Ring Forms in Red Blood Cells (RBCs) – *Babesia* ?

– from **Danish** Chronically Ill Patients, All Clinically Suspect of Having Persistent Active Borreliosis !

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Presentation in York, UK – June 2004

LymeRICK – lots of info on TBI: **http://lymerick.net**

Project website (Danish) : **http://kroun.ulmarweb.dk**

ILADS Website: **http://www.ilads.org**

First a Short Historic Introduction to **Babesia**
– a Large Group of Ring Formed Malaria-like Parasites Infecting RBCs

1893 Babesia was the first infection ever proven to be transmitted by TICKs (Smith & Kilbourne, USA)

1957 Skrabalo & Deanovic first published case description of human (bovine?) Babesiosis in a splenectomized farmer, fatal infection !

1970'ies Babesia was noticed as an into Denmark imported dog infection (Willeberg et al. 1973). Used Koch's postulate to prove the infection.
These Vets mention that "haemoglobinuria enzootica" [Babesia bovis infection?] was already - i.e. 30 years ago - endemic in wild and domestic animals (cattle) in Denmark ! They also mention the risk of importing new vectors, as well as the pathogens they may carry from endemic areas of the World, when there is a suitable environment, lack of knowledge and awareness, lack of information about preventive measures, when borders are open without control and people and their domestic animals are travelling fast, going hiking / camping abroad => **Increased risk in now MUCH BIGGER EU !?**

1996 Lebech et al. Conf. Abstract: WA1 SEROPOSITIVE found in Danish Borreliosis patients and in 2 healthy donors
– work not published, why not !?

Danish Tick Environment

- 1/5 - 1/10 of the tick population harbour *Borrelia burgdorferi sensu lato* species
- Known microbes also present: **Anaplasma, Ehrlichia, Rickettsia, Babesia, Bartonella, TBE virus** –other ??? – extent ???
- Often more family members have had multiple tick bites &/ erythema migrans &/ other signs of Borrelia +/- co-infection and usually live in or visit often bracken or wooded tick infested areas, may even have beautiful roe deer grassing in their backyard =>

Picture was taken by Marie Kroun in May 2003 in her own back yard !
– i.e. from home area of cases 1 & 18 !

Babesia Microscopy - looks 1:

Babesia WA1

Source: Kjemtrup et al. Int J Parasitol 2000 Nov 1;30(12-13):1323-1337.

