AOS
  - The probability of getting a seconversion in the first 4 weeks AOS is of 90%
  - Consequently the convalescent phase serum should be sampled between week 3 and 4
- Trouble shooting
  - Cross reaction, co-infection or co-exposure ?
    - These agents share the same vector

European guidelines

*Network for surveillance of Tick Borne diseases * EC QLRT-2001-01293*

- European referral center for serological diagnosis

Laboratory	IFA test	(Se /Sp)IgG- (Se /Sp)IgM	Antigen source	Commercially available
Unité des Rickettsies	IgG IgM	(80/92.7)-(40/94.1)	HGE Webster strain (human isolate)	no
Kalmar county hospital	IgG	Not evaluated	SMA 308 (Swedish isolate)	no
Medical faculty, Lubjana	IgG	Not evaluated	USG3 (tick isolate)	no
Zurich Swizz	IgG	Not evaluated	*A phagocytophila* (Swiss strain)	no
MRL diagnostic USA	IgG IgM	86.6/92.7-33/98	HGE 1 (Human isolate)	yes
Pettenkofer-Inst., Germany	IgG IgM	Not evaluated	HGE (Human isolate)	no

Specific diagnosis

- Immunohistochemistry

Specific diagnosis

- Cell culture
  - At least 25 strains including 3 Europeans (2 equine and one ovine)
  - P3 laboratory
    - Human leukemia cells HL60
    - RPMI 1640, 10% FBS, 2 mM L glutamine
    - 100 ml EDTA anti-coagulated blood fresh or frozen for 3ml HL60 at $5x10^5$ cell/ml
    - Cells maintained between $2x10^5$ and $6x10^5$
    - Alternative Tick cell line (Ixodes IE8 and ISE6)

Specific diagnosis

- Molecular detection
  - From blood (EDTA) and other fluid and tissues (CSF, …)
  - From Ticks
  - Mostly used genes
    - 16s r RNA, gltA, rpoB, Ank, Gro El, Gro Esl…..
  - Amplification methods
    - PCR « specific» (nested, multiplex, nested suicide, QRT nested or not ..)
    - PCR eubacterial or broad range
  - Sequence analysis
    - Sequencing and BLAST in GenBank®
    - RFLP

European guidelines

*Network for surveillance of Tick Borne diseases * EC QLRT-2001-01293*

- Molecular gene and primer sequence

European guidelines

*Network for surveillance of Tick Borne diseases * EC QLRT-2001-01293*

- Confirmed
  - Febrile illness with a history of a tick bite or tick exposure and
  - demonstration of *A. phagocytophilum* infection by seroconversion or = 4-fold change in serum antibody titre * or positive PCR result with subsequent sequencing of the amplicons demonstrating Anaplasma-specific DNA in blood or isolation of *A. phagocytophilum* in blood culture.

** by IFA using either intracellular or purified antigen in a reference laboratory or MRL diagnostic kit*
*** by using species specific primers*

European guidelines

*Network for surveillance of Tick Borne diseases * EC QLRT-2001-01293*

- Probable
  - Febrile illness with a history of a tick bite or tick exposure and
  - Presence of stable titre of *A. phagocytophilum* antibodies in acute and convalescent sera if titre > 4 times the cut off * or positive PCR result without sequence ** or presence of intracytoplasmic morulae in a blood smear.

** by IFA using either intracellular or purified antigen in a reference laboratory or MRL diagnostic kit*
*** by using species specific primers*

Antibiotic susceptibility

- Methods used for antibiotic susceptibility testing of *A.phagocytophilum*
  - Percentage of infected cellsin culture with and without antibiotics
    - Diff-Quick® , IFA, titration by serial dilution
  - RTQ PCR

Antibiotic susceptibility

- Experimental data

TABLE 1. Susceptibilities of six New York State isolates of *E. phagocytophila* to antibimicrobials

Antibiotic	MIC (μg/ml)	MBC (μg/ml)
Doxycycline	≤0.125	0.125–0.5
Rifampin	≤0.125	≤0.125
Ofloxacin	≤2	≤2
Levofloxacin	≤1	≤1
Trovafloxacin	≤0.032	≤0.032
Amoxicillin	≥32	≥32
Ceftriaxone	≥64	≥64
Chloramphenicol	>16	>8[b]
Erythromycin[a]	>8	>8
Azithromycin	>8	>8
Clarithromycin	>10	>10
Amikacin	>16	>16

[a] Erythromycin, erythromycin ethyl succinate.
[b] For a single isolate, the MBC was ≤8 μg/ml.

