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PREVALENCE AND CLINICAL CHARACTERISTICS OF EHRLICHIA CANIS INFECTION IN DOGS IN THUA THIEN HUE

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Abstract. The survey was carried out at OKADA PET Veterinary Center, Hue City, with 935 dogs of different ages and breeds. The results show that 39.6% of dogs have the clinical signs of *Ehrlichia canis* (*E. canis*) infection, 95.7% of which were serologically positive for *E. canis* antibody. The results also indicate that 54.9% of dogs have *E. canis* morulae in monocytes and/or neutrophils. Statistical analysis reveals that the prevalence of *E. canis* infection in dogs is not affected by breed, gender or age. The clinical symptoms of infected dogs are very complex, including fever, abortion, joint pain, breast tumours, short breathing, nasal haemorrhage, weakness, pale mucosa, skin inflammation, hair loss around the eyes, eye discharges, cloudy eyes, refuging to eat, diarrhoea, belly skin haemorrhage, anorexia, constipation, ascites, vomiting, depression, salivation, and metritis. About 37.9% of dogs are serologically infected with *Ehrlichia canis* with various symptoms.

Keywords: dogs, *Ehrlichia canis* infection, Okada Pet, morulae

1 Introduction

Erhlichia canis (*E. canis*) is an obligatory intracellular rickettsia of the anaplasmataceae family. This pathogen causes an infectious disease in dogs and can be transmitted from dogs to dogs by the tick *Rhipicephalus sanguineus*. The disease caused by *E. canis* is called Canine Ehrlichiosis. The first *E. canis* pathogen strain was discovered by Donetein in Algeria in 1935. However, it became a severe disease when military dogs returning from Vietnam during the 1970s were infected. It was, therefore, called "tracker dog disease" and "tropical canine pancytopenia". Then, the pathogen was detected in different parts of the world, such as the United States, South American countries, Africa, and Asia [16]. Nowadays, the disease receives increasing attention because of its worldwide occurrence, prevalence throughout the year, morbidity, and mortality. Furthermore, this disease attracted scientists from different parts of the globe, such as Brazil [1, 12, 13], Malaysia [11], and India [14], because of its complex dissemination.

In Vietnam, *E. canis* in dogs has not been widely and regularly studied since Nguyen et al. [9] reported a prevalence of *E. canis* on dog ticks collected in northern Vietnam. According to Tran et al. [3], the rate of positive tests for *E. canis* with rapid test at the Veterinary Clinic, Can Tho University, was 43.02%. Currently, in Thua Thien Hue, we have noticed the appearance of *E. canis*

pathogen with increasing frequency and complex developments that can seriously threaten dogs' and possibly human health. However, to our best knowledge, no studies on *E. canis* have been published in Thua Thien Hue and the central region of Vietnam. From that fact, this study was conducted to clarify the prevalence of *E. canis* infection and clinical symptoms of infected dogs and analyze the factors affecting *E. canis* infection in dogs.

2 Material and methods

2.1 Sample collection

The samples (peripheral blood) and animal patients (dogs) were collected at OKADA PET Veterinary Center, Hue City, from January 2020 to September 2021. The dogs suspected of *E. canis* infection were prescribed for blood collection [8]. The blood taken from peripheral veins was used to perform further *E. canis* rapid antibody detection tests and blood smear checking to find the presence of intracellular *E. canis* morulae within 10–30 min after the collection.

2.2 Blood smear and scanning

Blood smear and scanning were performed by veterinary doctors at OKADA PET Veterinary Center in Hue City. Blood from the dog's peripheral vein was collected and packed into a tube with an anticoagulant to perform the haemogram, serological antibody test, and blood smear. For blood smear preparation, the samples were taken into the hematocrit capillary tubes. One end of each tube was sealed with clay. The tubes were then centrifuged at 12,000 rpm for 30 min. The blood inside the tubes was separated into different layers after centrifugation. A white layer (buffy coat) in the middle was collected and dropped on a glass slide to smear the blood. The drop, mainly containing white blood cells, was thinly arrayed with another glass slide. The blood film was left to dry at ambient temperature and incorporated into a Quick Panoptic staining solution (Quick Panoptic kit, Quimica Clinica Aplicada S. A. UN 1230; www.qca.es). The procedure is as follows: First, dip the blood film into an absolute alcohol solution for 5 s, then wash with water. Next, dip the blood film into solution A (xanthene buffered aqueous solution of) for 5 s and rinse with water. After that, dip the blood film into solution B (buffered aqueous solution of dyes derived from thiazine) for 5 s, followed by washing with water. The blood film was then dried up. A drop of microscope immersion oil was dripped on top of the blood film, and then a lamen (coverslip) was covered on top, then examined under a microscope at an objective lens of 40× and 100× magnification. Upon examining with a 100× objective lens, also known as oil objective lens, a drop of microscope immersion oil was dripped onto the top of the lamen for observing at best vision. The light levels are customized so that the viewer can see the cells. The blood film was scanned with a zigzag motion to ensure all areas of the blood film were