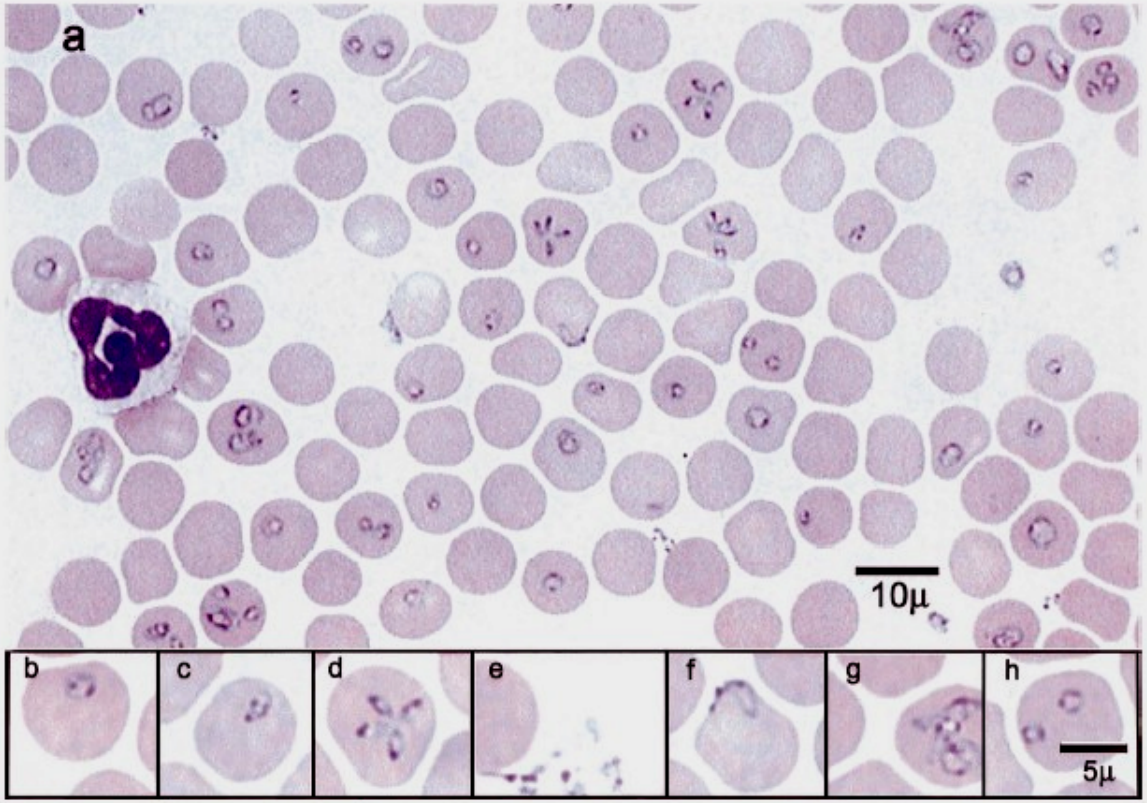

Fig. 1. Giemsa-stained thin blood smear of WA1-type parasites in hamster erythrocytes. Final magnification for (a) is 1112 ×. Final magnification for (b–h) is 2000 ×. (a) Oil-immersion field demonstrating severe parasitaemia; (b) ring form; (c) piriform; (d) tetrad or maltese-cross form; (e) exo-erythrocytic merozoites; (f,g) ameboid forms; (h) multiply-infected erythrocyte.

Babesia Microscopy - looks 2

Local Japanese variant of ***Babesia microti***

Source: Tsuji et al. J Clin Microbiol 2001 Dec;39(12):4316-22.

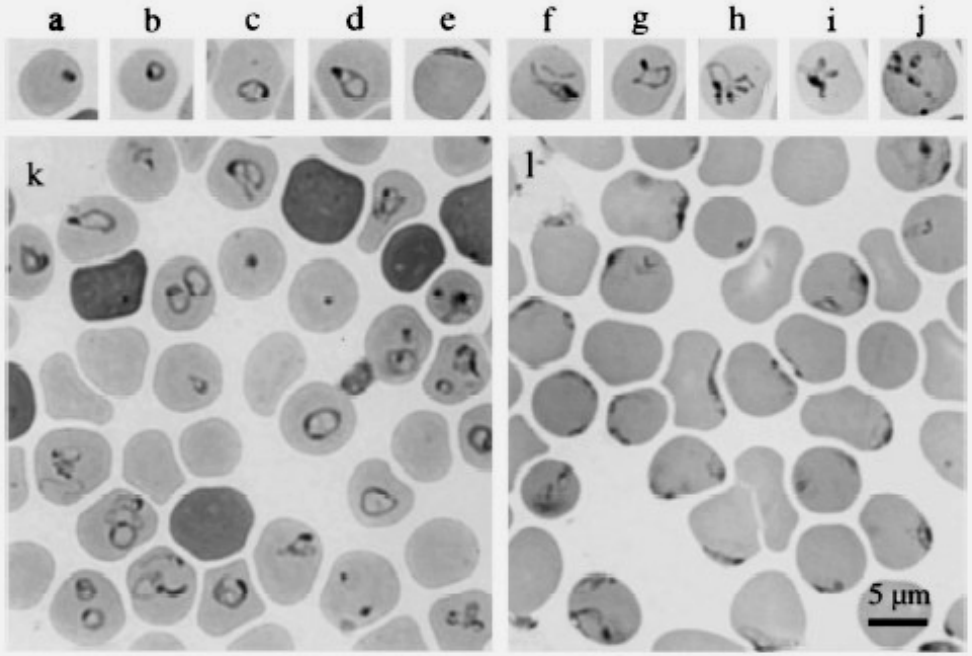

FIG. 5. Photomicrograph of Giemsa-stained thin-smear blood films showing various forms of piroplasms in hamster erythrocytes. (Upper Panels) a, dot form; b, ring form; c, ovoid form; d, pyriform; e, crescent arch form; f to i, ameboid forms; j, Maltese cross form. (Lower Panels) Selected microscopic views of strains Aw7 (k) and Ho234 (l), emphasizing the difference between Kobe- and Hobetsu-type parasites, respectively.

Babesia Like Ring Form Inclusions Found in RBCs of 33 Danish Chronically Ill Pilot Project Cases:

Bowen RTI (BRT) **10/33 = 30%**
Walter Tarello (WT) **11/15 = 73%**
Marie Kroun (MK) **15/31 = 48%**

Time used per microscopy 1000 X oil:
BRT 30-60 min, MK 160-300 min !

*) 1, 18 Found 2 times at Intervals !

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
BRT	+*	-	-	-	-	-	-	-	-	-	+	-	+	-	+	+	-
WT	+	-	+	+	+	+	+	-	-	+	+	/	/	/	/	/	/
MK	+*	-	-	+	+	/	-	-	-	/	+	-	+	+	+	+	-

	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
BRT	1*	-	-	-	-	-	+	-	-	-	+	-	-	+	-	+
WT	/	/	+	/	/	+	/	/	/	+	/	/	/	/	/	/
MK	1*	-	+	-	-	+	+	+	-	-	+	-	-	?	-	+

+ = found
– = not found
/ = not done

A Few Illustrative Danish Case Reports

- Case 1 & Case 18:
  illustrate the big risk of re-infection for people living in endemic areas –
  "*all the antibiotic you got last year did kill every bug in you* " – but previous treatment can't protect the patient for the rest of his/her life !
  Q: is it reactivated persistent infection or re-infection ?
- Case 4:
  illustrative change in symptoms during treatment for co-infections Borreliosis, Ehrlichiosis and Babesiosis ?
- Case 14:
  illustrating the difficulty in finding the parasites with microscopy even in BUFFY-COAT preparation !

