

Studi virulensi trypanosoma evansi isolat indonesia dengan penentuan marka molekular DNA mikrosatelit dan analisis profil sitokin pada mencit *Mus musculus* = Virulence study of trypanosoma evansi isolates from Indonesia and identification of molecular marker based on microsatellite DNA and cytokine profile analyses in mice *Mus musculus*

Dyah Haryuningtyas Sawitri, author

Deskripsi Lengkap: <https://lib.ui.ac.id/detail?id=20423702&lokasi=lokal>

Abstrak

ABSTRAK

Pendahuluan :Trypanosoma evansi adalah protozoa berflagella yang bersirkulasi di dalam darah secara ekstraseluler sebagai agen penyakit Surra serta menyerang seluruh hewan vertebrata, serta berpotensi sebagai zoonosis. Informasi virulensi isolat T. evansi sangat dibutuhkan untuk penentuan strategi pengobatan Surra di daerah wabah dan endemis. Penelitian ini bertujuan untuk mengetahui variasi virulensi isolat T. evansi yang dikoleksi dari berbagai wilayah di Indonesia termasuk memperoleh marka genetik serta mengetahui profil sitokin pada mencit. Disamping itu, dilakukan juga uji serologis pada peternak di daerah wabah Surra

Metode : Sebanyak 32 isolat lokal T. evansi dikonfirmasi dengan PCR multiplex (ITS-1; Te Ro Tat 1,2 VSG dan ESAG6/7), selanjutnya diuji virulensinya dengan menginfeksi 104 parasit pada mencit galur DDY. Studi genotyping populasi T. evansi dievaluasi dengan 8 marka mikrosatelit Tbb-1, Tbb-5, Tbb-9, Tbb-10, MORF2-CA, M6C8-CA, MEST-19AT, MT3033-AT. Dua isolat yang berbeda virulensi (tinggi-Bang87 dan rendah-Pml 287) dipilih untuk uji imunopatogenitas sedangkan serum peternak diuji dengan metode FELISA dan CATT T. evansi.

Hasil : Dari 32 isolat tersebut terbagi menjadi 17 isolat bervirulensi tinggi, 11 isolat bervirulensi moderat dan 4 isolat bervirulensi rendah dengan 8 pola tingkat parasitemia. Analisis Neighbour Joining (NJ) terhadap 8 lokus berdasarkan Multi Lokus Genotipe (MLG) mikrosatelit terbagi menjadi 4 populasi, yaitu MLG A, MLG B, MLG C dan MLG D. Analisis terhadap struktur populasi juga memberikan hasil yang sama dengan terbentuknya 4 klaster. Hasil ini juga membuktikan bahwa marka yang digunakan bersifat spesifik lokasi. Sebanyak tiga marka mengindikasikan adanya asosiasi antara virulensi dan MLG (Tbb-1, M6C8-CA dan MEST-19). Kadar IFN- γ meningkat secara tajam pada mencit yang diinfeksi isolat Bang 87 pada 4hpi berkorelasi negatif yang signifikan ($p < 0,05$) dengan kadar IL-10, sedangkan pada mencit yang diinfeksi isolat Pml 287, peningkatan kadar IFN- γ berkorelasi positif dengan kadar IL-10. Kematian dini pada mencit yang diinfeksi isolat Bang 87 disebabkan oleh sindrom respon inflamasi sistemik. Hasil uji serologis menunjukkan bahwa 4 dari 24 serum peternak (16,67%) di daerah wabah positif dan seluruh serum negatif untuk

daerah non wabah.

Kesimpulan : Variasi virulensi *T. evansi* isolat Indonesia memiliki karakter molekular yang berbeda serta menginduksi pola mediator sitokin pro dan antiinflamasi yang berhubungan dengan pola manifestasi patologi yang berbeda. Marka mikrosatelit pada studi ini mampu mengidentifikasi asal usul sumber infeksi, dan tingkat virulensi isolat yang sedang bersirkulasi. Surra berpotensi sebagai emerging zoonosis, terutama bagi peternak didaerah wabah dan endemis.

