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Journal of Parasitic Diseases

ISSN 0971-7196
Volume 45
Number 2

J Parasit Dis (2021) 45:502-511
DOI 10.1007/s12639-020-01328-z

Springer

J Parasit Dis (Apr-June 2021) 45(2):502–511
https://doi.org/10.1007/s12639-020-01328-z

ORIGINAL ARTICLE

Comparative pathology of mice infected with high and low virulence of Indonesian *Trypanosoma evansi* isolates

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Received: 11 April 2020 / Accepted: 25 November 2020 / Published online: 3 January 2021

Abstract Mice infected with *T. evansi* cause various clinical manifestations and histopathological changes. The aim of this study was to compare the histopathological lesions of mice infected with *T. evansi* Bang 87 isolates (high virulence) and Pml 287 isolates (low virulence). A total of 15 susceptible mice (DDY) were divided into three groups (five mice/group): Groups I and II each were infected with 10^4 *T. evansi* of high virulence (Bang87) and low virulence (Pml 287), respectively, whereas group III served as a control group. A total of three mice from group I, and one mouse from each group II and III were killed at 4 dpi. A total of two mice from each group II and III were killed at 24 dpi. Two remaining mice from each group were observed until succumb. Mice of group I and group II at 4 dpi showed no gross lesions. However, mice of group I showed very acute animal death at 5 dpi and showed mild to moderate histopathological lesions at 4 dpi, namely non-suppurative encephalitis, non-suppurative pneumonia, hepatitis non-suppurative with intravascular trypanosomiasis, tubular degeneration and necrosis. Group II showed chronic death at 26 dpi with significant gross pathological changes at 24 dpi in spleen (swelling 10 times than normal size) accompanied by severe non-suppurative encephalitis, cholangiohepatitis non-suppurative and bile duct proliferation, diffused splenic necrosis. The result of this study is expected to be used as a basis for improved treatment management in cattle infected with high virulence *T. evansi* isolates that are need to be handled appropriately to avoid fatal consequences.

Keywords *T. evansi* · High virulence · Low virulence · Mice · Histopathology

Introduction

Trypanosoma evansi is a hemoflagella protozoan that infects large numbers of livestock, pets and wild animals, such as horses, camels, cattle, buffaloes, pigs, dogs, cats (Salim et al. 2011; Desquesnes et al. 2013). These parasites caused 'surra' disease which is characterized by anemia, immunosuppression, emaciation, nerve symptoms, motor incoordination, paralysis and animal death (Gardiner and Mahmoud 1990; Desquesnes et al. 2013). Rodents are also very susceptible to surra disease which is indicated by hematological, biochemical and pathological changes associated with ataxia, tremor and terminal coma (De Menezes et al. 2004; Wolkmer et al. 2009). Although it is a hemoflagella parasite, *T. evansi* can live in intra- and extravascular fluids (Sharma et al. 2000). In contrast to other Trypanozoon subgenus, *T. evansi* is not only found in the bloodstream of the host but also has the ability to penetrate tissues or other body fluids including entering joint fluid or penetrating cerebrospinal fluid in the brain (Haines et al. 1990). These parasites can be observed in animal tissue, blood plasma and cavity fluids that initiate an infected host's immune response (Sharma et al. 2000). The pathogenicity of *T. evansi* varies significantly between different strains and animal species (De Menezes et al. 2004; Queiroz et al. 2000).

In horses and camels, *T. evansi* infection causes severe symptoms with high mortality. Other animals have mild to moderate clinical symptoms (Luckins 1988; Desquesnes et al. 2013). In laboratory animals (DDY strain mice)

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infected with high virulence *T. evansi*, clinical symptoms of ataxia and tremor occur shortly before the animal death (Sawitri 2016). According to De Menezes et al. (2004) and Wolkmer et al. (2009), rats are very susceptible to the disease, showing hematological, biochemical and pathological changes associated with ataxia, tremor and terminal coma in untreated animals. Sensitive mice infected with high virulence *T. evansi* have symptoms similar to those in horses.

Infection caused by *T. evansi* in horses develops in two stages, the initial stage (the hemolymphatic stage) and the final stage (encephalitis). Initial stage happened when the parasite multiplies and spreads in the blood and lymph nodes, whereas the final stage occurred when the parasite crosses the cerebrospinal fluid of the brain to attack the central nervous system. Brain lesions were reported in cattle and horses (Rodrigues et al. 2009). According to Enwezor and Sackey (2005), the main component of the cause of surra pathological lesions is anemia that leads to dysfunction in various organs. Anemia is caused by an increase in the sensitivity of red blood cells to an increase in oxidation stress as a result of a decrease in glutathione on the surface of red blood cells which will trigger acute hemolysis related to parasite multiplication (Sulaiman and Adeyemi 2010).

During early stage of *T. evansi* infection, the production of type I inflammatory components such as TNF, IFN-γ and nitric oxide (NO) was increased and consistent in different trypanosome models with different aspects of the disease such as parasitemia control, pathological conditions and overall survival time of the infected host (Baral et al. 2007). According to Antoine-Moussiaux et al. (2008), host immune response factors that determine the development of parasitemia and pathological changes related to Trypanosoma infections included nitric oxide, TNF-α, IFN-γ and IL-10. Magez et al. (2007) stated that TNF-α is a pleiotropic cytokine which has an important role in the cell-mediated inflammatory response of Trypanosoma infection. This cytokine has the activity of controlling parasitemia characterized by an increase in levels of *T. brucei* infection (Daulouede et al. 2001). Sensitivity to infection is reported in TNF-α deficiency mice infected with *T.congolense* (Naessens et al. 2004). Research carried out by Sawitri et al. (2017) showed increasing level of TNF, IFN-γ at the beginning of high pathogenic *T. evansi* infection in DDY mice. Moreover, TNF has been implicated in the infection-associated pathological changes (Magez et al. 1999) and trypanosome-elicited immunosuppression (Darji et al. 1996). Another study reported that TNF-α produced by macrophages has a direct and indirect role in eliminating parasites by NO induction. This substance has the effect of killing parasites and damaging tissue functions (spleen, liver, peritoneum and central nervous system) (Eckersall et al. 2001; Kitani et al. 2002; Reed et al. 1989).

