

## A Preliminary Study on The Effect of a Concurrent Infection with *Eperythrozoon wenyonii* on The Development of *Theileria annulata* in Calves<sup>1</sup>

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### Abstract

This work investigates the effect of concurrent calf infection with *E. wenyonii* on *T. annulata* development. In group I, calves simultaneously were inoculated with both parasites, *E. wenyonii* parasitaemia appeared 7 days post-inoculation (PI), peaked (62 peryth./rbc) 21 days PI and disappeared 31 days PI. *T. annulata* parasitaemia appeared 28 days PI, peaked (87%) 35 days PI and disappeared 50 days PI. *T. annulata* parasitaemia in calves infected only with *T. annulata* (control) appeared 15 days PI and peaked (90%) 30-35 days PI when the calves died. In group II calves, inoculated with *E. wenyonii* first, parasitaemia appeared one week PI, peaked (65 eperyth./rbc) 21 days PI and disappeared 35 days PI. Parasitaemia of *T. annulata*, inoculated 2 weeks later, appeared 25 days PI, reached a low peak (27%) 45 days PI and disappeared 65 days PI. After splenectomy (AS) of groups I and II calves, *E. wenyonii* parasitaemia reappeared 15 days AS, peaked (52 eperyth./rbc) 29 days AS and disappeared 57 days AS. *T. annulata* parasitaemia reappeared 27 days AS, reached a low peak (42%) 47 days AS and disappeared 87 days AS. Group I and II calves survived AS. In group III, calves, inoculated with *E. wenyonii* 5 days after the appearance of *T. annulata* parasitaemia (15%), *T. annulata* developed as in the control group and all calves died before *E. wenyonii* development. Alteration of *T. annulata* development may be attributed to modulation of the host immune system and/or competition with *E. wenyonii* for a certain substrate.

Keywords: Concurrent infections, *Eperythrozoon wenyonii*, *Theileria annulata*, blood parasites, calves.

### Introduction

Concurrent infections with parasites exhibit different expressions in the infected animals. They may not interfere with one another as in the case of vole coinfecting with *Babesia microti* and *Hepatozoon* spp. (Young, 1970; Hussein, 1973) or mice coinfecting with *Plasmodium berghei* and *P. vinckei* (Voza *et al.*, 2005).

On the other hand, one infection may enhance the development and/or pathogenicity of the concurrent infection as in the case of mice coinfecting with *Haemobartonella muris* and *P. berghei* (Hsu and Geiman, 1952), with mouse hepatitis virus and either of *E. coccoides* (Kraft, 1982) or *Trypanosoma cruzi* (Verinaud *et al.* 1998), with *Schistosoma mansoni* and *Leishmania major* (La Flamme *et al.*, 2002), with *Nippostrongylus brasiliensis* and *Try. brucei* (Chiejina *et al.*, 2003) and with *P. chabaudi* and either of *Litomosoides*

*sigmodontis* (Graham *et al.*, 2005) or *Heligmosomoides polygyrus* (Su *et al.*, 2005). The pathogenicity was also exacerbated in the case of birds coinfecting with *Ascaridia galli* and *Pasteurella multocida* (Dahl *et al.*, 2002) and in the case of cattle coinfecting with *Anaplasma marginale* and *Mycoplasma wenyonii* (Hofmann-Lehmann *et al.* 2004).

Coinfection with two parasites may also result in one infection ameliorating the development and/or pathogenicity of the other. This phenomenon was expressed in the case of mice coinfecting with *E. coccoides* and each of *P. berghei* (Peters, 1965), *P. chabaudi* (Ott *et al.* 1967), *B. microti* or

<sup>1</sup>From research project No. R-10-084, King Abdulaziz City for Science and Technology (KACST), Saudi Arabia. The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or as reflecting the views of KACST.

*B. hyalomysci* (Hussein, 1976). A similar phenomenon has been observed in the case of mice coinfecting with mouse hepatitis virus and *Salmonella typhimurium* (Fallon *et al.*, 1991) and in mice coinfecting with *P. yoelii* and either of *P. berghei* (Voza *et al.*, 2005) or *Mycobacterium tuberculosis* (Page *et al.*, 2005).

*Theileria annulata* is widespread throughout tropical and subtropical areas in the world and causes one of the most important diseases of cattle, tropical theileriosis (Uilenberg, 1981). This highly fatal disease is transmitted by ticks of the genus *Hyalomma* (Soulsby, 1986). Also, parasites of the genus *Eperythrozoon* [considered recently as *Mycoplasma* (Neimark *et al.*, 2001; 2004; 2005)] cause serious diseases in many animals. *E. wenyonii* causes an acute fever in cattle followed by emaciation and sometimes jaundice; however it is usually nonfatal (Soulsby, 1986). Both *T. annulata* and *E. wenyonii* have been detected in cattle in different localities in Saudi Arabia (Hussein *et al.* 1991; Al-Khalifa *et al.* 2007).

