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Review Article

Current updates on diagnostic methodologies for tick-borne hemoparasitic diseases in equids: A review

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ABSTRACT

Objective: The review is designed to draw up the specifics of diagnostic procedures in more convenient form for practitioners and researchers. Tick-borne diseases (TBDs) or otherwise called equine piroplasmosis (EP) are the foremost economic limitations to equids production. Thus, reducing the breeding capability and athletic performance of equids globally. Identification of these hemoparasites is crucial in understanding their distribution in the population and it is imperative to discern between species and subspecies that are responsible for the occurrence of the disease conditions.

Materials and methods: Conventional procedures such as microscopic and serological evaluations do not usually meet these prerequisites. Diagnostic contrivances, for instance complement fixation test (CFT), indirect fluorescent antibody test (IFAT) and enzyme linked immunosorbent assay (ELISA) were efficaciously adapted for many years. Furthermore, DNA-based investigations for identification, diversification and classification of dissimilar hemoparasites were also established. Molecular investigative procedures, for example DNA hybridization, polymerase chain reaction (PCR), transcriptomics, proteomics, metagenomics and metabolomics, permit the uncovering of hemoparasites or tissues with optimal specificity, sensitivity and consistency.

Results: Additionally, these procedures can be employed to detect definite species and subspecies. The prerequisite of these investigations must include proper premeditation and validation, these investigations provide an effective device for molecular studies, with greater benefits of flexibility to standard requirements. Application of these procedures for swotting TBDs or EP globally will be irreplaceable for a long period from now.

Conclusion: The bourgeoning transcriptomics, proteomics, metagenomic and metabolomics could immensely add to the diagnosis of tick-borne diseases found in the tropical, subtropical and some temperate region of the world.

KEYWORDS

Diagnosis, Equids, Hemoparasites, Metagenomics, Metabolomics, Proteomics, Tick-borne diseases, Transcriptomics

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INTRODUCTION

The diagnosis of hemoparasitic infections has remarkably been optimised as skills, expertise and diagnostic contraptions have greatly been improved over the years. Hierarchically, ticks were placed as the primary arthropod vectors of protozoa, rickettsiae, bacteria and viruses in horses, donkeys, mules and zebras (Salih et al., 2015). The prevalence of tick-borne diseases varies globally, their effect on equids breeding, athletic performances and precincts in meeting global needs connected to exportation or partaking in horse-riding sporting events is significant in the tropical, subtropical and in some temperate regions as they cause enormous peril to the well-being of equids in these areas (Baldani et al., 2010; Salih et al., 2015). The illness in equids is typified by fever, anemia, hepatosplenomegaly, icterus, intravascular hemoglobinuria, hemolysis, and death can ensue (Baldani et al., 2010). The essential tick-borne hemoparasites in equids comprise *Babesia caballi* and *Theileria equi* (*B. equi*), they share the same tick vectors, and they are also found in the same locations, and recurrently they infect horses. The genera *Babesia* and *Theileria* belong to the family *Piroplasmidae*.

The unswerving effect of these tick-borne microorganisms are revealed in breeding reduction, athletic performance, weight loss and considerable death in the population of the affected equids (Baldani et al., 2010). The eradication or reduction of tick-borne diseases in equids population will appreciably heighten the breeding and athletic performance of these animal species. Efficient control of tick-borne hemoparasitic illnesses needs speedy, consistent and extremely delicate diagnostic tests, which can also aid in monitoring the efficacy of the prophylactic and therapeutic measures (Salih et al., 2015). The conventional process of using history, clinical signs, blood or tissue smears examination, serology and post-mortem provide the rudimentary prerequisites for diagnosis, and have downside of specificity, sensitivity, and it requires the participation of experts and is labour intensive. The serodiagnosis do not vary between current and previous infection in equids that have cleared the microorganism, but still is seropositive (Baldani et al., 2007; Baldani et al., 2010). Nucleic acid based diagnostics, predominantly DNA based procedures were developed and certified for identification and characterization for numerous microorganisms (Baldani et al., 2010).

The polymerase chain reaction (PCR), ELISA, transcriptomics, proteomics, metagenomics and metabolomics based assessments allow for the identification of parasites at levels far-off below the detection limit used for parasitological procedures. Numerous procedures were developed disjointedly for detection of each species of tick-borne hemoprotozoa. In this review, a comprehensive dialogue on diagnostic techniques from conventional to molecular approaches comprising of transcriptomics, proteomics, metagenomics and metabolomics procedures are well-thought-out.