Gross pathological changes of mice infected with *T. evansi* have been carried out by Biswas et al. (2001); the comparison of gross and histopathological lesions of sensitive mice infected with low and high *T. evansi* virulence has not been reported. Virulence in this study was determined based on the number of animal deaths and parasitemia at a certain time period after infection (Subekti et al. 2013). This recent study was aimed to compare the gross and histopathological changes of various organs of mice infected with high and low virulence of. *T. evansi*. Gross and histopathological changes in various organs will provide an overview of the pathogenicity of *T. evansi* with different virulence.

Materials and methods

Trypanosoma evansi isolates

Two *T. evansi* isolates belong to the collection of BCC (Bblitvet Culture Collection) were used in this study. Those isolates were stored in cryopreservation using 10% glycerol as cryoprotectant (Table 1).

Laboratory animals

This study used fifteen DDY mice (males, aged 10–12 weeks with a body weight of about 25–30 g) from the Indonesian Food and Drug Testing Center (BPOM), Jakarta. The mice were adapted for 15 days before use. The mice were fed commercial pellets (Indofeed) 5–10 g per head/day, and drinking water was given ad libitum during the study.

T. evansi multiplication in mice

Two *T. evansi* isolates namely Bang 87 and Pml 287 isolates were stored in the form of cryopreservation. They were thawed and diluted using phosphate buffer saline glucose (PBSG) to 0.2 mL prior to be used. The solution was each injected to the mice by intraperitoneal (IP) route. The level of parasitemia of experimental animals was evaluated quantitatively every 2 days and observed using a

Table 1 Isolates origin used in the study

Isolate	BCC code	Isolate origin (subdistrict, district, province)	Host
Bang 87	P0176	Bangkalan, Bangkalan, East Java	Buffalo
Pml 287	P0232	Pemalang, Pemalang, Central Java	Buffalo

hemocytometer (*Naubeuer Improved*) (Subekti et al. 2013). When parasitemia reached its peak (around 10^7–10^8 Trypanosoma/mL blood), the blood was collected through the mice heart. Blood containing *T. evansi* was used as a source of infection in this experimental animal.

T. evansi experimental infection in mice

A total of 15 mice were divided into three groups (five mice/group): Group I: mice infected by 1×10^4 high virulence *T. evansi* (Bang 87 isolate), Group II: mice infected by 1×10^4 low virulence *T. evansi* (Pml 287 isolate) and Group III (negative control, not infected). All infected mice were examined for level of parasitemia quantitatively every 2 days. Three mice from group I and one mouse from group II were killed 4 days post-infection (dpi). Three mice from group II were killed at 24 dpi, the remaining mice from each group were observed until they were dying. Necropsy was carried out according to standard procedures. Gross changes were recorded, and pieces of lungs, heart, liver, kidney, spleen and brain were collected. About 0.5 cm thickness of tissues were fixed in 10% Buffered Neutral Formaline (BNF) with a ratio of the tissues volume and BNF of 1:10. The tissues were then processed according to the standard procedure for histopathological preparations and stained with hematoxylin and eosin (H and E) (Drury and Wallington 1980). Histopathological lesions were scored descriptively according to the degree of severity (mild, moderate and severe lesion).

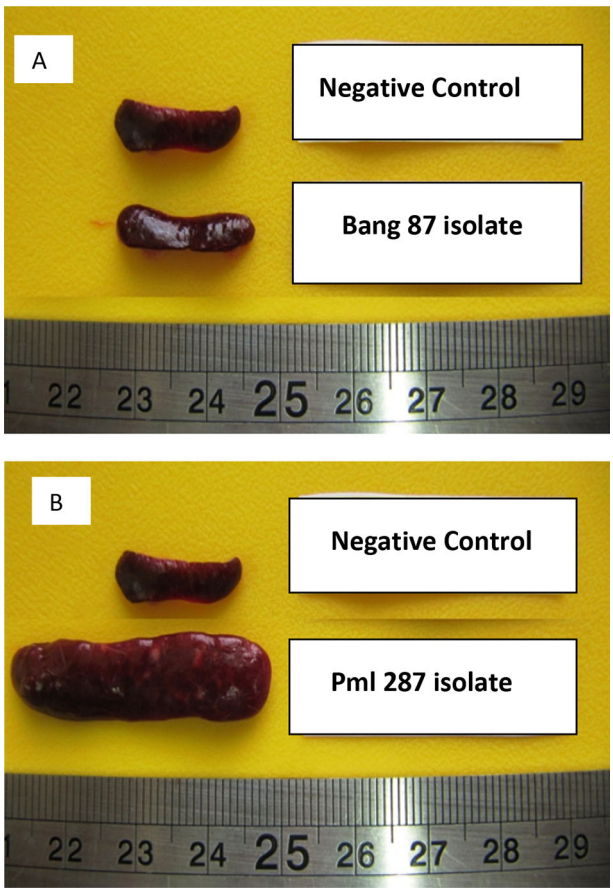

Fig. 1 **a** Mice spleen of group I at 4 dpi: spleen is relatively normal. **b** Mice spleen of group II at 24 dpi: spleen enlarged 10 times associated with multifocal white nodules