Further information about all pilot project cases …

http://lymerick.net/York2003/York2003.ppt

A Japanese Donor Transmitted Case of Babesiosis

- **Recipient**: Haemolysis 1 month after blood transfusion, steroids ! – later diagnosed with Babesia by **positive blood microscopy** + **positive US PCR for Babesia microti**, seronegative, later positive serology for a local Babesia microti like variant, sero-reaction disappeared after successful treatment; **persistent parasites, need for 12 wk. re-treatment !**
- **Donor**: ASYMPTOMATIC; negative blood microscopy, **positive US PCR for Babesia microti** at time of diagnosis, negative PCR a year later ! – **insignificant serology for American Babesia microti**, **positive serology for the local variant** also found in mice and blood recipient.
  Even though both microscopy and PCR were negative a year later **INOCULATION of the donors blood into immune suppressed mice resulted in infection, like at time of initial diagnosis when PCR pos. !**

- **Lesson:** blood from a healthy feeling, microscopy and PCR negative donor can be parasitaemic below level of detection but infectious, when blood is given to susceptible individuals; PCR & microscopy may not catch all cases nor can fully prevent against donor transmission, even if donors are screened!

Danish Measures for Prevention of Donor Transmitted Parasitic Infections – Sufficient ?

A true short case description (#18) was send to several Danish blood banks per email, asking "*Given these findings, can I become a blood donor?*" showed that:

+ Most blood banks knew the risk of transmission of such parasites by blood transfusion and answered correctly that
  – ***"this patient should never give blood !"***
– The possibility of a given donor having sub-clinical TBI is, however, not routinely explored:
  NO questions asked about previous TBI, NO tests done !
  – "we rely on our donors being honest enough to tell us about their previous test results and symptoms"

Experience So Far With **Ring Formed Parasites**

- **Out ruling presence of ring form parasites in blood is very difficult / impossible even on BUFFY-COAT smears.** Microscopy must be thorough, take hours and is very tedious / demanding.
- **Sub-clinical parasitism may persist long time before patient eventually become symptomatic (Krause) !** – immune suppression, high age !
- **Parasites may not be visible in blood smears initially**, especially when there are many 'GCS', **but may show up later in the disease course**, typically after Borrelia have been "gunned down" by antibiotic treatment ! **– these patients typically have a slower and lower than expected response to any usual Borrelia-treatment and may experience shift in symptoms to more 'Babesia-like'!**

Lack of or insufficient / slow clinical effect of conventional treatment for Borreliosis should always prompt for thorough investigations for other possible co-infections !

Treatment of Ring Forms in Denmark Is Difficult !
A Few Words About the ANTIMALARIALs Most Commonly Used:

- Combination therapy necessary to avoid development of resistance !
- A combo of Clindamycin and Quinine is available in DK and not very expensive, but the latter drug has serious side effects like decreased hearing and tinnitus that may prevent completion of treatment
- Lariam is available, but have serious psychic effects preventing use ..
- A new combo of Atovaquone suspension (for best absorbance) plus azithromycin is very tolerable and often effective, but atovaquone suspension is NOT available in Denmark, the drug was pulled from the market by the manufacturing company in circa 2001 (low sales?)
- Experimental Metronidazole plus Azithromycin, a “cheap” combo which also treat Borreliosis at the same time ! – has shown good clinical effect in some co-infected project patients that didn’t respond well to doxycycline alone, but unfortunately doesn’t help all … we need more treatment options !
- Persistence / Relapse after relevant beneficial treatment / Re-infection is possible as was seen in the Japanese donor case and Case 1 ! – does Babesia perhaps form hypnozoites like some Malaria?

Future Tasks:

1. Investigations
   – we need to learn much more about what we have to deal with of hitherto unrecognized infections in chronically ill people
2. Information
   – to doctors and patients on routes of transmission, modes of prevention, disease symptoms that need attention / testing, treatment options etc.
3. Routine Diagnostic Measures
   – need to be implemented, like doing BUFFY-COAT microscopy, PCR and serology for **local variants** of all possible tick borne infections; on all cases that do not get restored health after conventional short treatment for Borreliosis or who relapse after beneficial antibiotic treatment
4. All possible drug alternatives for treatment must be readily available in case of therapy failure / microbial resistance
5. Alternatives to antibiotic treatment ? …