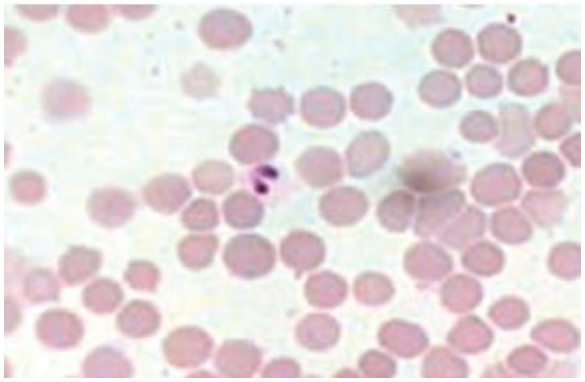

Figure 1. *B. caballi* merozoites and trophozoites within erythrocytes. Adopted from Baldani et al. (2007).

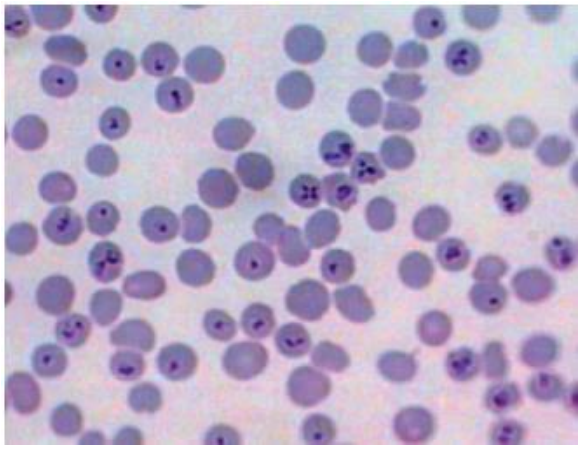

Figure 2. *B. equi* merozotes in "Maitese cross" formation. Adopted from Baldani et al. (2007).

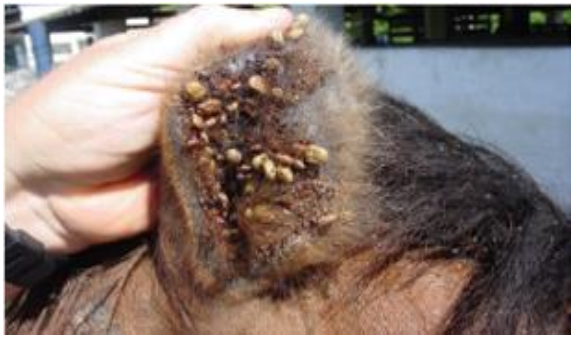

Figure 3. Horse heavily infected with *Dermacentor* (*Anocentor nitens*, one of the most common horse ticks capable of transmitting *B. caballi*. Adopted from Baldani et al. (2007).

Conventional Diagnostic Procedures

Microscopy

The hemoparasites in infected equids can be identified in blood smears stained with 10% Giemsa solution (Sumbria and Singla, 2015). Babesiosis is identified by examining organ or blood smears after staining with Romanowsky stain (Salih et al., 2015). In the acute phase of TBDs or EP, diagnosis by microscopic examination of blood smears is possible, and are still considered as "gold standard" (Salih et al., 2015). In subclinical carrier equids, nevertheless, the small number of piroplasms in circulation reduces the sensitivity of microscopy (Terkawi et al., 2011), and serologic diagnosis is more consistent (Sumbria and Singla, 2015). Parasitemia with *B. caballi* is usually very low, therefore, uncovering of parasites in thin blood smears is frequently challenging (Dave et al., 2012). The thick blood smear procedure is expedient. The merozoites of *B. caballi* within erythrocytes are pyriform in shape and differ between 2 and 5 µm × 1 and 1.5 µm in size and frequently form twosomes linked at their posterior ends (**Figure 1**). Trophozoites could similarly be detected in erythrocytes and are polymorphic in shape, changing from round to oval or elliptic, and is roughly between 1.5 to 3 µm in diameter. *T. equi* merozoites in erythrocytes characteristically seem as four pyriform parasites, almost 1.5 µm elongated and organised in a "Maltese cross" pattern (**Figure 2**). The trophozoites can assume an oval, round, elliptic, or spindle shape and is to 3 µm in diameter inside the erythrocytes. The conservative microscopy for identification is user friendly and doesn't need the procurement and upkeep of expensive contrivances. However, limitations of conventional microscopy techniques comprise enormous labour when bulky samples are to be analysed in a short time, proficient technicians are essential for accurate diagnosis and it is less effective in cases where the infection level is very low.