examined. Each blood sample was made of three films. The screening lasted for about 10 to 30 min per blood film. The images were captured with a digital camera mounted directly on the Olympus CX21 microscope and saved on a computer by using Amscope software. On the blood film, red blood cells are light- or dark-pink; platelets are pale or purple; neutrophils have dark-green nuclei. Meanwhile, the cytoplasm is pink with red-purple granules, and eosinophils have green nuclei and blue or red-orange cytoplasm. The basophils have dark-green nuclei with purple and black kernels; lymphocytes have purple nuclei and blue cytoplasm; monocytes have pale purple nuclei and blue cytoplasm. *E. canis* bacteria aggregated into mulberry-like clumps (morulae) are stained purple. The *E. canis* morulae are often found near the nucleus of white blood cells.

2.3 Rapid serological test

In this study, the rapid *Ehrlichia canis* antibody detection kits (*E. canis* AB test-Gen action, Green Age company) were used to determine *E. canis* infection by identifying the presence of antibodies against *E. canis* in the dog's blood. The kit achieved 98% sensitivity and specificity according to the manufacturer's instructions, and the testing procedure was performed as indicated. Briefly, about 1 mL of blood containing EDTA anticoagulant was inserted into an Eppendorf and centrifuged for 5 min at 2000 rpm. One drop of plasma was placed into the rapid test kit. One to two drops of the buffer solution of the kit were added. Test results came after 3 to 5 min. The sample is positive for *E. canis* when two purple lines appear on the T (Test line) and C (Control line) positions of the test strip. If there is no line, it means that the test is invalid or damaged, and the test needs to be retaken. If only the purple line appears in position C, it is negative. If the purple line appears in position T but not in position C, the test has no diagnostic value and needs to be repeated with a new test trip.

Evaluation of clinical and subclinical symptoms of the disease: Antibody-positive dogs for *E. canis* were monitored for clinical and subclinical symptoms. Within the scope of this paper, clinical signs were collected and analyzed.

2.4 Data analysis

Data were collected, stored and analyzed by using Excell 2010 software and compared statistically with Chi-square tests on the Minitab 18.0 software with a 95% reliability. Some parameters, including breed, sex, age, and geographical distribution, were also analyzed.

3 Results and discussion

3.1 Prevalence of *E. canis* infection in dogs

The prevalence of *E. canis* infection in dogs in Thua Thien Hue is presented in Table 1.

Table 1. Prevalence of *E. canis* infection in dogs

Variable	Number (dog)	Percentage (%)	Percentage as total suspected (%)
Surveyed	935	100	
Suspected *E. canis* infection	370	39.6	100
E. canis antibody-positive test	354	37.9	95,7
E. canis found in blood smear	203	21.7	54.9

After examining 935 dogs brought to OKADA PET Veterinary Center, we found 370 dogs (39.6%) suspected of *E. canis* infection. From these 370 cases, we tested for *E. canis* with the rapid test and realized that 354 dogs were positive, accounting for 95.7% and 37.9% of the suspected and total dogs. These rates are much higher than those of Tran et al. [3] when reporting 37/86 (43.02%) dogs with clinical signs of *E. canis* infection. In another study, Rodríguez et al. [13] also found that 53/120 dogs showed signs of *E. canis* infection (44.1%). Our infected rate was also higher than that of Erdeger et al. [5] reporting 91 dogs with a positive rate of 50.55%. Therefore, it is possible to accurately diagnose the dogs infected with *E. canis* from clinical examination. This high infected rate is possibly because we surveyed in the summer when the ticks *Rhipicephalus sanguineus*, a vector bone for *E. canis* transmission, have the best condition for reproduction and spreading pathogens. Another explanation might be that the dogs brought to OKADA PET Center already have some health problems.