Results and discussion

Level of parasitemia and gross lesion of mice

The pathological aspect of *T. evansi* in the host can vary according to the strain virulence and host susceptibility. Susceptible mice (DDY strain) which are infected with Indonesian *T. evansi* isolates caused death simultaneously before 7 dpi were categorized as high virulence and mice that died more than 14 dpi were categorized as low virulence (Sawitri 2016). Observation in the level of parasitemia in group I mice infected with Bang 87 isolates (high virulence) showed peak parasitemia at 4 dpi (10^8 *T. evansi*/mL blood) and died simultaneously at 5 dpi. However, mice infected with Pml 287 isolates (low virulence) had a fluctuating parasitemia pattern, i.e, the peak of parasitemia occurred at 6 dpi (10^8 *T. evansi*/mL blood) and subsequently decreased at 8 dpi (10^6 *T. evansi*/ml of blood) and then increase in return was at 14 dpi (10^8 *T. evansi*/ml of blood) until the mice death at 26 dpi. Mice from group III (control) showed parasitemia negative until the end of the study.

There were no clinical symptoms in group I and II mice up to 4 dpi. In group II, the clinical symptoms of mice were dull hair, clustering in the cage corner and decreased appetite starting at 20 dpi and dying occurred at 26 dpi. In group I, one mouse has observed death at 5 dpi and showed clinical symptoms of ataxia few minutes before death and no symptoms showed in the other mice. There was no gross lesion observed in mice in groups I and II. These findings were in accordance with Garba et al. (2017) that showed *T. evansi* infection in donkeys was showing no gross lesions in the acute stage. This finding was in contrast reported by Sivajothi et al. (2015) which infected 5×10^5 *T. evansi* South Indian isolate in Swiss albino mice, resulted in splenomegaly, hepatomegaly, marked congestion of lungs and presence of fluid in peritoneal cavity at 3–4 dpi.

Although group II (Pml 287 isolate) was categorized as a low virulence, this isolate at 24 dpi caused swelling 10 times bigger than the normal size of the spleen with white nodules on its surface (Fig. 1). Paleness and swelling of the liver (1.5 times the normal size) were detected. The lungs, heart and kidneys were paler than the control group. Enlarged spleen was probably caused by increased activity

of the mononuclear phagocytic system which destroyed red blood cells coated with Trypanosoma antigen (splenic hemosiderosis). Splenomegaly followed by hyperplasia and hypersplenism seemed very clear in disease progression (Singla et al.2001). In addition, according to Mekata et al. (2013), the death of mice infected with high-virulent *T. evansi* Philippines isolates occurred after the 10th dpi with severe organ damage. However, different result was found with *T. evansi* from Indonesia (Bang 87 isolates) which suggest more pathogenic than Philippines isolates because they were able to cause the mice death on the 5th dpi but organ damage was not severe. According to our previous study (Sawitri et al. 2017), this condition occurred as a result of failure immune response to the disease characterized by elevated proinflammatory cytokine (IFN-γ) levels that were not adequately compensated by anti-inflammatory cytokine (systemic inflammation syndromes) in early 7 days of infection. Sivajothi et al. (2015) stated that cellular damage is caused by toxicants released by the parasite or immunological reaction.

Histopathological lesions

Histopathological examination showed mild lesions in group II at 4 dpi, in the form of *T. evansi* infiltration in the intravascular area of the liver, heart, lungs and kidneys. In control mice, there were no lesions found. Histopathological lesions at group I and group II are shown as follows:

Brain

Significant histopathological lesions namely non-suppurative encephalitis were characterized by reactions in the form of glia cell proliferation (gliosis), moderate severity of mononuclear inflammatory cell infiltration in group I at 4 dpi. Severe mononuclear inflammatory cell infiltration was found to occur in group II at 24 dpi (Fig. 2a–c).

Berlin et al. (2009) stated that the infiltration and dissemination of *T. evansi* in the central nervous system have been reported to cause severe and potentially fatal clinical symptoms in the second stage of the disease, but this phenomenon is not detected in group II at 24 dpi. According to Singla and Singh (2013), utilization of tryptophan by Trypanosoma in the brain has been proposed as basis for functional disturbances of CNS. Malfunction in CNS is due to generation of phenyl pyruvate and indole–3–ethanol by the trypanosomes in the brain. The use of tryptophan by trypanosome in the brain was proposed as basis for functional disorder of CNS. CNS failure is due trypanosome in the brain producing phenyl pyruvate and indole–3– ethanol. The immunological injury mechanism often produces substances that induce direct inflammatory changes via a delayed hyper-sensitivity reaction.