<hr>
ABSTRACT

Introduction: *Trypanosoma evansi* is an extracellular homoflagellate of protozoan blood causing Surra. The disease attacks all vertebrates and potentially as zoonosis. Virulence analysis of *T. evansi* is a fundamental knowledge to determine treatment strategies of Surra in both outbreak and endemic areas. The aims of this study was to determine virulence variation of *T. evansi* isolates collected from various regions in Indonesia and to obtained genetic markers as well as cytokine profile in mice. In addition, serological test was also carried out to farmers living in a Surra outbreak area

Methods: Total of 32 isolates of *T. evansi* confirmed with multiplex PCR (ITS-1; Te Ro Tat 1.2 VSG and ESAG6 / 7), were further tested with inoculation 104 parasite in DDY mice strain. The population genotype study of *T. evansi* was evaluated with 8 microsatellite markers (Tbb-1, Tbb-5, Tbb-9, Tbb-10-CA MORF2, M6C8-CA, MEST-19AT, MT3033-AT). Two different virulence isolates, high-Bang87 and low-PML287 was selected to cytokine profile analysis using ELISA, while farmers sera were tested using CATT and FELISA kits

Results: A total of 32 local isolates of *T. evansi* tested were divided into three different virulences, i.e. 17 high virulence isolates, 11 moderate virulence and 4 low virulence isolates forming 8 pattern parasitemia levels. Based on Neighbour Joining (NJ) on 8 Microsatellite Multilocus Genotype (MLGs) was grouped into 4 populations (MLG A, MLG B, MLG C and MLG D). Structure population analysis also provided the similar result generating 4 clusters. These results indicated that the markers used in this study had a specific location property. Three markers (TBB-1, and MEST M6C8-CA-19) showed an association between virulence and MLG. IFN- γ levels increased significantly in mice infected with Bang 87 isolate on 4th day post infection (dpi) having a significant negative correlation ($p < 0.05$) with increased IL-10 levels, whereas in mice infected by PML 287 isolate, IFN- γ levels were positively correlated with IL- 10 levels. Early death in mice infected with Bang87 isolates was caused by systemic inflammatory response syndrome (SIRS). Result of serological test showed that 4 out of 24 farmers sera (16.67%) from outbreak areas are positive and all sample from free area are negative.

Conclusion: Virulence variation of *T. evansi* isolates from Indonesia has different molecular character and induces cytokine pattern of pro and antiinflammatory

mediators associated with distinct patterns of pathological manifestations. The microsatellite markers found in this study are able to identify origin of infection sources and determine virulence of isolates that circulate on the outbreak area. Surra is potential new emerging disease, particularly for farmers or immunosuppressed individuals who living in both endemic and outbreak areas; Introduction: *Trypanosoma evansi* is an extracellular homoflagellate of

protozoan blood causing Surra. The disease attacks all vertebrates and potentially as zoonosis. Virulence analysis of *T. evansi* is a fundamental knowledge to determine treatment strategies of Surra in both outbreak and endemic areas. The aims of this study was to determine virulence variation of *T. evansi* isolates collected from various regions in Indonesia and to obtain genetic markers as well as cytokine profile in mice. In addition, serological test was also carried out to farmers living in a Surra outbreak area

Methods: Total of 32 isolates of *T. evansi* confirmed with multiplex PCR (ITS-1; Te Ro Tat 1.2 VSG and ESAG6 / 7), were further tested with inoculation 104 parasite in DDY mice strain. The population genotype study of *T. evansi* was evaluated with 8 microsatellite markers (Tbb-1, Tbb-5, Tbb-9, Tbb-10-CA MORF2, M6C8-CA, MEST-19AT, MT3033-AT). Two different virulence isolates, high-Bang87 and low-PML287 was selected to cytokine profile analysis using ELISA, while farmers sera were tested using CATT and FELISA kits

Results: A total of 32 local isolates of *T. evansi* tested were divided into three different virulences, i.e. 17 high virulence isolates, 11 moderate virulence and 4 low virulence isolates forming 8 pattern parasitemia levels. Based on Neighbour Joining (NJ) on 8 Microsatellite Multilocus Genotype (MLGs) was grouped into 4 populations (MLG A, MLG B, MLG C and MLG D). Structure population analysis also provided the similar result generating 4 clusters. These results indicated that the markers used in this study had a specific location property.