Indirect Diagnostic Procedures

When hemoparasites are detected at concentrations lower than the sensitivity of unswerving procedure or is not able to be established in tissue sample as a result of the stage in the life cycle of the parasite in the host, indirect procedures of identification are adapted, which comprise serological tests that detect antibodies or antigens. Numerous serological tests are available and include complement fixation test (CFT), indirect fluorescent antibody technique (IFAT) and enzyme-linked immuno-sorbent assay (ELISA) among others.

Complement Fixation Test

The complement fixation test (CFT) is hitherto the authorised normal test for equine piroplasmosis and is the universal test for incoming equine in EP-free nations. The fundamental standard for CFT is the fixation of complement during the reaction between specific antigen and antibody. Equine sera that react at a dilution of 1:5 are assumed to be positive. CFT identifies antibody titers as from day 8 of post infection, and the titers decreases at 2 to 3 months of post infection. CFT reactions may perhaps become transitorily negative within 24 months of treatment of *T. equi* infected horses and within 3 to 15 months for *B. caballi*–infected horses. Consequently, retesting horses that were treated should be done 4 to 6 weeks post treatment.

Numerous downsides are divulged using CFT, comprising the need for the generation of large quantities of antigens, the manifestation of false-negative results, and cross reactivity between *B. caballi* and *T. equi* sera. CFT is a very specific test; nonetheless, because of the presence of IgG (T) antibodies (non-complement fixing) it indicated low sensitivity in chronic cases.

Indirect Immunofluorescent Antibody Test

The indirect immunofluorescent antibody test (IFAT) has greater sensitivity compared to CFT and has been indicated as an additional assessment when CFT results are questionable. In this test, test sera react with antigens of parasites are fixed to glass slides. The antibodies are observable using ultraviolet light after binding of a fluorescein-labeled antiequine serum. Sera are assumed to be positive if they express resilient fluorescence of the parasites at a dilution of 1:80 and higher (Baldani et al., 2007). The earliest antibody responses in equines experimentally inoculated with *B. caballi* and *T. equi* in one study were at 3 to 20 days of post infection, with titers still demonstrable in the covert period of infection. IFAT titers are identified reliably than CFT titers, and sera continue to be positive using IFAT compared to CFT (Baldani et al., 2007). To upsurge the specificity with IFAT, serum should be diluted, which concomitantly results in forfeiture of sensitivity (Rothschild and Knowles, 2007). IFAT is laborious, needs colossal amounts of antigen, and due to subjectivity in interpreting fluorescence, is challenging to normalize. IFAT is used as an additional test to aid in the analysis of CFT results, and is the most significant approved tests for horse piroplasmosis as endorsed by the OIE.

Enzyme-Linked Immunosorbent Assay

Enzyme-linked immunosorbent assay (ELISA) is indicated in the identification of dominant antibodies to both *T. equi and B. caballi*, although cross-reactivity may ensue. Using *T. equi* EMA-1 and specific monoclonal antibodies (Knowles et al., 1991; Battsetseg et al., 2002; Ueti et al., 2003; Bhoora et al., 2009) developed a competitive inhibition ELISA (cELISA) for *T. equi* infection. EMA-1 is a specific *T. equi* surface erythrocyte stage protein that has an epitope shown to be both immunodominant and conserved universally (Cacciò et al., 2000; Nicolaiewsky et al., 2001; Rampersad et al., 2003; Heim et al., 2007; Baldani, 2008; Fritz, 2010). This cELISA was well along upgraded by adapting a recombinant protein instead of culture-derived whole parasites. This assay overwhelms the difficulty of antigen purification due to specificity which depends on monoclonal antibody. ELISA has enhanced the ability to perform compared with CFT and IFAT and has identified covert infections of experimentally inoculated equines not identified by CFT (Rothschild and Knowles, 2007). The adoption of a recombinant protein simplifies normalization of the test and overwhelms the need for *in vitro* cultivation of the parasite or the artificial inoculation of equines for antigen production, making cELISA an ideal test for screening of *Babesia* infection (USAHA, 2011). In a field survey, this test identified 25% more sera as positive for *B. caballi* as the CFT (Rothschild and Knowles, 2007). In 2004, OIE (http://www.oie.int) approved the cELISA for both *T. equi* and *B. caballi* as the approved assay for international equine trading. A single-dilution ELISA using the whole-merozoite antigen was validated in the field in India by Kumar et al. (2003) in 2003 to detect antibodies to *T. equi*. These authors reported the assay to be economical and sensitive, with no cross-reaction with *B. caballi*, and suitable for large epidemiologic studies.