As mentioned above, some members of the genus *Eperythrozoon* have been found to interfere with the development of several pathogens. The present work is a preliminary investigation of the effect of concurrent infection with *E. wenyonii* on the course of development of infection with *T. annulata* in calves.

## Materials and Methods

### Maintenance of calves

Newly borne Holstein Friesian-Herefordshire calves, obtained from dairy farms in Riyadh, were maintained in the laboratory on reconstituted milk that was gradually replaced by solid animal feed. The calves were maintained under strict tick-proof conditions. Each calf was placed in a separate metal cage kept in a concrete-floored room, with the cage floor being 50cm above the ground. Personnel entering the rooms had to step in a wide shallow tray containing an acaricide solution and placed in front of the door of each room. When required, the calves were splenectomized using standard surgical procedures.

### Preparation of *T. annulata* and *E. wenyonii* for infection

Blood smears from several cows at Al-Majma'a were stained with Leishman stain and examined for infection with parasites in the field using a field microscope. Blood was then collected from the jugular veins of cows with the highest parasitaemia of *T. annulata* or *E. wenyonii* in

vacutainers coated with silicon and EDTA and kept over ice in an icebox. Immediately on arrival to the laboratory, splenectomized calves were inoculated via the jugular vein with 5ml of blood infected with either of the parasites. Blood smears, collected daily from the infected calves, were examined microscopically until parasitaemia of each of the infections reached its peak. Blood from these infected calves was used to infect naïve calves, as described below.

### Experimental design

Naïve calves were inoculated via the jugular vein with 5ml of blood infected with *T. annulata* (parasitaemia of 90%) and/or *E. wenyonii* (parasitaemia of 65 eperythrozoa/ rbc) as described below. Blood smears from each of the infected calves were daily examined microscopically to monitor the parasitaemia of each parasite.

Four groups, each of two calves, were inoculated with the parasites according to the regimen suggested by Hussein (1976). These were a) group I calves inoculated simultaneously with *E. wenyonii* and *T. annulata*, b) group II calves inoculated with *E. wenyonii* first and then with *T. annulata* two weeks after *E. wenyonii* had disappeared from their blood, c) group III calves inoculated with *T. annulata* and then five days after the development of detectable parasitaemia (ca 15%) they were inoculated with *E. wenyonii*, and d) group IV calves inoculated with *T. annulata* only to act as a control group.

Following recovery of groups I and II calves, they were splenectomized and the parasitaemia in each calf was microscopically monitored daily. All experiments were carried out in accordance with King Saud University Ethical Committee Acts.

## Results

### Simultaneous inoculation with both parasites

In group I calves, *E. wenyonii* parasitaemia appeared first on day 7 post-inoculation (PI) (Fig. 1). It then rose gradually reaching a peak of 62 eperythrozoa/rbc by day 21 PI, declined rapidly and disappeared by day 31 PI. On the other hand, *T. annulata* parasitaemia appeared 28 days PI, rose rapidly reaching a peak of 87% on day 35 PI, declined gradually and disappeared by day 50 PI. In the control group IV, *T. annulata* parasitaemia appeared on day 15 PI and rose to a peak of 90% 30 to 35 days PI when the calves died (Fig. 1).

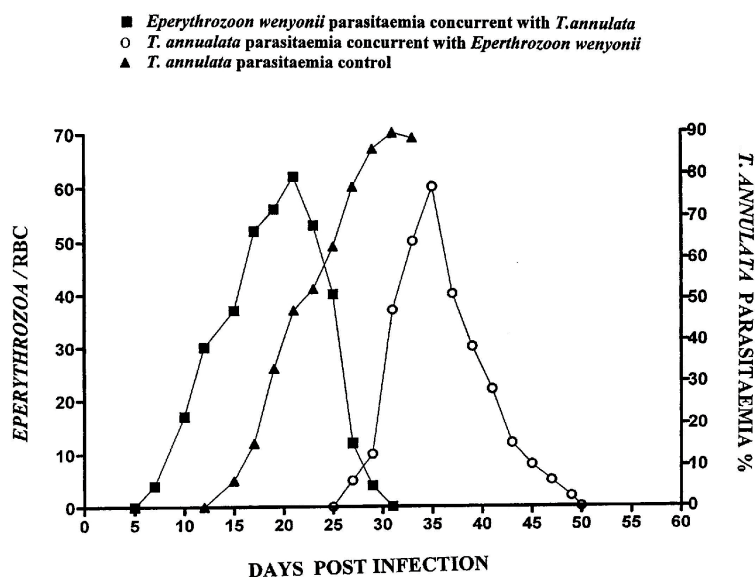


Fig 1. Concurrent *Eperythrozoon wenyonii* and *Theileria annulata* infections in calves.