Three markers (TBB-1, and MEST M6C8-CA-19) showed an association between virulence and MLG. IFN- γ levels increased significantly in mice infected with Bang 87 isolate on 4th day post infection (dpi) having a significant negative correlation ($p < 0.05$) with increased IL-10 levels, whereas in mice infected by PML 287 isolate, IFN- γ levels were positively correlated with IL-10 levels. Early death in mice infected with Bang87 isolates was caused by systemic inflammatory response syndrome (SIRS). Result of serological test showed that 4 out of 24 farmers sera (16.67%) from outbreak areas are positive and all sample from free area are negative.

Conclusion: Virulence variation of *T. evansi* isolates from Indonesia has different molecular character and induces cytokine pattern of pro and antiinflammatory mediators associated with distinct patterns of pathological manifestations. The microsatellite markers found in this study are able to identify origin of infection sources and determine virulence of isolates that circulate on the outbreak area. Surra is potential new emerging disease, particularly for farmers or

immunosuppressed individuals who living in both endemic and outbreak areas; Introduction: *Trypanosoma evansi* is an extracellular homoflagellate of protozoan blood causing Surra. The disease attacks all vertebrates and potentially as zoonosis. Virulence analysis of *T. evansi* is a fundamental knowledge to determine treatment strategies of Surra in both outbreak and endemic areas. The aims of this study was to determine virulence variation of *T. evansi* isolates collected from various regions in Indonesia and to obtained genetic markers as well as cytokine profile in mice. In addition, serological test was also carried out to farmers living in a Surra outbreak area

Methods: Total of 32 isolates of *T. evansi* confirmed with multiplex PCR (ITS-1; Te Ro Tat 1.2 VSG and ESAG6 / 7), were further tested with inoculation 104 parasite in DDY mice strain. The population genotype study of *T. evansi* was evaluated with 8 microsatellite markers (Tbb-1, Tbb-5, Tbb-9, Tbb-10-CA MORF2, M6C8-CA, MEST-19AT, MT3033-AT). Two different virulence isolates, high-Bang87 and low-PML287 was selected to cytokine profile analysis using ELISA, while farmers sera were tested using CATT and FELISA kits

Results: A total of 32 local isolates of *T. evansi* tested were divided into three different virulences, i.e. 17 high virulence isolates, 11 moderate virulence and 4 low virulence isolates forming 8 pattern parasitemia levels. Based on Neighbour Joining (NJ) on 8 Microsatellite Multilocus Genotype (MLGs) was grouped into 4 populations (MLG A, MLG B, MLG C and MLG D). Structure population analysis also provided the similar result generating 4 clusters. These results indicated that the markers used in this study had a specific location property. Three markers (TBB-1, and MEST M6C8-CA-19) showed an association between virulence and MLG. IFN- γ levels increased significantly in mice infected with Bang 87 isolate on 4th day post infection (dpi) having a significant negative correlation ($p < 0.05$) with increased IL-10 levels, whereas in mice infected by PML 287 isolate, IFN- γ levels were positively correlated with IL-10 levels. Early death in mice infected with Bang87 isolates was caused by systemic inflammatory response syndrome (SIRS). Result of serological test showed that 4 out of 24 farmers sera (16.67%) from outbreak areas are positive and all sample from free area are negative.