Polymerase Chain Reaction

Uncovering of hemoparasitic deoxyribonucleic acid (DNA) using PCR is highly sensitive when compared to microscopic recognition of parasites in blood smears and is superior for the detection of carrier infections (Salim et al., 2008). The PCR systems may be a cherished tool in the expeditious uncovering and identification of *T. equi* and *B. caballi* in blood, as substitute to microscopy and serology for enhancing diagnostic results; at the moment, these contrivances are only used for research purposes (Baldani et al., 2010). Investigations of horses in endemic regions are essential for the valuation of diagnostic sensitivity and specificity of these tests. Primary PCR tests have been developed to identify both *T. equi* and *B. caballi* DNA in equids. In one investigation, PCR was able to identify calculated parasitemias as low as 0.0083% for *B. equi* and 0.017% for *B. caballi*. Nevertheless, many equines with positive PCR results also had positive microscopic investigation of blood smears. A nested PCR for *T. equi* based on the sequence of the EMA-1 gene has amplified sensitivity and may be more consistent for the diagnosis of subclinical infection, identifying an equivalent calculated parasitemia of 0.000006% (Nicolaiewsky et al., 2001; Baldani, 2008). In a field study using nested PCR for *T. equi*, the test was able to identify 3.6 times more infections than microscopic investigation and 2.2 times more than with primary PCR. Many subclinical infections in apparently healthy equines that could not be identified with primary PCR were identified by nested PCR. The same assay has been efficaciously used to identify *B. caballi*–infected and *T. equi* infected ticks in Mongolia and to identify infected ticks and equines in Brazil. A PCR-based hybridization test for *B. caballi* using a specific biotin-labeled DNA probe has been developed with excellent results but has not yet been verified in field conditions. PCR testing is objectively forthright and becoming more inexpensive and soon may become commercially obtainable.

In-vitro Organism Cultivation

In vitro culture of blood samples from suspected equids for the identification of *B. caballi* and *T. equi* in carrier horses and zebras has been described. This is a substitute to the traditional in vivo testing in which blood from a suspected infected horse is injected into a susceptible splenectomized horse, which is observed for development of characteristic clinical signs and clinicopathologic indication of disease. Regardless of the substantial benefit of disregarding the use of live animals, in vitro culture procedures are arduous, costly, and unreliable and thus not suitable for commercial use.

Transcriptomics, Proteomics, Metagenomics and Metabolomics as Promising Diagnostic Procedures for Equids Tick-Borne Diseases

Transcriptomics and Proteomics

Due to the current methodological advances in transcriptomics and proteomics analyses, a general assessment of the various expressed genes and proteins of an organism has become accessible. Transcriptomics and proteomics analysis is an essential step in changing genetic data to protein function and cell biology. Complete genome transcriptomic analysis revealed the expression of roughly 70% of bacterial gene transcripts (Nelson et al., 2008). Proteomics analyses based on 1-D

and 2-D gel identified one-fourth of the total ORFs from human and tick cell-derived pathogen cultures (Singu et al., 2005; Seo et al., 2008). Nevertheless, there are major technical hitches in proteomic studies regarding obligate intracellular organisms; due to high-purified sample is not definitely obtainable, and the manifestation of a huge amount of host proteins decreases the identification and sensitivity scores of microbial proteins (Li and Lostumbo, 2010). The development of highly sensitive nano-liquid chromatography coupled with tandem MS/MS (nano-LC–MS/MS)-based proteomic methodology improves protein analysis of obligate intracellular organisms, as low levels of proteins can be identified in samples mixed with a huge amount of host proteins (Zimmer et al., 2006). Moreover, label-free protein quantitation based on LC–MS peptide peak intensity data becomes possible due to the sensitivity and reproducibility of intensity data measurements, and multiple samples from different conditions can be compared directly without stable isotope labelling (Old et al., 2005; Zimmer et al., 2006; Shi et al., 2009). Here, we review the efficiency of transcriptomics and proteomic approach in the diagnosis of tick-borne diseases by their relative protein expression abundances. The determination of protein expression profiles of tick-borne diseases in equids erythrocytes will help advance understanding cell biology, physiology of these ticks, and complex interplay between ticks and their host, and enhance the opportunities for investigation of novel targets for chemotherapy or blocking of pathogenic pathways.