The blood film is an effective diagnostic tool in daily veterinary practice. Examining a well-prepared and properly stained blood smear allows veterinary doctors to evaluate cell morphology, including erythrocyte, leukocyte, and platelet. Changes in sizes, shapes, and colours of the cells occur with numerous disorders, including infectious disease, inflammation, and anemia. These cellular changes may go unnoticed when relying on automated analyzers alone. Furthermore, the information gained from blood smear examination (images) is strong evidence of the cause of the disease. Therefore, in our study, we prepared a blood smear to examine the presence of *E. canis* in infected dogs. We found that 203 of 354 dogs (57.3%) with a specific antibody to *E. canis* have *E. canis* morulae in monocytes and/or neutrophils. The high detection rate of *E. canis* morulae in infected dogs in this study is consistent with that reported by Freire et al. [6].

3.2 *E. canis* infection by age

Table 2 reveals that all dogs were at risk of *E. canis* infection with the highest rate at ages one to five years (39.4%) and lowest at ages six months to one year (31.7%). However, this discrepancy is not statistically significant ($p > 0.05$), indicating that the infection of *E. canis* in dogs does not

Table 2. *E. canis* infection in dogs by age

Age group	Number (dog)	Number of possitive cases	Percentage (%)
≤6 months	61	20	32.8
6 months to ≤1 year	60	19	31.7
1 to ≤5 years	439	173	39.4
>5 years	375	142	37.9
Sum	**935**	**354**	**37.9**

depend on age. Our results are similar to that of Tran et al. [3], who stated that age does not affect *E. canis* infection. *E. canis* infection can occur at any age if dogs are exposed to pathogenic vectors or pathogens [4, 10].

3.3 *E. canis* infection by breed

In Vietnam, dog breeds are very diverse, including domestic, imported, and crossed breeds, and they are infected with *E. canis* (Table 3).

Although the incidence of *E. canis* infection in the native breed (36.9%) is lower than in the exotic breed (38.7%) and the crossed breed (38.1%), this difference is not statistically significant ($p > 0.05$). This result demonstrates that the risk of *E. canis* infection does not depend on the breed. Our finding is also consistent with that reported by Tran et al. [3], in which the authors compared native and exotic dogs. Our results are also similar to those published by Malik et al. [10]. The authors surveyed of *E. canis* infection in various dog breeds, including German, Boxer, Labrador, Bully, Pug, and Russian. They found that breeds did not affect the rate of *E. canis* infection ($p > 0.05$).

3.4 *E. canis* infection by gender

Dog gender may have different physiological behaviours, and they might affect the infection rate of *E. canis*. Table 4 shows a higher rate of *E. canis* infection in male dogs (39.5%) than in females (36.8%). However, this difference is statistically insignificant ($p > 0.05$), indicating that the risk of *E. canis* infection in dogs does not depend on gender. This result is in line with the study of Malik

Table 3. *E. canis* infection by dog breed

Breed group	Number (dog)	Number of positive dogs	Percentage (%)
Native	350	129	36.9
Exotic	359	139	38.7
Crossed	226	86	38.1
Sum	935	354	37.9

Table 4. The incidence of *E. canis* infection in dogs by gender

Gender	Number (dog)	Number of possitive dogs	Percentage (%)
Male	380	150	39.5
Female	555	204	36.8
Sum	935	354	37.9

et al. [10], in which the rate of *E. canis* infection in male dogs (32.9%) is higher than that of bitches (31.7%) ($p > 0.05$). Similarly, Tran et al. [3] and Medina et al. [4] also reported that the rate of *E. canis* infection in the dog did not depend on gender.