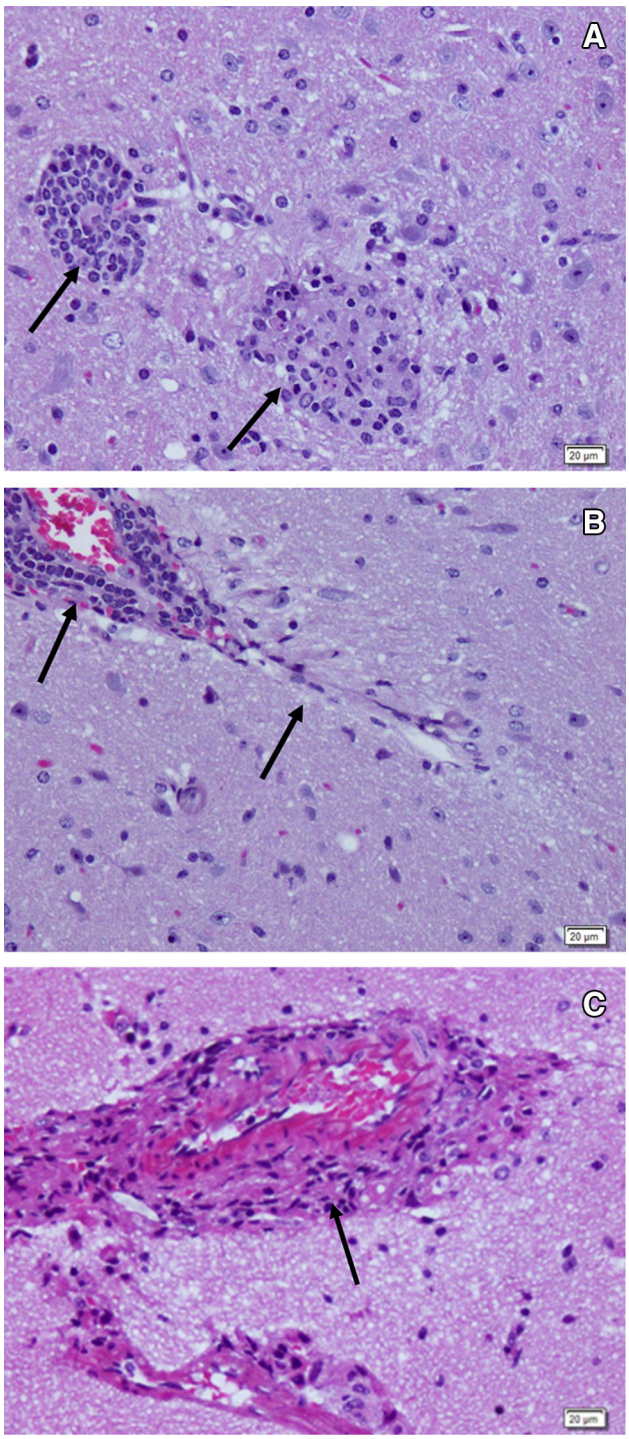

Fig. 2 **a** Gliosis of the mice brain in group I at 4 dpi. **b** Mononuclear cell infiltration associated with *perivascular cuffing* of the mice brain in group I at 4 dpi. **c** Group II resulted in mononuclear cell infiltration forming *perivascular cuffing* in mice brain

Histopathological changes in organs in mice infected by *T. evansi* Bang 87 and Pml 287 isolates (group I) support the results of our previous studies on cytokine profiles that high levels of TNF-α can cause organ damage with various

severity. Rapid increase in TNF-α and IFN-γ level (sevenfold) compared with control group on the peak of parasitemia (4dpi) on mice group infected by Bang87 isolate causes simultaneous mice death at 5 dpi with mild or moderate organ damage. However, both of cytokine on mice group infected by Pml 287 isolate increase gradually reaching fivefold cytokine level at the peak of parasitemia at 16 dpi which cause severe organ damage (Sawitri et al. 2017). According to Baral et al. (2007), Magez et al. (1999), Mertens and Taylor (1999) and Taylor (1998), during infection with Trypanosoma, TNF-α will be involved in controlling parasitemia and infections associated with pathology such as anemia, central nerve damage, fever and emaciation in animals and humans. In this study, the brains of mice group I at 4 dpi and group II at 24 dpi found proliferation of glia cells (gliosis) and perivascular cuffing (lymphocyte infiltration at the edges of blood vessels) in response to central nervous system damage (Fig. 2a–c). This result similar with research by Ghaffar et al. (2016) which experimentally infected mice with *T. evansi* indian isolate and sacrifice the mice at 20 dpi. Further changes develop in the organs either due to cellular damage caused by toxicants released by the parasite, or this damage is thought to be due to high levels of TNF-α in acute infections by Bang 87 isolate and persistent certain TNF-α levels in chronic infections (Sawitri 2016).

Amin et al. (2012) stated that mice infected with *T. brucei* showed that signals mediated by TLR-MyD88 would stimulate TNF-α expression which initiates T cells and parasites to penetrate the brain and activate microglia. A similar statement was given by Masocha et al. (2004) and Amin et al. (2012) that IFN-γ originating from T cells and induced chemokine CXCL10 causes penetration of T cells and parasites in the brain. According to Masocha et al. (2004), the mechanism of Trypanosoma can penetrate the brain's blood barrier involving laminin, IFN-γ and lymphocytes in the process of infiltration of *T. brucei brucei* into the brain parenchyma. Parasitic invasion resulted from penetration of intracerebral vessels did not originate by the spread of parasites from circumventricular organs through cerebrospinal fluid. IFN-γ originated from lymphocytes facilitates the penetration of Trypanosoma in all brain blood vessels, while the location of penetration is determined by the composition of the basement membrane of the intracerebral blood vessels. Interactions between Trypanosoma and host responses can increase tissue damage and facilitate the penetration of parasites into the central nervous system (Londsdale-Eccles 2002).

Liver

Mice liver of group I at 4 dpi showed congestion, hepatocyte cell cytoplasm degeneration, mononuclear inflammatory cell infiltration (non-suppurative hepatitis) with moderate severity accompanied by *T. evansi* infiltration in the intravascular area (Fig. 3a, b). Lesions in mice of group II at 24 dpi were very severe non-suppurative hepatitis accompanied by damage to hepatocyte cell structure, bile duct proliferation and mononuclear cell infiltration (non-suppurative cholangitis) (Fig. 3c, d).