Splenectomy of group I calves following the disappearance of the relatively mild *T. annulata* infection resulted in recrudescence of *E. wenyonii* parasitaemia 15 days after splenectomy (AS) (Fig. 2). *E. wenyonii* parasitaemia then rose to a peak of 52 eperythrozoa/rbc by day 29 AS, declined afterwards and disappeared by day 57 AS (Fig 2). On the other hand, *T. annulata* parasitaemia appeared on day 27 AS, rose gradually reaching a relatively low peak of 42% by day 47 AS, gradually declined afterwards and disappeared by day 87 AS (Fig 2).

#### Infection with *T. annulata* after *E. wenyonii* had disappeared from the blood

Group II calves developed *E. wenyonii* parasitaemia that followed a course similar to that of group I (Fig. 3). The parasitaemia appeared one week PI, reached a peak of 65 eperythrozoa/rbc by day 21 PI, declined and completely subsided by day 35 PI. When *T. annulata* was inoculated into these calves two weeks later (on day 50 PI with *E. wenyonii*), the parasitaemia started to appear on day 25 PI (75 days PI with *E. wenyonii*), rose gradually reaching a low peak of 27% by day 45 PI (95 days PI with *E. wenyonii*), declined afterwards and disappeared by day 65 PI (115 days PI with *E. wenyonii*) (Fig. 3). When these calves were later splenectomized, *E. wenyonii* appeared first followed by *T. annulata* and the parasitaemia of each

of them followed a course similar to that occurring in group I calves AS (Fig. 2).

*T. annulata* infections in calves either simultaneously injected with *E. wenyonii* (group I) or after *E. wenyonii* infection has subsided (group II) was quite mild and all calves survived the infection AS.

#### Infection with *E. wenyonii* after *T. annulata* parasitaemia

In group III calves, inoculated with *E. wenyonii* 5 days after the appearance of *T. annulata* parasitaemia (at ca 15% parasitaemia), *T. annulata* developed normally as in the control group and was not affected by the subsequent *E. wenyonii* infection. All calves died before *E. wenyonii* was able to develop.

## Discussion

The present work investigates the phenomenon of alteration of the course of parasitaemia development by a concurrent infection of *E. wenyonii* on *T. annulata* in calves according to the model formulated by Hussein (1976). The results obtained are different from those obtained by Hussein (1976) in mice using each of *B. microti* and *B. hylomyisci* with *E. coccoides*. *T. annulata*, is as fast multiplying as the highly pathogenic *B. hylomyisci* (Hussein, 1976, 1990). However, it has not suppressed the development of *E. wenyonii* when both are simultaneously

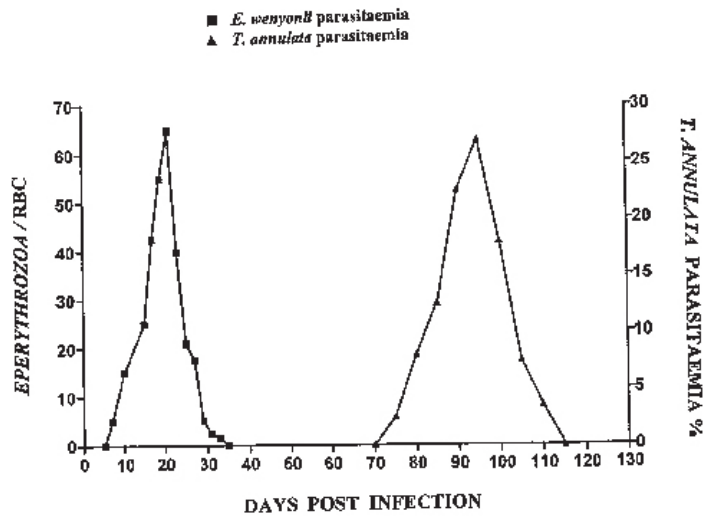


Fig 2. *Theileria annulata* infections in calves after recovery from *Eperythrozoon wenyonii* infections.

