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Prevalence of *Eperythrozoon* spp. infection and congenital eperythrozoonosis in humans in Inner Mongolia, China

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SUMMARY

Eperythrozoon is an obligate parasitic bacteria found in many species of animals. A large scale investigation of the prevalence of *Eperythrozoon* spp. in humans, was conducted in a developing country using light, electron microscope and animal inoculation. Samples were collected in undeveloped areas of Inner Mongolia in China over a 2-year period of 1994–6. Of the 1529 investigated samples, 35·3% were found to be *Eperythrozoon* spp. positive. The prevalence of infection was associated with occupation and seasonal variations. The infections were mainly mild, in 89·6% of cases (excluding pregnant women and their children). Of 74 pregnant women tested in the areas of high prevalence, 44 were confirmed *Eperythrozoon* spp. positive. Similarly, eperythrozoa were found in all 44 umbilical cords tested and in the neonatal peripheral blood samples taken at birth. These data suggest that eperythrozoa can be transmitted via the placenta.

INTRODUCTION

Eperythrozoon is a genus of blood-born epicellular parasitic organism found in different species of vertebrate, including rodents, ruminants, and pigs [1–4]. Eperythrozoa are found attached to erythrocytes and may be observed free in the plasma. In Wright's or Giemsa stained blood films, the organisms are identified as pinkish-violet or blue coccoid- or ring-shaped basophilic organisms respectively. Under scanning electron microscopy, bacteria attached to membrane surfaces appear initially to cause no membrane deformation, but eventually result in significant deformation leading to anaemia [3, 4]. The first description of the human disease caused by *Eperythrozoon* was reported by V. Puntaric and colleagues in Yugoslavia in 1986 [5]. Since 1991, the acute clinical disease has been seen sporadically in China [6, 7].

Inner Mongolia, a half pastoral and half agricultural province in China, is home to many kinds of livestock and wild animals. Due to poor sanitation and insufficient medical care, *E.* spp. infection in humans has been reported in some areas. In this large scale survey, we would like to clarify *E.* spp. epidemiology in different seasons, and in population groups of different occupations and ages.

Although the exact mode of transmission is unknown, close contact with infected individuals or insects bites, particularly lice, fleas, mosquitoes, midges and stable flies, are thought to be the principal sources of transmission between animals, or from animals to humans [1, 7–12]. When acquired by immuno-competent adults, eperythrozoonosis in humans is usually asymptomatic [13], although it may

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cause anaemia, haemolytic jaundice, fever, lymphadenopathy [5–7] and in severe cases, haemoglobinuria [7]. Also a member of the family Anaplasmataceae [14], rickettsia is very easily transmitted from mothers to 86% offsprings via the placenta and have been shown to cause congenital disease in animal experimental models [15]. However, published literature about *Eperythrozoon* does not record the possibility of transplacental infection in humans or other animals. Confirmation of *E.* spp. infection in pregnant women and their newborn babies will not only identify the possibility of transplacental transmission in humans, but will also help clinicians in the management of suspected cases.

MATERIALS AND METHODS

Large scale investigation samples

From July 1994 to June 1996, 1529 peripheral blood or bone marrow samples were collected from volunteers throughout Inner Mongolia in different seasons. The age range was 1–70 years old. A total of 961 blood samples during winter–spring and 568 during summer–autumn seasons were collected: 634 of the collected samples were male and 895 female. The number of samples from livestock farmers and veterinarians was 257, from agriculture farmers was 855, and from nursery children, full-time students, teachers and other white-collar workers was 417.

Pregnant woman and neonatal samples

During the 2-year period, peripheral blood samples from pregnant woman and neonate were collected from several hospitals in the pastoral area. All pregnant women were livestock farmers or veterinarians. As a hospital routine test and with consent of the mothers, 50–100 μl of peripheral blood was taken immediately after birth for blood smear tests. At the same time, one ml blood sample from the umbilical cord and one ml from the mother were taken for investigation by electron microscopy and for animal inoculation. Great care was taken to avoid the contamination of umbilical cord blood with mother's blood. A total of 74 blood samples each were obtained from mothers and umbilical cords.

Light microscopy investigation

Blood smears obtained from representative people during the 2-year period, were stained with Wright–Giemsa Stain (Sigma, Sino-American Biotechnology Company, Beijing, China).

Scanning electron microscopy studies

The electron microscopy investigation procedures were conducted following Zachary's and Basgall's methods [16].