Liver of group I showed congestion and degeneration accompanied by accumulation of Trypanosoma (Fig. 3a, b). According to Uche and Jones (1992), the liver was the most important visceral organ that affects *T. evansi* infection and resulted in necrosis. Fat degeneration followed by hepatocyte necrosis was considered as a general cytological change associated with *T. evansi* infection as a result of nutritional disorders. Lipid accumulation inside hepatocytes due to tissue hypoxia resulted from anemia and vascular damage (Derakhshanfar et al. 2010). According to Biswas et al. (2001), congestion, hemorrhage and hepatocyte degeneration were likely due to hypoglycemia which causes deficiency of energy cells (starvation) and anoxia due to anemia in animals infected with *T. evansi*. In contrast to group II, mice at 24 dpi showed bile duct proliferation enormous monocyte cell infiltration, lysis hepatocytes and severely damaged polyhedral structures (Fig. 3c, d). This finding was similar to the observation made by Ghaffar et al. (2016) using *T. evansi* Egypt strain in mice. According to Morrison et al. (1983), hepatocyte degeneration and necrosis were caused by Trypanosoma activity while severe anemia occurred from lysosomes release by Kupffer cells and this exacerbates tissue damage. Bile duct hyperplasia indicates a local obstruction in bile flow (Dargantes et al. 2005).

Spleen

Among lymphatic tissues, the spleen is the most important organ that serves as the first line of defense mechanism for parasitic infections. Swelling of the spleen is followed by changes in hemorrhagic lesions, blockages, depletion of the germinal center, hemosiderosis, increased follicular cells and focal necrosis (Biswas et al. 2001; Uche and Jones 1992). Histopathological spleen showed active proliferation of lymphocyte cells characterized by widespread area of white pulp in group I mice at 4 dpi (Fig. 4a), whereas in group II at 24 dpi, there was a severe degree of multifocal to diffuse necrosis in which most of the lysis network structure was totally lost (Fig. 4b). These findings were also reported in *T. evansi* infection in experimentally infected Indonesian swamp buffalo (Damayanti et al. 1994). However, Ghaffar et al. (2016) stated that mice infected by Indian isolate *T. evansi* at 20 dpi showed mild congested red pulp with a few scattered parasites, multinucleated giant cells and many histiocytes. This finding