Metagenomics and metabolomics

Metagenomics and metabolomics procedures have emerged and could be promising in identifying tick-borne diseases, bringing novel potential for the development of diagnostics methodologies. The field of metagenomics, and the study of microbes' populations and their contributions to health and disease could tremendously be enhanced using this procedure. Metagenomic evaluations are usually undertaken by sequencing the microbial 18S and 16S ribosomal RNA (rRNA) subunit or the complete metagenome shotgun sequencing, characteristically on an enormously analogous pyrosequencing podium (Dave et al., 2012; Bahrami et al., 2014). These skills have extended the possibility of culture-reliant microbiological procedures and have improved our understanding of the microbial populations that dwell in the blood streams, intestine, skin, oral cavity, and genitourinary tract and how these microbes interact with the host (Dave et al., 2012).

Entire metagenome or metatranscriptome shotgun (WMS) sequencing includes all nucleotides in a sample, classifying all microorganisms that may be existing, to a species or even strain level whereas providing functional based information on genome content. Downsides of WMS comprise exorbitant cost in terms of nucleotide number and capitals required to analyze the larger data set and contamination with host nucleic acid. WMS methods have an intrinsic nucleic acid extraction bias because some microorganisms are lysed far more easily than others. It is more effective than Sanger sequencing, of the "next-generation" technologies which aligns shorter reads and as a result are more liable to sequencing inaccuracies (Morgan and Huttenhower, 2014).

Autonomously, the field of metabolomics emerged as logical, non-subjective examination of all low molecular weight small molecules, or metabolites, produced by the body in response to an ecological stimulus. Metabolites are released into body fluids by host and microbial cells, measured by mass spectrometry based methodologies, and aligned alongside libraries of known biochemicals. These procedures have been employed to understand the mechanisms of pathogenesis and to detect novel biomarkers of disease. Metabolomics similarly indicates the presence and function of microbes living in difficult crannies and highlights the multifarious relationship between microbes, host metabolism, and comparative health or disease (Geoffrey and Peter, 2015).

Metabolites formed by microbial and host cells consist of an unusual pool of physicochemical properties, these may be existing in almost all body tissues or fluids, and are found in varying concentrations. Consequently, no particular metabolomics podium is capable of identifying all metabolites in a sample, and a collection of approaches is usually employed (Dettmer et al., 2007; Yozwiak et al., 2012). Mass spectrometry coupled to gas chromatography (GC-MS) identifies volatile, thermally stable metabolites with less than millimolar sensitivity, while liquid chromatography (LC-MS) is used to identify nonvolatile polar and nonpolar compounds with nanomolar resolution. These methods are dependent on sample preparation procedures that present bias and unavoidably metabolite losses (Geoffrey and Peter, 2015). Otherwise, nuclear magnetic resonance (NMR) spectroscopy does not need prior separation of compounds in a sample, though curtailing sample preparation diminishes the resolution; NMR can typically identify compounds at or above a millimolar level. After alignment to libraries of identified biochemical, multivariate statistical methods present metabolite contingency tables and principal component analyses. The major downside to all metabolomics methods is cost, both in terms of data procurement and labor intensity of data analysis. Moreover, a fully interpreted, complete metabolite library,

particularly for microbial-derived compounds, is still far-flung (Geoffrey and Peter, 2015).

Transcriptomics, proteomics, metagenomics and metabolomics are the most speedily progressing "omics" tools, transcriptomics and proteomics are essential in changing genetic information to protein function and cell biology while, metagenomics classifies the genetic potential of a microbial population, and metabolomics accounts for the specific mechanism that yields a phenotype. These fields have contributed positively to numerous animal conditions. Furthermore, there exist unprecedented prospects in transcriptomics, proteomics, metagenomic and metabolomics in understanding the host parasite relationship. Thus, the bourgeoning transcriptomics, proteomics, metagenomic and metabolomic could immensely contribute to the diagnosis of tick-borne diseases found in the tropical, subtropical and some temperate region of the world.