Blood samples were diluted 1:50 in phosphate buffered saline (pH 7·4) and applied dropwise to 13-mm polycarbonate membranes (Sino-American Biotechnology Company, Beijing, China) in a support apparatus. The samples were then fixed in 2% phosphate buffered glutaraldehyde (Beijing Chemical Industry Company, Beijing, China), rinsed in phosphate buffered saline (pH 7·4), post-fixed in 1% osmium tetroxide (Sigma, Sino-American Biotechnology Company, Beijing, China) and dehydrated. Prior to examination, the samples were dried, mounted on aluminium stubs and coated with gold palladium (Sino-American Biotechnology Company, Beijing, China).

Definitions of degree of infection

There is no internationally accepted clinical case definition of eperythrozoonosis in adults or of congenital infection. In this study, one or more erythrocytes (RBC) infected by *E.* spp. in every 100 RBC tested were recorded as a positive result. The infection degree was recorded according to Gulland's method for animals [17]; less than 30 erythrocytes infected in every 100 RBC was classed as mild, between 30 and 60 termed moderate and more than 60 recorded as a severe infection degree. Lesser numbers of echinocytes and erythrocytes with other abnormal shapes (artifacts) were also observed in some blood samples.

Animal inoculation

Normal and healthy inbred mice (L615 mouse, Institute of Haematology, Tianjing, China) were inoculated intramuscularly with 0·1 ml fresh anticoagulated blood. The mice were bled at the time of inoculation and 1 week later at the peak of bacteraemia. In samples with less than one erythrocyte infected in every 100, if no eperythrozoa were identified in the inoculated animals at one week, a negative (non-infected) result was recorded.

RESULTS

Prevalence of *E.* spp. in humans

Morphology of Eperythrozoon *in humans*

Examination of peripheral blood smears revealed variably-sized coccoid, discoid, and ring parasitic forms attached to cell membranes singularly or in clusters centred around a single membrane focus. Some erythrocytes were not infected, whilst others were severely invaded. The delicate fibrils of the organism could be seen even under a light microscope; the organisms floating free in plasma were bigger and stained darker (Fig. 1a).

As described in the Introduction, erythrocytes were infected by one or several small epicellular coccoid forms that measured between 0·2 and 0·5 μm in diameter. The bacteria attached to the RBC membrane surface or invaded the RBC, which caused different levels of RBC deformation (Fig. 1b).

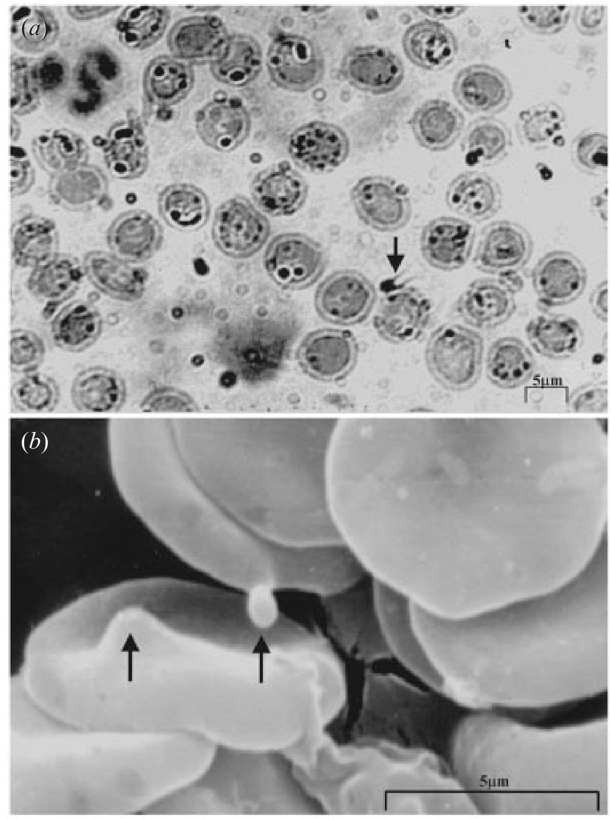

Fig. 1. Morphology of *Eperytherozoon* in humans. A newly infected samples with *E.* spp. the blood smear was stained with Wright–Giemsa Stain. A large quantity of variably-sized organisms attach to or invade erythrocytes. The arrow shows the delicate fibrils of the organism floating free in the plasma (**a**, light microscope, ×1600). The arrows show that the bacteria attach to RBC membrane surface and cause deformation (**b**, scanning electron microscope, ×5600).

E. *spp. infection in humans during different seasons*

The incidence of infections during different seasons was recorded and the results are shown in Table 1 (excluding pregnant women and neonates). During winter and spring, the level of infection was 24·6%; and during summer and autumn, this rose to 53·5%. The variation in these results was statistically significant, $P < 0{\cdot}01$.