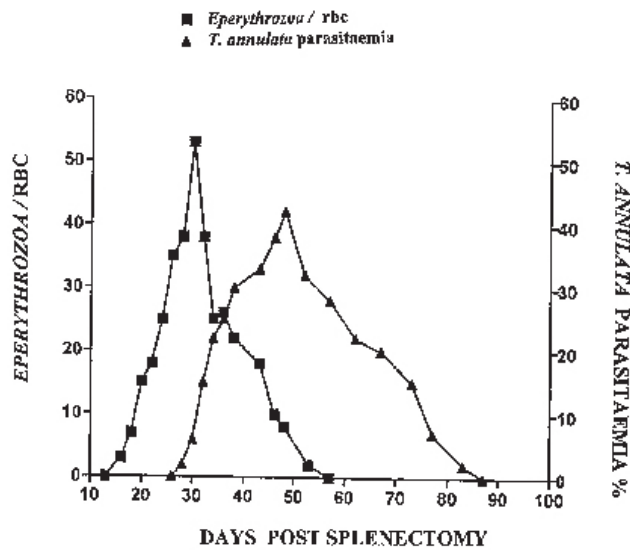


Fig 3. Recrudescence of *Eperythrozoon wenyonii* and *Theileria annulata* infections in calves after splenectomy.

injected into calves as did *B. hylomysci* to *E. coccoides* in mice. On the contrary, development of *T. annulata* has been hampered by that of *E. wenyonii*. It has been proposed by Peters (1965) and Hussein (1976) that both parasites in a concurrent infection may compete for the same complex substrate. Hussein (1976) has suggested that *B. hylomysci*,

which develops faster than *E. coccoides*, uses much of that substrate before the latter has reached its growth phase and thus, its development is suppressed by its faster counterpart. If this hypothesis of competition for a specific substrate is applied in the case of calf infection with *T. annulata* and *E. wenyonii*, then it may be assumed that when *T. annulata*

is inoculated first, it develops normally presumably using considerable quantity of that substrate and thus causing complete suppression of *E. wenyonii*. On the other hand, in case of simultaneous inoculation with *T. annulata* and *E. wenyonii*, it seems that neither of the two is able to use much of that substrate before the other can reach its growth phase. Thus, complete suppression of the development of either parasite by the other was not observed. On the other hand, *T. annulata* commencing its development after that of *E. wenyonii* might have not found enough of that substrate to develop excessively as it normally does. However, its development has been greatly modified by *E. wenyonii* to the benefit of the host that has survived the infection. The same thing seems to happen when these calves are splenectomized. In that case, neither parasite has used up large enough quantities of the supposed substrate in order to inhibit totally the development of the other as has been seen in combinations of certain *Babesia* and *Eperythrozoon* species (Hoyte, 1961; Barnett, 1963; Peters, 1965; Hussein, 1976). The biochemical characterization of the supposed substrate requires investigation. Such characterization, if carried out, is going to be of importance in the formulation of new chemotherapeutic agents against these dangerous parasites of domestic ungulates.

On the other hand, the results of more recent investigations have construed that the altered course of parasitic infections in the case of concurrent infections in mice has been due to mechanisms involved in modulation of the host immune response. Such modulations were exhibited by *Try. brucei* which prolonged the infection with *N. brasiliensis* (Chiejina *et al.*, 2003), by *S. mansoni* which delayed *L. major* lesion resolution (La Flamme *et al.*, 2002), by mouse hepatitis virus type 3 which increased the pathogenicity of *Try. cruzi* owing to depletion of thymocytes (Verinaud *et al.*, 1998), by *Helig. polygyrus* which impaired the development of protective antimalarial immunity (Su *et al.*, 2005) and by *Lit. sigmodontis* which altered gamma interferon responsiveness to *P. chabaudi chabaudi* (Graham *et al.*, 2005). However, modulation of the immune response was for the benefit of the host in other coinfections. Using splenic microarray analysis, *Mycobac. tuberculosis* was reported to induce potentiation of type 1 immune responses in mice (strain C57BL/6), which resulted in protection against a concurrent lethal *P. yoelii* infection (Page *et al.*, 2005). Splenocytes from mice coinfecting with both parasites were found to produce higher levels of gamma

interferon and tumor necrosis factor alpha than splenocytes from mice infected with either parasite. In the present study, the course of infection with concurrent *T. annulata* and *E. wenyonii* seemed to be similar to that in the case of mice coinfection with *Mycobac. tuberculosis* and *P. yoelii*. The mild relapse of parasitaemia of both *T. annulata* and *E. wenyonii* following splenectomy of the infected calves might be attributed to competition of the two parasites for the supposed substrate.