Approach to protozoan parasite systems

In recent times metabolomics has been employed for the study of trypanosomatid, specifically for the biology and the host parasite interactions (Kafsack and Llinas, 2010; Timothy et al., 2013). The Metabolism of *Leishmania* have been studied (Creek et al. 2011; Scheltema et al. 2010; Timothy et al., 2013) including *Trypanosoma* (Vincent et al. 2012; Creek et al. 2011; Timothy et al., 2013). Ancillary studies were carried out on *Plasmodium* (Besteiro et al. 2010; Lakshmanan et al. 2011; Timothy et al., 2013). However, studies on *Entamoeba* was performed to illustrate how metabolomics can be used to evaluate crucial features of metabolism in the parasite. The effect of L-cysteine on *E. histolytica* was studied using metabolomics (Husain et al. 2010; Timothy et al., 2013). Capillary electrophoresis (CE), mass spectrometry (MS) and DNA microarray-based expression were used to examine metabolic and transcriptomic alterations that ensue during encystation in *Entamoeba invadens,* a family member of *E. histolytica* from reptiles but distinct from *E. histolytica*, will encyst in *in vitro* culture (Jeelani et al. 2012; Timothy et al., 2013). As encystations advanced, the levels of most metabolites involved in glycolysis and nucleotides decreased significantly, signifying energy cessation. Likewise, glucose utilization is transformed from energy production to chitin wall biosynthesis (Timothy et al., 2013). Furthermore, proteomics has abetted in supporting this hypothesis. Additionally, Mingqun et al. (2011) in their study presented a comprehensive proteomics data examination of *A. phagocytophilum* and *E. chaffeensis* proteomes, and indicated a quantifiable human host protein expression profiles measured by bacterial infection.

CONCLUSION

The diagnosis of tick-borne diseases of equids could be greatly enhanced by employing these nascent procedures. This review provides the significant procedural tools for the diagnosis of tick-borne diseases of equids. Thus, the bourgeoning transcriptomics, proteomics, metagenomic and metabolomics could immensely contribute to the diagnosis of tick-borne diseases found in the tropical, subtropical and some temperate region of the world.

CONFLICT OF INTEREST

Nothing to declare.

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REFERENCES

Bahrami S, Ghadrdan AR, Pourmahdi BM, Vafayi Salarpur M (2014). Epidemiology of *Theileria equi* in Persian Arab horses from Iran. Veterinarni Medicina, 59: 409-414.

Baldani CD (2008). In vitro culture, PCR, and nested PCR for the detection of *Theileria equi* in horses submitted to exercise. Arquivo Brasileiro de Medicina Veterinária eZootecnia, 60: 550-558.

Baldani CD, Andrea CH, Nakaghi R., Zacarias M (2010). Occurrence of *Theileria equi* in horses raised in the Jaboticabal microregion, São Paulo State, Brazil. Brazillian Journal of Veterinary Parasitology, 19: 228-232.

Baldani CD, Rosangela ZM, Tânia FR, Aramis AP (2007). Serodiagnosis of *Babesia equi* in horses submitted to exercise stress. Pesquisa Veterinária Brasileira, 27: 179-183.

Battsetseg B, Lucero S, Xuan XN, Claveria FG, Inoue N, Alhassan A, Kanno T, Igarashi I, Nagasawa H, Mikami T, Fujisaki K (2002). Detection of natural infection of Boophilus microplus with *Babesia equi* and *Babesia caballi* in Brazilian horses using nested polymerase chain reaction. Veterinary Parasitology, 107: 351-357.

Besteiro S, VoDuy S, Perigaud C, Lefebvre-Tournier I, Vial H (2010). Exploring metabolomic approaches to analyze phospholipid biosynthetic pathways in Plasmodium. Parasitology, 137: 1343-1356.

Bhoora R, Franssen L, Oosthuizen MC, Guthrie AJ, Zweygarth E, Penzhorn BL, Jongejan F, Collins NE

(2009). Sequence heterogeneity in the 18S rRNA gene within *Theileria equi* and *Babesia caballi* from horses in South Africa. Veterinary Parasitology, 159: 112-120.

Cacciò S, Cammà C, Onuma M, Severini C (2000). The beta-tubulin gene of *Babesia* and *Theileria* parasites is an informative marker for species discrimination. International Journal for Parasitology, 30: 1181-1185.