Table 1. *The number of people with* E. *spp. infection during different seasons*

Test period	Negative	Positive	Total	% of positive
Winter–spring	725	236	961	24·6
Summer–autumn	264	304	568	53·5
Total	989	540	1529	35·3

$\chi^2 = 122{\cdot}30$, $P < 0{\cdot}01$.

E. *spp. infection in different occupation groups*

The volunteers were divided into three groups. Group one had close contact with livestock or wild animals, such as pig, cattle, chicken and sheep farmers and veterinarians. Group two had occasional contact with animals, such as agriculture farmers in villages. Group three had no significant contact with the animals, including nursery children, full-time students, teachers, nurses, doctors and other white collar workers in towns. Table 2 shows that there is a statistically significant high level of infection in the first group, $P < 0{\cdot}01$ (excluding the samples from pregnant women and neonates).

Congenital infection of *E.* spp.

Of the 74 blood samples taken from pregnant women, 44 showed evidence of *E.* spp. infection. The infection (59·0%) was close to that of the average infection rate (55·3%) in the pastoral area. There was evidence of vertical transmission to the foetus during the mother illness, confirmed by the presence of *E.* spp. in the 44 neonatal peripheral blood smears (Fig. 2), and in the corresponding umbilical blood samples used for animal inoculation. The vertical transmission was 100%.

Of the 44 mothers, 8 of whom had severe infection, all showed a fever, lympadenopathy and haemolytic

Table 2. E. *spp. infection in different occupation groups*

Groups	Negative	Positive	Total	% of positive
Group one	115	142	257	55·3
Group two	527	328	855	38·4
Group three	347	70	417	16·8
Total	989	540	1529	35·3

$\chi^2 = 64{\cdot}06$, $P < 0{\cdot}01$.

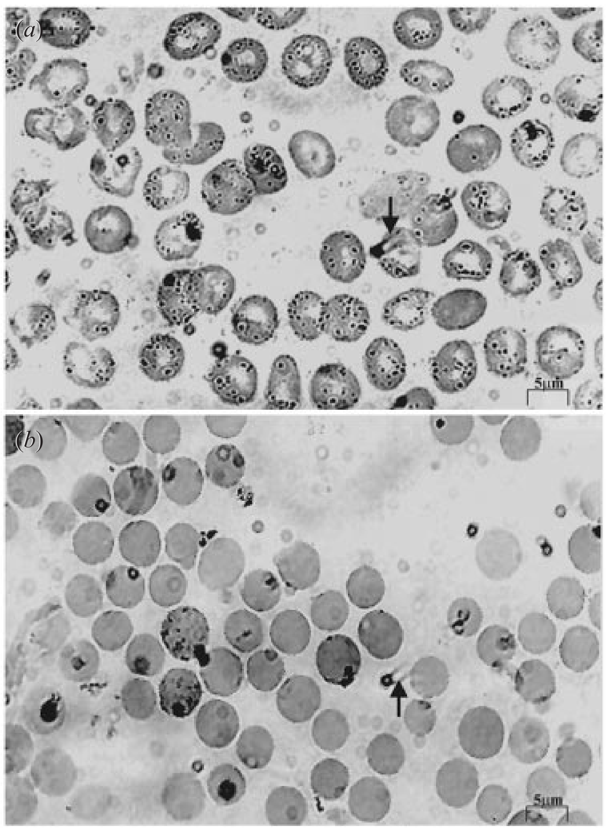

Fig. 2. Blood smear films of infected mother and neonate. Mother's blood smear (**a**) shows a large amount of *E.* spp. infected RBC. Compared with the neonatal (**b**), each RBC in mother is infected with more bacteria, and the size of the organisms looks smaller. Arrows show the fibrils of *E.* spp. floating in plasma (light microscope, ×1600). (Peripheral blood and bone marrow smears indicated that the woman was severely infected by the bacteria at her fifth month pregnancy. As she worried about the side effects of medication, she refused medicine during the pregnancy.)

jaundice. Similarly, 8 of the 44 neonates also had severe infection, all showing clinical evidence of hyper-haemolytic jaundice. Of the 16 moderate infected neonates, 2 had severe jaundice, but their mothers looked healthy. As the jaundice was severe and persistent in the newborn babies, it was suspected that the jaundice might be caused by *E.* spp. infection. Tetracycline is the recommended drug for eperythrozoonosis, but it could not be used on the infected children and their mothers due to its side effects on child bone and teeth development. Instead erythromycin was administered for both mothers and their children for 3 weeks. The treatment resulted in a vast improvement of jaundice in the children and the disappearance of the *E.* spp. from peripheral blood samples as seen under light microscope.