The results of the present study provide preliminary basic data on concurrent infection with *E. wenyonii* and *T. annulata* in their natural ungulate host. These data are important for further detailed research on this phenomenon since the use of experimental animals may provide different results with different strains; Page *et al.* (2005) have reported that the immune system of different mice strains respond differently to coinfection with *Mycobact. tuberculosis* and *P. yoelii*. Elucidation of the mechanism controlling this phenomenon may contribute to the use of new trends in the treatment and/or control of these blood parasites.

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## دراسة مبدئية علي أثر إبريثروزون وينيوني علي تطور ثايليريا أنيولاتا عند الإصابة شتركة يهما في العجول

محمد الخليفة وجلييلة خليل و حسين حسين و فتحي مسلم

قسم علم الحيوان - كلية العلوم - ص.ب. ٢٤٥٥ - جامعة الملك سعود - الرياض ١١٤٥١ - المملكة العربية السعودية

### المخلص

تمت الدراسة علي أربع مجموعات من العجول. في المجموعة الأولى تم حقن *Theileria annulata* و *Eperythrozoon wenyonii* معا في نفس الوقت فبدأ ظهور الطفيلي الأول في الدم بعد سبعة أيام من الحقن وبلغ عدده حدا أقصى (٦٢ طفيلي لكل خلية دموية حمراء) بعد ٢١ يوما، ثم تراجع العدد حتى اختفى الطفيلي من الدم بعد ٣١ يوما من الحقن. أما إصابة خلايا الدم الحمراء بـ *Thaileiria* فقد ظهرت بعد ٢٨ يوما من الحقن وبلغ عدد الطفيلي أقصاه (٨٧٪) بعد ٣٥ يوما، ثم تراجع العدد حتى اختفى الطفيلي بعد ٥٠ يوما من الحقن. أما في المجموعة الضابطة التي تم حقنها بـ *Thaileiria* فقط فقد بدأ ظهور الطفيلي في الدم بعد ١٥ يوما من الحقن ثم ارتفع عدده حتى بلغ حدا أقصى (٩٠٪) بعد ٣٠-٣٥ يوما من الحقن، و عندها نفقت العجول. وفي المجموعة الثانية تم حقن العجول أولا بـ *Eperythrozoon* حيث ظهرت إصابة كرات الدم بعد أسبوع من الحقن وازداد عدد الطفيلي بالغا حدا أقصى (٥٦ طفيلي لكل خلية حمراء) بعد ٢١ يوما من الحقن، ثم تراجع العدد حتى اختفى الطفيلي بعد ٣٥ يوما من الحقن. وبعد أسبوعين من اختفاء الإصابة تم حقن العجول بـ *Thaileiria*، و ظهرت إصابة الخلايا الحمراء بالطفيلي بعد ٢٥ يوما، ثم ارتفع عدد الطفيلي قليلا بالغا حدا أقصى منخفضا (٢٧٪) بعد ٤٥ يوما من الحقن، ثم تراجع العدد حتى اختفى الطفيلي تماما بعد ٦٥ يوما من الحقن. وبعد اختفاء الطفيليين تماما تم استئصال الطحال من عجول المجموعتين الأولى والثانية فعاد إبريثروزون إلى الظهور بعد ١٥ يوما من الاستئصال، وازداد عدده بالغا حدا أقصى (٥٢ طفيلي لكل خلية حمراء) بعد ٢٩ يوما ثم تراجع العدد حتى اختفى الطفيلي تماما من الدم بعد ٥٧ يوما من الاستئصال. أما طفيلي *Thaileiria* فقد ظهر في الدم بعد ٢٧ يوما من الاستئصال وارتفع عدده بالغا حدا أقصى منخفضا نسبيا (٤٢٪) بعد ٤٧ يوما، ثم تناقص حتى اختفى تماما بعد ٨٧ يوما من الاستئصال. أما عجول المجموعة الثالثة فقد تم حقنها بالطفيلي *Eperythrozoon* بعد خمسة أيام من ظهور إصابة خلايا الدم بـ *Thaileiria* (١٥٪). وفي هذه العجول كان تطور الإصابة بـ *Thaileiria* مماثلا لذلك في المجموعة الضابطة، ونفقت العجول قبل أن تظهر أي إصابة بـ *Eperythrozoon* في الدم. و ربما يعزى التغير في نهج تطور الإصابة بـ *Thaileiria* في حالة الإصابة المشتركة مع إبريثروزون إلى تغير في استجابة جهاز العائل المناعي أو إلى تنافس *Thaileiria* مع إبريثروزون على مكون واحد في الدم، أو إلى كلا السببين معا.