Conclusion: Virulence variation of *T. evansi* isolates from Indonesia has different molecular character and induces cytokine pattern of pro and antiinflammatory mediators associated with distinct patterns of pathological manifestations. The microsatellite markers found in this study are able to identify origin of infection sources dan determine virulence of isolates that circulate on the outbreak area. Surra is potential new emerging disease, particularly for farmers or immunosuppressed individuals who living in both endemic and outbreak areas; Introduction: *Trypanosoma evansi* is an extracellular homoflagellate of protozoan blood causing Surra. The disease attacks all vertebrates and potentially as zoonosis. Virulence analysis of *T. evansi* is a fundamental knowledge to

determine treatment strategies of Surra in both outbreak and endemic areas. The aims of this study was to determine virulence variation of *T. evansi* isolates collected from various regions in Indonesia and to obtained genetic markers as well as cytokine profile in mice. In addition, serological test was also carried out to farmers living in a Surra outbreak area

Methods: Total of 32 isolates of *T. evansi* confirmed with multiplex PCR (ITS-1; Te Ro Tat 1.2 VSG and ESAG6 / 7), were further tested with inoculation 104 parasite in DDY mice strain. The population genotype study of *T. evansi* was evaluated with 8 microsatellite markers (Tbb-1, Tbb-5, Tbb-9, Tbb-10-CA MORF2, M6C8-CA, MEST-19AT, MT3033-AT). Two different virulence isolates, high-Bang87 and low-PML287 was selected to cytokine profile analysis using ELISA, while farmers sera were tested using CATT and FELISA kits

Results: A total of 32 local isolates of *T. evansi* tested were divided into three different virulences, i.e. 17 high virulence isolates, 11 moderate virulence and 4 low virulence isolates forming 8 pattern parasitemia levels. Based on Neighbour Joining (NJ) on 8 Microsatellite Multilocus Genotype (MLGs) was grouped into 4 populations (MLG A, MLG B, MLG C and MLG D). Structure population analysis also provided the similar result generating 4 clusters. These results indicated that the markers used in this study had a specific location property. Three markers (TBB-1, and MEST M6C8-CA-19) showed an association between virulence and MLG. IFN- γ levels increased significantly in mice infected with Bang 87 isolate on 4th day post infection (dpi) having a significant negative correlation ($p < 0.05$) with increased IL-10 levels, whereas in mice infected by PML 287 isolate, IFN- γ levels were positively correlated with IL- 10 levels. Early death in mice infected with Bang87 isolates was caused by systemic inflammatory response syndrome (SIRS). Result of serological test showed that 4 out of 24 farmers sera (16.67%) from outbreak areas are positive and all sample from free area are negative.

Conclusion: Virulence variation of *T. evansi* isolates from Indonesia has different molecular character and induces cytokine pattern of pro and antiinflammatory mediators associated with distinct patterns of pathological

manifestations. The microsatellite markers found in this study are able to identify origin of infection sources dan determine virulence of isolates that circulate on the outbreak area. Surra is potential new emerging disease, particularly for farmers or immunosuppressed individuals who living in both endemic and outbreak areas;

Introduction: *Trypanosoma evansi* is an extracellular homoflagellate of

protozoan blood causing Surra. The disease attacks all vertebrates and potentially as zoonosis. Virulence analysis of *T. evansi* is a fundamental knowledge to determine treatment strategies of Surra in both outbreak and endemic areas. The aims of this study was to determine virulence variation of *T. evansi* isolates collected from various regions in Indonesia and to obtained genetic markers as well as cytokine profile in mice. In addition, serological test was also carried out

to farmers living in a Surra outbreak area

Methods: Total of 32 isolates of *T. evansi* confirmed with multiplex PCR (ITS-1; Te Ro Tat 1.2 VSG and ESAG6 / 7), were further tested with inoculation 104 parasite in DDY mice strain. The population genotype study of *T. evansi* was evaluated with 8 microsatellite markers (Tbb-1, Tbb-5, Tbb-9, Tbb-10-CA MORF2, M6C8-CA, MEST-19AT, MT3033-AT). Two different virulence isolates, high-Bang87 and low-PML287 was selected to cytokine profile analysis using ELISA, while farmers sera were tested using CATT and FELISA kits