3.5 *E. canis* infection by keeping types

Table 5 reveals the highest rate of *E. canis* infection is in captive dogs (44.9%), followed by free-ranging dogs at 40.1% and the lowest in semi-captive dogs (32.1%). Statistical analysis shows that the rate of *E. canis* infection in the semi-captive group is statistically different ($p < 0.05$) from the captive and free-ranging groups. In contrast, no statistical significance is found in the difference between the captive and free-ranging groups. According to Medina et al. [4], the infection due to *E. canis* depends directly on the exposure of the hosts to the vector parasites (*Rh. Sanguineus*). Torres [18] suggests that the ticks, including *Rh. Sanguineus* infestation on dogs, can vary widely, depending on geographical origin, seasons, population density, the proportion of dogs treated with ectoparasiticides or tick repellents within a population, and dog individual levels (e.g., age, breed, and lifestyle). Therefore, further studies on tick biology and ecology in Thua Thien Hue and the analysis of other factors, such as tick infestation, need to be carried out in the future.

3.6 Clinical symptoms of *E. canis* infected dogs

Typical symptoms of the dogs infected with *E. canis* are presented in Table 6.

Symptoms of *E. canis* infection in dogs are varied, but most pronounced are fever (>39.5 °C), abortion or stillbirth, joint pain, breast tumours, short breathing, nasal haemorrhage,

Table 5. *E. canis* infection in dogs by keeping type

Type of keeping	Number (dog)	Number of infected dogs	Percentage (%)
Captive	98	44	44.9[a]
Semi-captive	321	103	32.1[b]
Free ranging	516	207	40.1[a]
Sum	**935**	**354**	**37.9**

Note: The values with different letters are significantly different ($p < 0.05$).

Table 6. Clinical symptoms of *E. canis* infected dogs

Symptoms	Number (dog)	%	Symptoms	Number (dog)	%
Presence of ticks	121	34.2	Refuging to eat	100	28.3
Fever	119	33.6	Diarrhea	100	28.3
Abortion, stillbirth	115	32.5	Belly skin hemorrhage	99	28.0
Joint pain	113	31.9	Anorexia	98	27.7
Breast tumors	112	31.6	Constipation	98	27.7
Short breathing	111	31.4	Coughing	97	27.4
Nasal haemorrhage	111	31.4	Ascites	96	27.1
Weakness	110	31.1	Vomitting	96	27.1
Pale mucosa	109	30.8	Hematuria	96	27.1
Skin inflammation	107	30.2	Depression	91	25.7
Hair loss around the eyes	105	29.7	Salivation	90	25.4
Eye discharges	104	29.4	Metritis	59	16.7
Cloudy eyes	101	28.5			

weakness, pale mucosa, skin inflammation, hair loss around the eyes, eye discharges, cloudy eyes, refuging to eat, diarrhoea, belly skin haemorrhage, anorexia, constipation, ascites, vomiting, depression, salivation, and metritis.

The ticks can transmit several diseases, including canine ehrlichiosis. Some ticks can release toxins, leading to tick-paralysis conditions. Once attached to a host, the ticks feed voraciously. As a tick feeds for extended periods, it interacts with its vertebrate host. It can suppress the host's immune system by dampening down the immune response and binding up antibodies that the host might have made in an attempt to get rid of the blood-sucking parasite.

These attributes ensure that a pathogen can be acquired from or transmitted to a bite site that is suppressed and immunologically inactive [17]. In this study, the rate of dogs with ticks on their skin is 34%. Some dogs may not have a tick bite at the clinical examination, but they might have been bitten previously. Moreover, *E. canis* can also be transmitted through the placenta, and newborn puppies can be infected without being bitten by ticks.

Fever is also the most common symptom in infected dogs. The body temperature ranges from 39.5 to 41 °C. Fever is usually a sign that the body is trying to fight against bacteria, including *E. canis*. Fever can also lead to secondary symptoms, such as anorexia or depression.

Previously, ehrlichiosis was called "nasal haemorrhagic disease" because of the high prevalence of this symptom in infected dogs. However, in our study, the nasal haemorrhage rate is only 31.4%. This result shows that the symptoms of each infection change over time, and therefore scientists should update their studies regularly. In this research, the signs indicated above were found in dogs infected with *E. canis*, and some are difficult to explain. For example, joint pain in *E. canis* infected dogs has so far remained unexplainable. Obviously, hair loss around the eyes has not been explained satisfactorily in the scientific literature.

3.7 *E. canis* intracellular detection

Upon performing a blood film scan under a microscope at 400× and 1000× magnification, the *E. canis* morulae can be seen intracellularly (Table 7).