Table 3. E. *spp. infection degree in different age groups*

Groups (years)	Mild	Moderate	Severe	% of severe infection
0	20	16	8	18·2*
1–7	28	13	2	4·7
> 7–70	483	47	11	2·0
Total	531	76	21	3·3

* $P < 0{\cdot}01$.

E. spp. infection degree in different age groups

Of the 1529 people plus 74 pregnant women and 74 neonates, 628 cases were infected by *E.* spp. The positive cases were divided into three age groups arbitrarily. The neonates were recorded as 0 year group; 1–7 year old children in nursery, were marked as 1–7 year group; children above 7 years who had already started school, were put in the category of 7–70 year group. According to the number of RBC invaded by *E.* spp., the infection degree was recorded as mild, moderate and severe. There were 21 with severe, 76 with moderate and 531 with mild infection. Table 3 shows details of the relationship between age and infection degree. Of the neonate group 18·2% had severe infection, compared with 4·7% in the 1–7 year group, and 2·0% in the 7–70 year group, the difference is satistically significant ($P < 0{\cdot}01$).

DISCUSSION

There were two striking findings from this survey of clinical and laboratory reports of eperythrozoonosis in humans.

Firstly, the number of eperythrozoonosis in humans was originally thought to be very rare [6–8], but the actual prevalence was considerably higher than expected. In this survey of 1529 subjects, including 74 pregnant women and 74 neonates, total of 1677 samples were analysed, 628 were found to be *E.* spp. positive; an overall infection rate of 37·5%. There were 11 adult cases, 2 in 1–7 year old children and 8 in neonates with severe infection. This corresponds to 3·3% of the total infected population and 1·2% of the total population of the area. All severely infected children and the majority of adults were clinically symptomatic, whereas the mild cases were asymptomatic; only 2 neonates and 1 adult with moderate infection showed evidence of haemolysis. This finding also strengthens the previous finding [6] that most *E.* spp. infections are mild cases, and the clinical symptoms are fundamentally related to the infection degree.

The prevalence of infection with *E.* spp. in humans was obviously higher during summer-autumn than during winter-spring. A possible explanation is that the distribution and number of insects, which may act as a vector for disease transmission, was much greater during warm and wet summer-autumn seasons. Due to freezing cold, dry winter-spring weather in this province, the insect population would be markedly reduced, and the infection rate would consequently decrease. Although the prevalence of *E.* spp. dropped during the cold season, there was still, however, 24·6% *E.* spp. positive. The persistence of infection in individuals may explain the repeated annual outbreaks of this disease combined with the continuous contact with infected animals producing parasitic carriers.

The infection rate of *E.* spp. varied in the different occupation groups. The highest infection (55·3%) was found in the farmers of livestock, veterinarians and the people living in the pastoral zone. This may be because these people have much more direct or indirect contact with infected animals.

The transmission of bacteria between animals or from animals to humans can occur via insect bites or by direct contact with infected individuals [1, 8, 9, 18, 19]. However, published papers and textbooks do not mention *E.* spp. transplacental transmission.

The second principal finding of this survey was the possibility of congenital *E.* spp. infection via placenta. We found 44 samples infected with *E.* spp. bacteria in 74 pregnant women, and all of the 44 neonates and umbilical cords tested were *E.* spp. positive. Transplacental infection was found in 100% of foetuses in humans, which was much higher than other congenital infectious diseases, such as hepatitis B virus, rickettsia and toxoplasma etc. [5, 20, 21]. This suggests that *E.* spp. can be transplacentally transmitted in humans.

There is a closely causative relationship between mother and child infection degree and clinical manifestations. Of the 44 infected mothers, 8 had severe infection, and all showed clear clinical symptoms. This percentage (18·2%) is much higher than the average adult group (3·3%). Similarly, 8 of the 44 neonates with severe infection had severe haemolytic jaundice; even 2 neonates with only moderate congential infection still showed clinical symptoms. We believe that pregnant women and foetuses are highly vulnerable to the bacteria.

More than 100000 female tourists from western countries visit Inner Mongolia China every summer in recent years. Assuming the average annual 2000 pregnancies during the study period (1994–6), 1180 would have been expected to be infected each year. During pregnancy, 18·2% of infected women would be classed as severe infectious cases, which means at least 200 severely infected neonates would have occurred. Although severely haemolytic anaemia due to *E.* spp. infection is not often reported in adults, it is worthwhile to note that neonate hyperhaemolytic jaundice, and pregnant woman haemolytic anaemia, may occur as a result of *E.* spp. infection in the high prevalent areas.

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