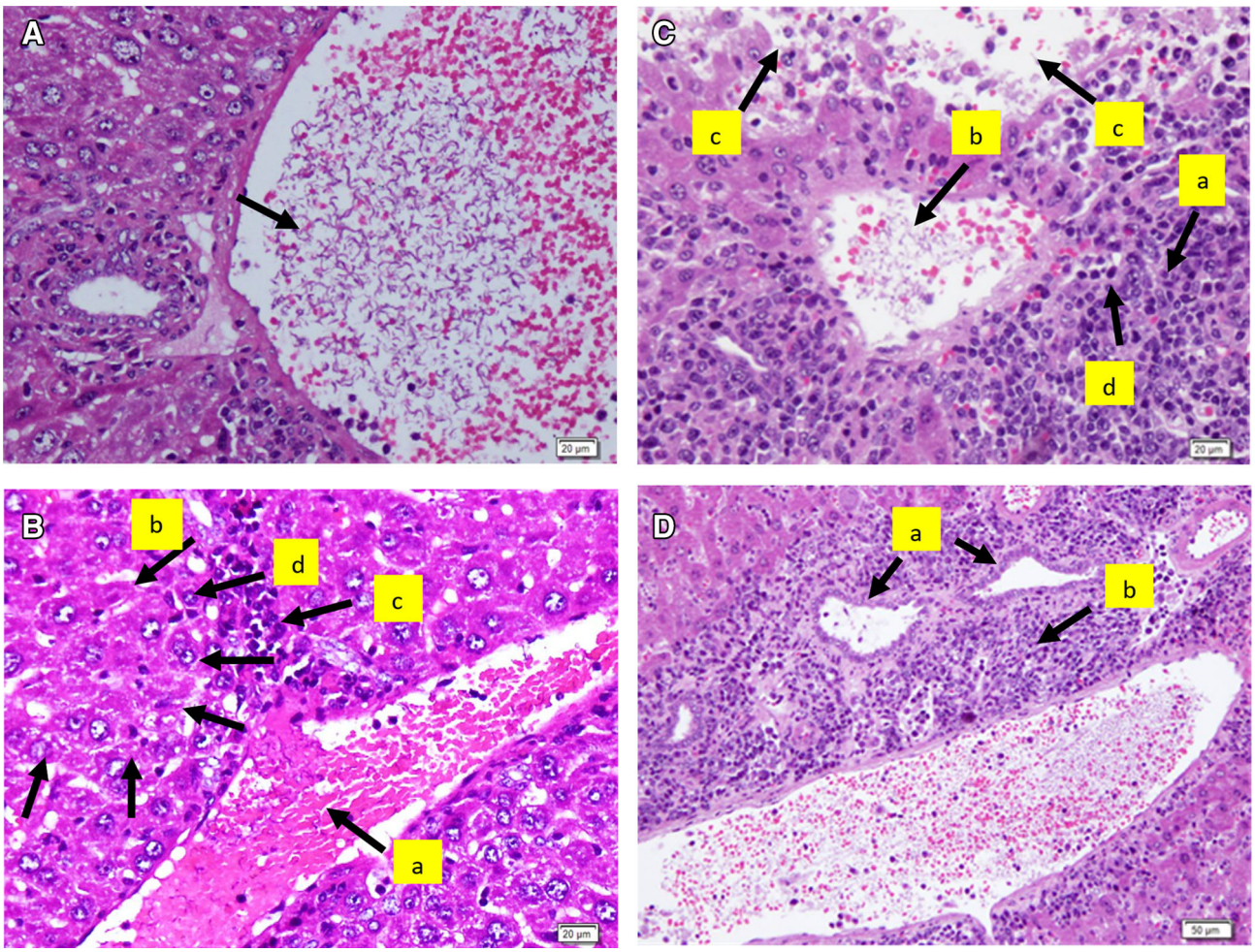

Fig. 3 **a** Mice liver of group I at 4 dpi. *T. evansi* was numerous in the central vein lumen (intravascular) of the liver. **b** Mice liver of group I at 4 dpi showing (a) congestion, (b) degeneration of hepatocyte, (c) mononuclear cell infiltration (d) Kupffer's cell of the liver. **c** Mice liver of group II at 24 dpi showing severe hepatitis non-suppurative (a) mononuclear cell infiltration (b) intravascular *T. evansi* (c) degeneration of hepatocytes (d) Kupffer's cell of the liver. **d** Mice liver of group II at 24 dpi. (a) The liver showed severe cholangiohepatitis non-suppurative and bile duct proliferation association with (b) mononuclear cell infiltration

showed that low virulence Indonesian *T. evansi* isolate was more pathogenic than Indian isolate. Granulomatous lesion formation due to histiocyte aggregation was considered as the initial response to *T. evansi* infection (Biswas et al. 2001; Uche and Jones 1992).

Mice spleen of group I at 4 dpi showed no pathological changes but only very active lymphocyte cell proliferation appeared (Fig. 4a). This condition indicates that the spleen works hard to meet the needs of lymphocytes due to infection by *T. evansi*. This result was different from the results of a study conducted by Bal et al. (2012) in Swiss albino mice infected with *T. evansi* relevance of Indian isolates at 8 dpi showed extensive hemorrhage and congestion with segregation of lymphoid follicles with hyperplasia and hypertrophy. In group I, the mice death occurred very acutely/sudden death at 5 dpi due to high-level proinflammatory cytokine (Sawitri et al.2017). In this case, the anti-inflammatory cytokine (IL-10) level both on Pml 287 and Bang87 group showed an increase of three-fold and fourfold at 4 dpi compared with the control group, respectively. It means that IL-10 production cannot compensate the production of high IFN-γ level in mice infected by Bang87 isolate lead to organ failure and had not reached the stage to severe damage the network (Sawitri et al. 2017). Thus, it was suggested that *T. evansi* Bang 87 isolates were more virulent than Indian isolate. However, in group II, the mice at 24 dpi had severe diffuse necrosis of the spleen. In such damage, the spleen failed to produce T cells and B cells resulting in death at 26 dpi.

Lung

Histopathologically, the lung had non-suppurative interstitial pneumonia characterized by mononuclear cell

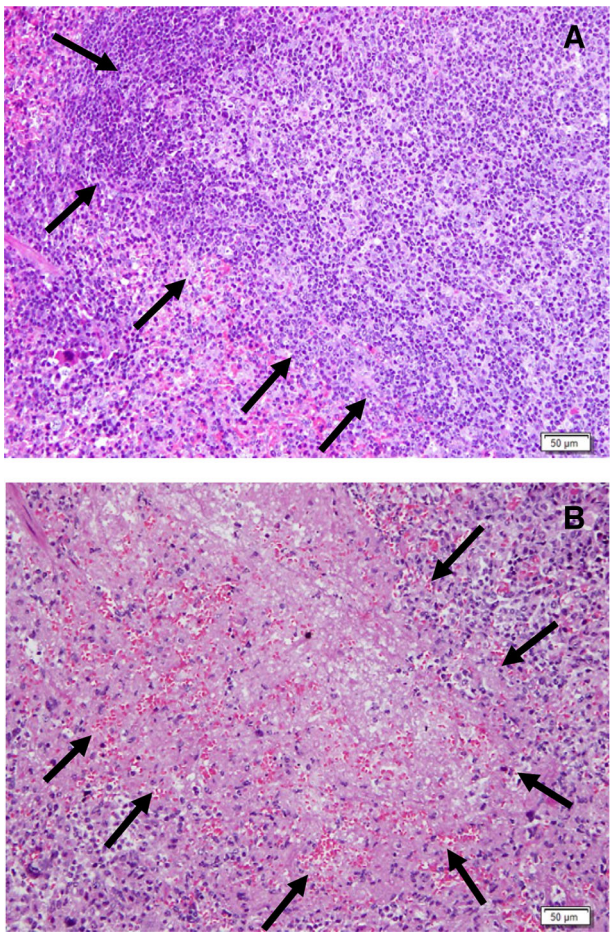

Fig. 4 **a** Mice spleen of group I at 4 dpi showed active proliferation of splenic lymphocytes characterized by large area of white pulp. **b** Mice spleen of group II at 24 dpi showed severe diffused splenic necrosis