Results: A total of 32 local isolates of *T. evansi* tested were divided into three different virulences, i.e. 17 high virulence isolates, 11 moderate virulence and 4 low virulence isolates forming 8 pattern parasitemia levels. Based on Neighbour Joining (NJ) on 8 Microsatellite Multilocus Genotype (MLGs) was grouped into 4 populations (MLG A, MLG B, MLG C and MLG D). Structure population analysis also provided the similar result generating 4 clusters. These results indicated that the markers used in this study had a specific location property. Three markers (TBB-1, and MEST M6C8-CA-19) showed an association between virulence and MLG. IFN- γ levels increased significantly in mice infected with Bang 87 isolate on 4th day post infection (dpi) having a significant negative correlation ($p < 0.05$) with increased IL-10 levels, whereas in mice infected by PML 287 isolate, IFN- γ levels were positively correlated with IL-10 levels. Early death in mice infected with Bang87 isolates was caused by systemic inflammatory response syndrome (SIRS). Result of serological test showed that 4 out of 24 farmers sera (16.67%) from outbreak areas are positive and all sample from free area are negative.

Conclusion: Virulence variation of *T. evansi* isolates from Indonesia has different molecular character and induces cytokine pattern of pro and antiinflammatory mediators associated with distinct patterns of pathological

manifestations. The microsatellite markers found in this study are able to identify origin of infection sources dan determine virulence of isolates that circulate on the outbreak area. Surra is potential new emerging disease, particularly for farmers or immunosuppressed individuals who living in both endemic and outbreak areas; **Introduction:** *Trypanosoma evansi* is an extracellular homoflagellate of protozoan blood causing Surra. The disease attacks all vertebrates and potentially as zoonosis. Virulence analysis of *T. evansi* is a fundamental knowledge to determine treatment strategies of Surra in both outbreak and endemic areas. The aims of this study was to determine virulence variation of *T. evansi* isolates collected from various regions in Indonesia and to obtained genetic markers as well as cytokine profile in mice. In addition, serological test was also carried out to farmers living in a Surra outbreak area

Methods: Total of 32 isolates of *T. evansi* confirmed with multiplex PCR (ITS-1; Te Ro Tat 1.2 VSG and ESAG6 / 7), were further tested with inoculation 104 parasite in DDY mice strain. The population genotype study of *T. evansi* was

evaluated with 8 microsatellite markers (Tbb-1, Tbb-5, Tbb-9, Tbb-10-CA MORF2, M6C8-CA, MEST-19AT, MT3033-AT). Two different virulence isolates, high-Bang87 and low-PML287 was selected to cytokine profile analysis using ELISA, while farmers sera were tested using CATT and FELISA kits

Results: A total of 32 local isolates of *T. evansi* tested were divided into three different virulences, i.e. 17 high virulence isolates, 11 moderate virulence and 4 low virulence isolates forming 8 pattern parasitemia levels. Based on Neighbour Joining (NJ) on 8 Microsatellite Multilocus Genotype (MLGs) was grouped into 4 populations (MLG A, MLG B, MLG C and MLG D). Structure population analysis also provided the similar result generating 4 clusters. These results indicated that the markers used in this study had a specific location property. Three markers (TBB-1, and MEST M6C8-CA-19) showed an association between virulence and MLG. IFN- γ levels increased significantly in mice infected with Bang 87 isolate on 4th day post infection (dpi) having a significant negative correlation ($p < 0.05$) with increased IL-10 levels, whereas in mice infected by PML 287 isolate, IFN- γ levels were positively correlated with IL-10 levels. Early death in mice infected with Bang87 isolates was caused by systemic inflammatory response syndrome (SIRS). Result of serological test showed that 4 out of 24 farmers sera (16.67%) from outbreak areas are positive and all sample from free area are negative.