Creek D, Anderson J, McConville M, Barrett M (2012). Metabolomic analysis of trypanosomatid protozoa. Molecular and Biochemical Parasitology, 181: 73-84.

Dave M, Higgins PD, Middha S, Rioux KP (2012). The human gut microbiome: current knowledge, challenges, and future directions. Translational Researh: The Journal of Laboratory and Clinical Medicine, 160: 246-257.

Dettmer K, Aronov PA, Hammock BD (2007). Mass spectrometry-based metabolomics. Mass Spectrometry Reviews, 26: 51-78.

Fritz D (2010). A PCR study of piroplasms in 166 dogs and 111 horses in France (March 2006 to March 2008). Parasitology Research, 106: 1339-1342.

Geoffrey AP, Peter JH (2015). The Newest "Omics"-Metagenomics and Metabolomics-Enter the Battle against the Neglected Tropical Diseases. PLoS Neglected Tropical Diseases, 9: e0003382. Doi: 10.1371/journal.pntd.0003382.

Heim A, Passos LM, Ribeiro MF, Costa-Júnior LM, Bastos CV, Cabral DD, Hirzmann J, Pfister K (2007). Detection and molecular characterization of *Babesia caballi* and *Theileria equi* isolates from endemic areas of Brazil. Parasitology Research, 102: 63-68.

Husain A, Sato D, Jeelani G, Mi-ichi F, Ali V, Suematsu M, Soga T, Nozaki T (2010). Metabolome analyses revealed increase in S-methylcysteine and phosphatidylisopropanolamine synthesis upon L-cysteine deprivation in the anaerobic protozoan parasite Entamoeba histolytica. Journal of Biological Chemistry, 285: 39160-39170.

Jeelani G, Sato D, Husain A, Escueta-de Cadiz A, Sugimoto M, Soga T, Suematsu M, Nozaki T (2012). Metabolic profiling of the protozoan parasite Entamoeba invadens revealed activation of unpredicted pathway during encystation. PLoS One, 7: e37740. DOI: 10.1371/journal.pone.0037740.

Kafsack B, Llinás M (2010). Eating at the table of another: metabolomics of host-parasite interactions. Cell Host and Microbe, 7: 90-99. DOI: http://dx.doi.org/10.1016/j.chom.2010.01.008.

Knowles DP Jr, Perryman LE, Goff WL, Miller CD, Harrington RD, Gorham JR (1991). A monoclonal antibody defines a geographically conserved surface protein epitope of *Babesia equi* merozoites. Infection and Immunity, 59: 2412-2417.

Kumar S, Kumar Y, Malhotra DV, Dhar S, Nichani AK (2003). Standardisation and comparison of serial dilution and single dilution enzyme linked immunosorbent assay (ELISA) using different antigenic preparations of the *Babesia (Theileria) equi* parasite. Veterinary Research, 34: 71-83.

Lakshmanan V, Rhee K, Daily J (2011). Metabolomics and malaria biology. Molecular and Biochemical Parasitology, 175: 104-111.

Li Q, Lostumbo G (2010). Proteomic analyses of a variety of intracellular bacterial species infecting different host cell lines. Current Proteomics, 7: 222-232.

Mingqun L, Takane K, Heather M, Brewer A, Norbeck D, Yasuko R (2011). Global proteomic analysis of two tick-borne emerging zoonotic agents: *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis*. Frontier in Microbiology. 2: 1-19.

Morgan XC, Huttenhower C (2014). Meta'omic analytic techniques for studying the intestinal microbiome. Gastroenterology 146: 1437–1448 e1431. Doi: 10.1053/j.gastro.2014.01.049.

Nelson CM, Herron MJ, Felsheim RF, Schloeder BR, Grindle SM, Chavez AO, Kurtti TJ, Munderloh UG (2008). Whole genome transcription profiling of *Anaplasma phagocytophilum* in human and tick host cells by tiling array analysis. BMC Genomics, 9: 364. Doi: 10.1186/1471-2164-9-364.

Nicolaiewsky TB. Richter MF, Lunge VR, Cunha CW, Delagostin O, Ikuta N (2001). Detection of *Babesia equi* (Laveran, 1901) by nested polymerase chain reaction. Veterinary Parasitology, 101: 9-21.