In veterinary practice, the diagnostic procedure usually consists of taking the history of illness, physical examination, and laboratory investigations. The literature reveals that as much as 70% of clinical decisions and diagnoses are supported by laboratory investigation. Peripheral blood film (PBF) is an essential and highly informative haematological tool at the clinician's disposal to screen, diagnose, and monitor the disease progression and therapeutic response.

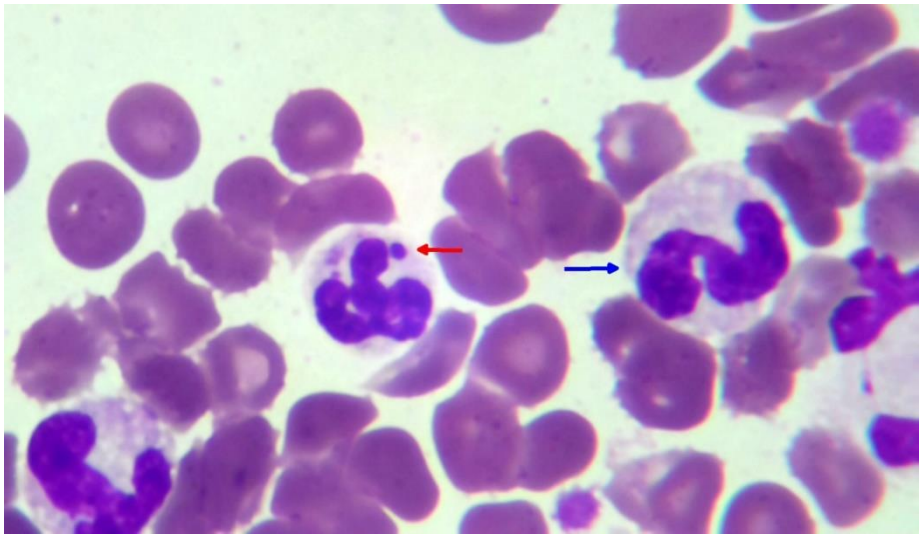
A deep understanding of peripheral blood interpretation is essential for a successful clinical practice. The diagnostic relevance of a PBF is enormous. The PBF exposes the morphology of peripheral blood cells and can be used for morphologic diagnosis of various primary and secondary blood and blood-related diseases. Its diagnostic relevance has not been lessened by advances in haematology automation and molecular techniques [2].

Since *E. canis* parasitizes white blood cells, the highest chance of detecting *E. canis* morulae can be achieved by performing a blood smear from a buffy coat (a layer of white blood cells separated by centrifugation). Using Quick Panoptic dye for staining, we found that *E. canis* morulae mainly multiplies in monocytes (75.8%) and neutrophils (40%) or may appear in both

Table 7. Location of *E. canis* pathogen detected with blood staining method

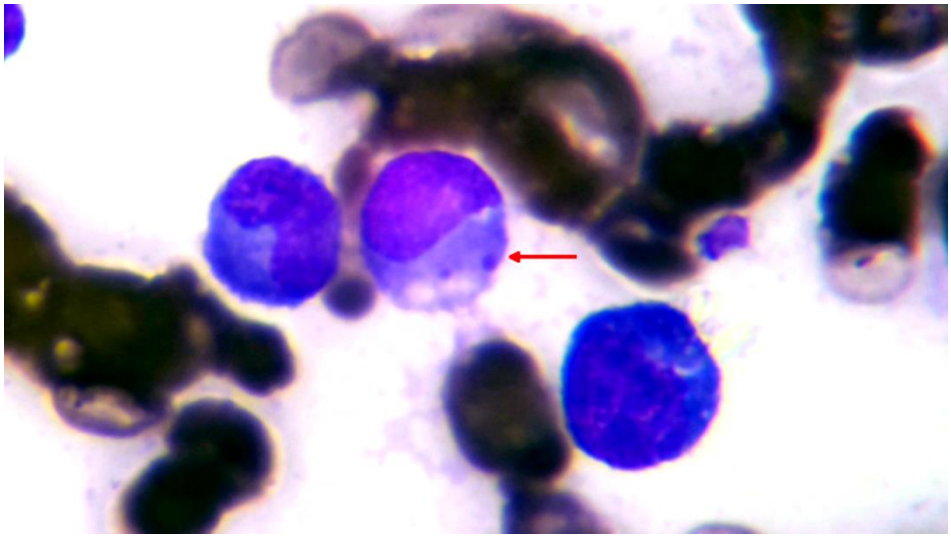
Index	Number (dog)	Percentage (%)
Number of positive cases by blood staining	203	100
Number of dogs with *E. canis* found in monocytes	154	75.9
Number of dogs with *E. canis* found in neutrophils	83	40.9
Number of dogs with *E. canis* found in lymphocytes	0	0
Number of dogs with *E. canis* found in eosinophils	0	0
Number of dogs with *E. canis* found both in monocyte and in neutrophils	54	26.6