Conclusion: Virulence variation of *T. evansi* isolates from Indonesia has different molecular character and induces cytokine pattern of pro and antiinflammatory mediators associated with distinct patterns of pathological manifestations. The microsatellite markers found in this study are able to identify origin of infection sources and determine virulence of isolates that circulate on the outbreak area. Surra is potential new emerging disease, particularly for farmers or immunosuppressed individuals who living in both endemic and outbreak areas;

Introduction: *Trypanosoma evansi* is an extracellular homoflagellate of protozoan blood causing Surra. The disease attacks all vertebrates and potentially as zoonosis. Virulence analysis of *T. evansi* is a fundamental knowledge to determine treatment strategies of Surra in both outbreak and endemic areas. The aims of this study was to determine virulence variation of *T. evansi* isolates collected from various regions in Indonesia and to obtain genetic markers as well as cytokine profile in mice. In addition, serological test was also carried out to farmers living in a Surra outbreak area

Methods: Total of 32 isolates of *T. evansi* confirmed with multiplex PCR (ITS-1; Te Ro Tat 1.2 VSG and ESAG6 / 7), were further tested with inoculation 104 parasite in DDY mice strain. The population genotype study of *T. evansi* was evaluated with 8 microsatellite markers (Tbb-1, Tbb-5, Tbb-9, Tbb-10-CA MORF2, M6C8-CA, MEST-19AT, MT3033-AT). Two different virulence isolates, high-Bang87 and low-PML287 was selected to cytokine profile analysis using ELISA, while farmers sera were tested using CATT and FELISA kits

Results: A total of 32 local isolates of *T. evansi* tested were divided into three different virulences, i.e. 17 high virulence isolates, 11 moderate virulence and 4 low virulence isolates forming 8 pattern parasitemia levels. Based on Neighbour Joining (NJ) on 8 Microsatellite Multilocus Genotype (MLGs) was grouped into 4 populations (MLG A, MLG B, MLG C and MLG D). Structure population analysis also provided the similar result generating 4 clusters. These results indicated that the markers used in this study had a specific location property. Three markers (TBB-1, and MEST M6C8-CA-19) showed an association between virulence and MLG. IFN- γ levels increased significantly in mice infected with Bang 87 isolate on 4th day post infection (dpi) having a significant negative correlation ($p < 0.05$) with increased IL-10 levels, whereas in mice infected by PML 287 isolate, IFN- γ levels were positively correlated with IL-10 levels. Early death in mice infected with Bang87 isolates was caused by systemic inflammatory response syndrome (SIRS). Result of serological test showed that 4 out of 24 farmers sera (16.67%) from outbreak areas are positive and all sample from free area are negative.

Conclusion: Virulence variation of *T. evansi* isolates from Indonesia has different molecular character and induces cytokine pattern of pro and antiinflammatory mediators associated with distinct patterns of pathological manifestations. The microsatellite markers found in this study are able to identify origin of infection sources and determine virulence of isolates that circulate on the outbreak area. Surra is potential new emerging disease, particularly for farmers or immunosuppressed individuals who living in both endemic and outbreak areas; Introduction: *Trypanosoma evansi* is an extracellular homoflagellate of protozoan blood causing Surra. The disease attacks all vertebrates and potentially as zoonosis. Virulence analysis of *T. evansi* is a fundamental knowledge to determine treatment strategies of Surra in both outbreak and endemic areas. The aims of this study was to determine virulence variation of *T. evansi* isolates collected from various regions in Indonesia and to obtain genetic markers as well as cytokine profile in mice. In addition, serological test was also carried out to farmers living in a Surra outbreak area

Methods: Total of 32 isolates of *T. evansi* confirmed with multiplex PCR (ITS-1; Te Ro Tat 1.2 VSG and ESAG6 / 7), were further tested with inoculation 104 parasite in DDY mice strain. The population genotype study of *T. evansi* was evaluated with 8 microsatellite markers (Tbb-1, Tbb-5, Tbb-9, Tbb-10-CA MORF2, M6C8-CA, MEST-19AT, MT3033-AT). Two different virulence isolates, high-Bang87 and low-PML287 was selected to cytokine profile analysis using ELISA, while farmers sera were tested using CATT and FELISA kits

Results: A total of 32 local isolates of *T. evansi* tested were divided into three different virulences, i.e. 17 high virulence isolates, 11 moderate virulence and 4 low virulence isolates forming 8 pattern parasitemia levels. Based on Neighbour Joining (NJ) on 8 Microsatellite Multilocus Genotype (MLGs) was grouped into