- Resistant to Chloramphénicol, co-trimoxazole, macrolides and beta lactams

Treatment

- In humans
  - Death have been reported in patients treated with Chloramphénicol
  - Rifampicin used with success in two pregnant women and one child
    - Care with emergence of resistance
  - Quinolones : no data in humans but not allowed in pregnancy and in children
    - Gyr A mutation reported in *E canis* and *E chaffeensis*

Treatment

- Be attentive to co-infections
  - Lyme borrelioses / Anaplasmosis
- Staining of teeth
  - elevated doses and prolonged therapy
- Recommended treatment (A II)
  - Adults and children
    - doxycycline
      - 100 mg X 2 / day for 7 à 10 days
  - During pregnancy
    - Rifampicin

History

George Hoyt Whipple
1878-1976

In 1905 he was appointed Assistant in Pathology at the Johns Hopkins Medical School and remained at Johns Hopkins University until 1914.

In 1907 (29 yo) he report "A hitherto undescribed diseases characterized by deposit of fat in the intestinal and mesenteric tissue" that he named "intestinal lypodystrophy" or "Whipple's disease"

1961 : Bacterial etiology

2000

The Agent

1- 2 µ , trilamellar cell wall, intracellular location

Gram positive *Actinomycetes* clade

Epidémiologie

- Maladie rare
- Homme de 50 ans
- Source et mode de contamination?
- Présence de l 'ADN de la bactérie dans des eaux d 'égout
- Distribution chez l 'homme:
  - Salive, liquide gastrique, duodénum de sujets sains?

Système immunitaire et maladie de Whipple

- Immunité cellulaire
  - Diminution de la prolifération des cellules T en réponse aux mitogènes
  - Détérioration de l 'hypersensibilité de type retardée
  - Diminution de la production d 'Il-12
  - Diminution de la sécrétion de l 'interféron-gamma

The disease

- Rare
  - < 1000 reported cases
- Male 80%
- More frequent 40-50 year-old
- Extremely rare in child

Diagnosis of Whipple's disease

- Non specific diagnosis
  - Signs of chronic inflammation
    - Elevated ESR and C reactive protein
    - Anemia
  - Hyper eosinophilia
  - Signs of malabsorption

Specific diagnosis

- Pathological examination

PAS Staining of foamy macrophages

Immunohistochemistry with mabs

Specific diagnosis

- PCR Gene amplification

QRT PCR useful to differentiate
environmental contamination from active infection

Quantitative Detection of *Tropheryma whipplei* DNA by Real-Time PCR

Specific diagnosis

- Cultivation
  - Human fibroblast HEL or MRC5 cell lines
  - Generation time 18 days
  - Culture delay 6 weeks
    - cytopathic effect
  - Molecular detection +++

IFA labeling of *T.whipplei*
in human fibroblast

Traitement

- Empirique
- Pénicilline G et Streptomycine IV pendant 14 j
- puis Bactrim Forte*, 2 cp/j PO pour une durée de 1 à 2 ans
- Efficacité rapide du traitement
- Rechute
- Evolution réfractaire aux antibiotiques:
  - Association à de l'interféron-gamma
- Suivi des patients

Lyme disease

- Spirochete
  - *Borelia burgdorferii* senso lato
    - *B afezelii*
    - *B garinii*
    - *B burgdorferii sens stricto*
- Repartition
  - USA northern Europe
- Vector
  - Ixodes spp
- Primary clinical manifestation :EM

Lyme disease

- Secondary manifestation
  - Myocarditis
    - *B burgdorferi ss*
  - Arthritis
    - *B burgdorferi ss*
  - Meningoencephalitis
    - *B garinii*
- Tertiary manifestation
  - Dermatitis chronica atrophicans
    - *B afzelii*
  - Lymphocytoma
    - *B afzelii*