Old WM, Meyer-Arendt K, Aveline-Wolf L, Pierce KG, Mendoza A, Sevinsky JR, Resing KA, Ahn NG (2005). Comparison of label-free methods for quantifying human proteins by shotgun proteomics. Mol. Cell. Proteomics, 4: 1487-1502.

Rampersad J, Cesar E, Campbell MD, Samlal M, Ammons D (2003). A field evaluation of PCR for the routine detection of *Babesia equi* in horses. Veterinary Parasitology, 114: 81-87.

Rothschild C, Knowles D (2007). Equine piroplasmosis. In Equine Infectious Diseases, ed. Sellon DC, Long Mt. Saunders, Elsevier, St. Louis; pp 465-473.

Salih DA, El Hussein, Singla LD (2015). Diagnostic approaches for tick-borne haemoparasitic diseases in livestock. Journal of Veterinary Medicine and Animal Health. 7: 45-56. Doi: 10.5897/JVMAH2014.0345.

Salim BO, Hassan, SM, Bakheit MA, Alhassan A, Igarashi I, Karanis P, Abdelrahman MB (2008). Diagnosis of *Babesia caballi* and *Theileria equi* infections in horses in Sudan using ELISA and PCR. Parasitology Research, 103: 1145-1150.

Scheltema R, Decuypere S, T'Kindt R, Dujardin J, Coombs G, Breitling R (2010). The potential of metabolomics for Leishmania research in the post-genomics era. Parasitology, 137:1291-1302. DOI: http://dx.doi.org/10.1017/S0031182009992022.

Seo GM, Cheng C, Tomich J, Ganta RR (2008). Total, membrane, and immunogenic proteomes of macrophage- and tick cell-derived *Ehrlichia chaffeensis* evaluated by liquid chromatography-tandem mass spectrometry and MALDI-TOF methods. Infection and Immunity, 76: 4823-4832.

Shi L, Chowdhury SM, Smallwood HS, Yoon H, Mottaz-Brewer HM, Norbeck AD, Mcdermott JE, Clauss TR, Heffron F, Smith RD, Adkins JN (2009). Proteomic investigation of the time course responses of RAW 264.7 macrophages to infection with *Salmonella enterica*. Infection and Immunity, 77: 3227-3233.

Singu V, Liu H, Cheng C, Ganta RR (2005). *Ehrlichia chaffeensis* expresses macrophage- and tick cellspecific 28-kilodalton outer membrane proteins. Infection and Immunity, 73: 79-87.

Sumbria D, Singla LD (2015). Recent diagnostic and control approaches in equine piroplasmosis. Veterinaria. 2: 29-32.

Terkawi MA, Thekisoe OM, Katsande C, Latiff AA, Mans BJ, Matthee O, MKize N, Mabogoane N, Marais F, Yokoyama N, Xuan X, Igarashi I (2011). Serological survey of *Babesia bovis* and *Babesia bigemina* in cattle in South Africa. Veterinary Parasitology, 182: 337-342.

Timothy P, Nicolas H, Sushmita B, Edward J (2013). Metabolomics and Protozoan Parasites. Acta Parasitologica, 58: 127-131.

Ueti MW, Palmer GH, Kappmeyer LS, Scoles GA, Knowles DP (2003). Expression of equi merozoite antigen 2 during development of *Babesia equi* in the midgut and salivary gland of the vector tick *Boophilus microplus*. Journal of Clinical Microbiology, 41: 5803-5809.

USAHA (2011). One hundred and fourteenth annual meeting of the United States Animal Health Association: 11-17 November 2011. Minneapolis: Richardson Printing; pp 412-413.

Vincent I, Creek D, Burgess K, Woods D, Burchmore R, Barrett M (2012). Untargeted metabolomics reveals a lack of synergy between nifurtimox and eflornithine against *Trypanosoma brucei*. PLoS Neglected Tropical Disease, 6: e1618. DOI: 10.1371/journal.pntd. 0001618.

Yozwiak NL, Skewes-Cox P, Stenglein MD, Balmaseda A, Harris E (2012). Virus identification in unknown tropical febrile illness cases using deep sequencing. PLoS Neglected Tropical Diseases, 6: e1485. Doi: 10.1371/journal.pntd.0001485.

Zimmer JS, Monroe ME, Qian WJ, Smith RD (2006). Advances in proteomics data analysis and display using an accurate mass and time tag approach. Mass Spectrometry Reviews, 25: 450-482.

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