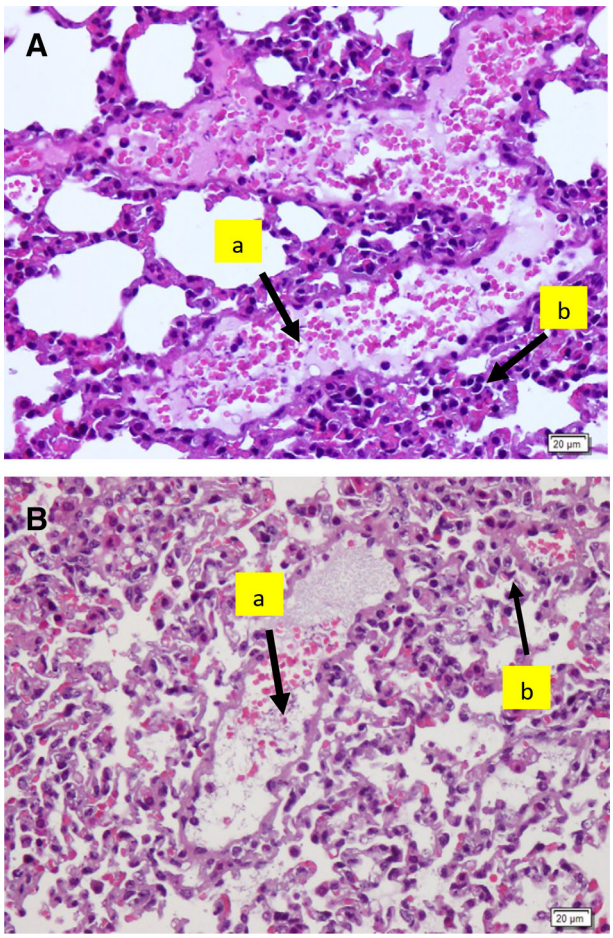

Fig. 5 **a** Mice lung of group I mice at 4 dpi showing (a) *T. evansi* intravascular, (b) swollen of alveolar wall by moderately mononuclear cellular infiltration. **b** Mice lung of group II at 24 dpi showing (a) intravascular *T. evansi* (b) swollen of alveolar wall by moderately mononuclear cellular infiltration

infiltration, with moderate severity in group I at 4 dpi and group II at 24 dpi. In addition, *T. evansi* infiltration of the vascular lumen was obviously detected (Fig. 5a, b)

Mice lung showed non-suppurative pneumonia at moderate severity either in group I at 4 dpi (Fig. 7a) or group II at 24 dpi (Fig. 7b). According to Bal et al. (2012), the inflammatory response of the lungs to parasites causes lung congestion and edema which results in vasodilation and exudation

Heart

Histopathological lesions of the heart showed degeneration of myocardium cells with mild (+) to moderate severity (++). Besides *T. evansi* was detected in the blood vessel lumen (intravascular). Figure 6a, b shows histopathological lesions in the heart. The heart of group I mice and group II had mild-moderate degeneration changes (Fig. 5a, b). Similar results were reported by Biswas et al. (2001) in their research of Indian *T. evansi* isolate in albino Swiss mice. This was probably caused by anemia and hypoglycemia.

Kidney

Histopathologically, the kidneys showed degeneration to necrosis in tubular cytoplasm (nephropathy), with moderate severity in group I mice at 4 dpi (Fig. 7a) and severe lesion in group II at 24 dpi (Fig. 7b).

Kidneys of group I and group II showed degenerative changes and necrosis of renal tubules with different severity. Mice in group II at 24 dpi showed severe damage (Fig. 7b), while mice in group I at 4 dpi showed moderate severity (Fig. 7a). Kidneys revealed tubular degeneration, congestion and cellular infiltration in mice infected by *T. evansi Egypt* isolate (Ghaffar et al. 2016). Dilatation of proximal and distal glomeruli associated with hemorrhage might be caused by the toxins released by trypanosomes

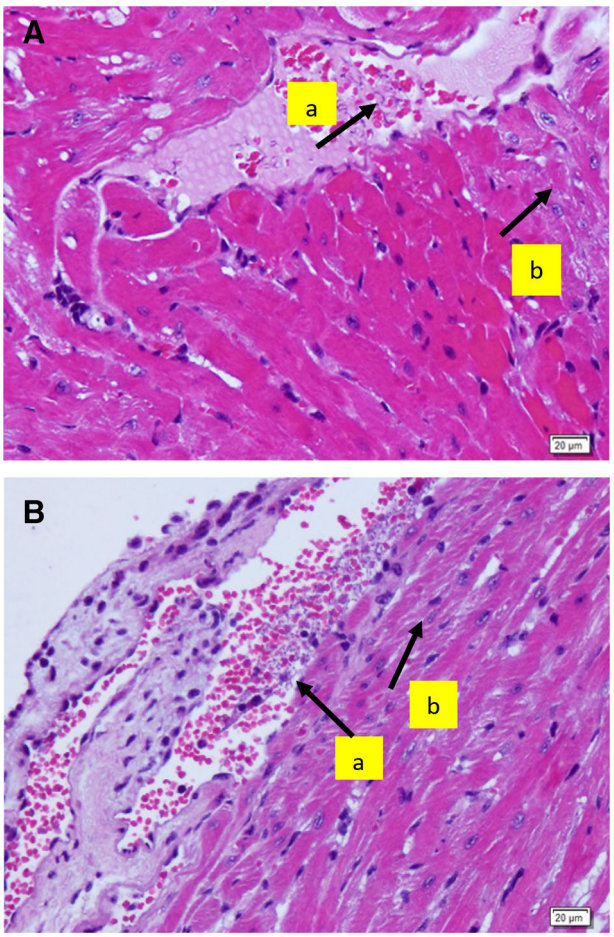

Fig. 6 **a** Mice heart of group I at 4 dpi showing (a) intravascular *T. evansi* and (b) mild myocard degeneration of the heart. **b** Mice heart of group II at 24 dpi showing (a) intravascular *T. evansi* and (b) mild myocard degeneration of the heart