neutrophils and monocytes in the same blood sample. Therefore, when examining a blood film to find E. canis, one should focus on monocytes and neutrophils instead of other white blood cells type, such as lymphocytes and eosinophils.



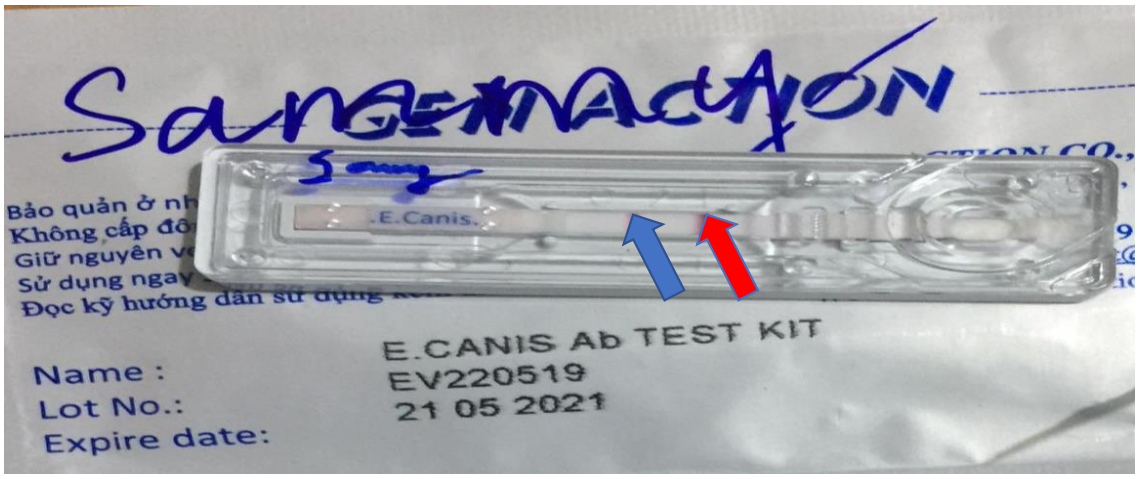
Picture 1. Microscopic image of *E. canis* morula (red arrow, a small purple particle near to the nuclear of the host cell) in neutrophil cytoplasm of an infected dog (1000× magnification, Quik Panoptic dye). The blue arrow refers to intact neutrophils without the presence of *E. canis*.

Photo: Vu Van Hai



Picture 2. Microscopic image of *E. canis* morulae (red arrow) in monocyte cytoplasm of a serological infected dog (1000× magnification, stained with Quick Panoptic dye)

Photo: Vu Van Hai



Picture 3. Blood sample with *E. canis* AB positive results (red arrow is positive line, blue arrow is control line)

Photo: Vu Van Hai

4 Conclusion

Our study indicates that the prevalence of dogs infected with *E. canis* is 37.9% of the total surveyed dogs. The positive serological rate is 95.7% of all suspected infections, revealing that the diagnosis of the *E. canis* infection based on the clinical signs is relatively precise. The rate of dogs infected with *E. canis* does not depend on age, gender, and breed. The prevalence of *E. canis* infection in native breeds is higher than that of exotic breeds, but there is no statistical difference. The dogs infected with *E. canis* have very diverse clinical signs. Still, the most common is the presence of ticks (34%), fever (33%), abortion (32%), joint pain (31%), breast tumours (31%), nasal haemorrhage (31.36%), pale mucus (30%), skin inflammation (30%), hair loss around the eyes (29%), cloudy eyes (29.38%), refuging to eat (28%), belly skin haemorrhage (27%), anorexia (27%), and coughing (27%).

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