4 populations (MLG A, MLG B, MLG C and MLG D). Structure population analysis also provided the similar result generating 4 clusters. These results indicated that the markers used in this study had a specific location property. Three markers (TBB-1, and MEST M6C8-CA-19) showed an association between virulence and MLG. IFN- γ levels increased significantly in mice infected with Bang 87 isolate on 4th day post infection (dpi) having a significant negative correlation ($p < 0.05$) with increased IL-10 levels, whereas in mice infected by PML 287 isolate, IFN- γ levels were positively correlated with IL-10 levels. Early death in mice infected with Bang87 isolates was caused by systemic inflammatory response syndrome (SIRS). Result of serological test showed that 4 out of 24 farmers sera (16.67%) from outbreak areas are positive and all sample from free area are negative.

Conclusion: Virulence variation of *T. evansi* isolates from Indonesia has different molecular character and induces cytokine pattern of pro and antiinflammatory mediators associated with distinct patterns of pathological manifestations. The microsatellite markers found in this study are able to identify origin of infection sources and determine virulence of isolates that circulate on the outbreak area. Surra is potential new emerging disease, particularly for farmers or immunosuppressed individuals who living in both endemic and outbreak areas;

Introduction: *Trypanosoma evansi* is an extracellular homoflagellate of protozoan blood causing Surra. The disease attacks all vertebrates and potentially as zoonosis. Virulence analysis of *T. evansi* is a fundamental knowledge to determine treatment strategies of Surra in both outbreak and endemic areas. The aims of this study was to determine virulence variation of *T. evansi* isolates collected from various regions in Indonesia and to obtain genetic markers as well as cytokine profile in mice. In addition, serological test was also carried out to farmers living in a Surra outbreak area

Methods: Total of 32 isolates of *T. evansi* confirmed with multiplex PCR (ITS-1; Te Ro Tat 1.2 VSG and ESAG6 / 7), were further tested with inoculation 104 parasite in DDY mice strain. The population genotype study of *T. evansi* was evaluated with 8 microsatellite markers (Tbb-1, Tbb-5, Tbb-9, Tbb-10-CA MORF2, M6C8-CA, MEST-19AT, MT3033-AT). Two different virulence isolates, high-Bang87 and low-PML287 was selected to cytokine profile analysis using ELISA, while farmers sera were tested using CATT and FELISA kits

Results: A total of 32 local isolates of *T. evansi* tested were divided into three different virulences, i.e. 17 high virulence isolates, 11 moderate virulence and 4 low virulence isolates forming 8 pattern parasitemia levels. Based on Neighbour Joining (NJ) on 8 Microsatellite Multilocus Genotype (MLGs) was grouped into 4 populations (MLG A, MLG B, MLG C and MLG D). Structure population analysis also provided the similar result generating 4 clusters. These results indicated that the markers used in this study had a specific location property. Three markers (TBB-1, and MEST M6C8-CA-19) showed an association

between virulence and MLG. IFN- γ levels increased significantly in mice infected with Bang 87 isolate on 4th day post infection (dpi) having a significant negative correlation ($p < 0.05$) with increased IL-10 levels, whereas in mice infected by PML 287 isolate, IFN- γ levels were positively correlated with IL-10 levels. Early death in mice infected with Bang87 isolates was caused by systemic inflammatory response syndrome (SIRS). Result of serological test showed that 4 out of 24 farmers sera (16.67%) from outbreak areas are positive and all sample from free area are negative.

Conclusion: Virulence variation of *T. evansi* isolates from Indonesia has different molecular character and induces cytokine pattern of pro and antiinflammatory mediators associated with distinct patterns of pathological manifestations. The microsatellite markers found in this study are able to identify origin of infection sources and determine virulence of isolates that circulate on the outbreak area. Surra is potential new emerging disease, particularly for farmers or immunosuppressed individuals who living in both endemic and outbreak areas