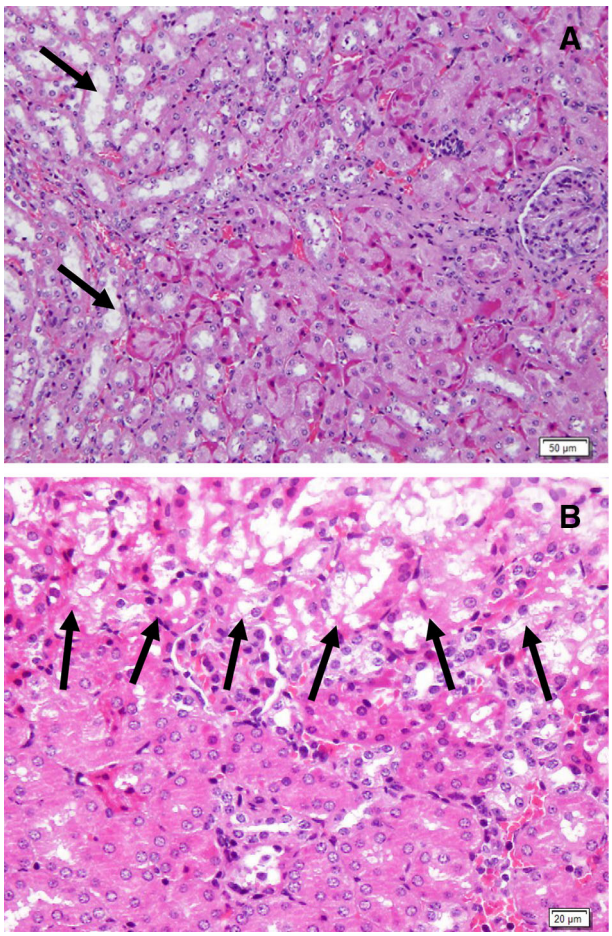

Fig. 7 **a** Mice kidney of group I at 4 dpi had moderate degeneration and tubular necrosis. **b** Mice kidney of group II at 24 dpi had severe degeneration and tubular necrosis

(Ghaffar et al. 2016). Morrison et al. (1981) and Ngeranwa et al. (1993) reported that changes in the kidneys were mainly caused by toxicity of parasites and accumulation of immune complexes that disrupted kidney structure and function. Toxins released by parasites tend to damage kidney function. In such cases, renal cast formation and granulomatous lesions show non-functional kidneys especially in the late stages of *T. evansi* infection (Uche and Jones 1992).

Based on the present findings, the differences result of gross lesion and histopathological changes found in early death mice infected with high virulence *T. evansi* isolate (Bang 87- Group I) support our previous studies (Sawitri et al. 2017) that was caused by high levels of proinflammatory cytokines (IFN -γ and TNF-α) and had not reached the stage of severe tissue damage of the organ. The pathogenicity of *T. evansi* varies among different strain and animal species (Queiroz et al. 2000; De Menezes et al. 2004). Mekata et al. (2013) stated that the rate of parasite growth and the length of the prepatent periods in high and low virulence isolates of *T. evansi* isolated from cattle in Philippines were found to be similar and consistent with those observed in mice, whereas the cattle infected with the highly pathogenic strain developed anemia and a marked decrease in leukocyte counts while in mice showed excessive increase in leukocytes at 8 days post-infection and marked splenomegaly. According to Sawitri and Wardhana (2019), in an outbreak area, cattle that were infected by high virulence of Indonesian *T. evansi* isolate showed fever, anemia, depression and weakness which lead to high mortality but in endemic area, most of the cattle that were infected with moderate or low virulence of *T. evansi* showed no clinical sign with rare or no mortality. According to Mekata et al. (2013), the pathogenicity *T. evansi* had different clinical manifestations in cattle and mice. Damayanti et al. (1994) reported that buffaloes infected with Indonesian *T. evansi* isolate originated from West Java caused fluctuating pyrexia after the peak of parasitemia, emaciation accompanied by serous atrophy of

fat, hydropericardium, and edema of lymph nodes with varying degree of severity among the animals. Histopathological changes showed that the severity of the disease increased from 1 to 7 weeks after infection. The lesions mild to severe were interstitial pneumonia, interstitial myocarditis, splenic multifocal necrosis, interstitial myositis and hyperplastic bone marrow. Unfortunately, research on mice with this isolate was not carried out.

Conclusion

The high pathogenic Indonesian *T. evansi* isolate (Bang87-Group I) caused very acute/sudden death at 5 dpi in susceptible mice. This isolate showed no gross pathological lesions at 4 dpi and produced mild to moderate histopathological changes namely non-suppurative encephalitis, non-suppurative pneumonia, hepatitis non-suppurative associated with intravascular trypanosomiasis, tubular degeneration and necrosis. However, mice infected with low virulence Indonesian *T. evansi* isolate (Pml 287-Group II) caused chronic animal death at 26 dpi with significant pathological changes in spleen (swelling 10 times than normal size) accompanied by severe non-suppurative encephalitis, cholangiohepatitis non-suppurative and bile duct proliferation, and diffused splenic necrosis. The result of this study is expected to be used as a basis for improved treatment management in cattle infected with high virulence *T. evansi* isolates that are need to be handled appropriately to avoid fatal consequences.

Acknowledgement The authors gratefully thank to Prof. Dr. dr Mohamad Sadikin DSc and Dr. drsHeri Wibowo MBiomed from University of Indonesia for the support during the study. This study was funded, in part, by Indonesian Agency for Agricultural Research and Development, Ministry of Agriculture in National Budget.

Author contribution DHS conducted the research and analyzed the parasitology data while RD analyzed the histopathological findings. DHS and RD are equally contributed in preparing the manuscript writing. Both authors had read and approved the final manuscript.

Compliance with ethical standard

Conflict of interest The authors declare that they have no conflict of interest in this study.

Ethical approval The research work was conducted at the Indonesian Research Centre for Veterinary Science, Bogor, Indonesia. This study was approved by the ethics Committee of the Faculty of Medicine, University of Indonesia number 124/H2.F1/ETIK/2013.

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