

Dept. of Vet. Med.,  
Faculty of Vet. Med., Cairo Univ.,  
Head of Dept. Prof. Dr. A.A. Salem.

**FURTHER STUDIES ON THE EFFICACY OF THE LOCALLY  
PREPARED BABESIA EQUI VACCINE ON EQUINE  
PIROPLASMOSIS COMPARED WITH IMPORTED ONE**  
(With 6 Tables)

By

**A.A. SALEM; SAMIA, A.H.; M.A. EL-SEIFY\*  
and G.H. EL-BATTAWY\*\***

(Received at 3/7/1989)

دراسات متقدمة عن مدى كفاءة لقاح البابينزيا الكواى المحضر محلياً على مرض ملاريا  
الخيول مقارناً بآخر مستورد

عبد المنعم سالم ، سامية عبد الحفيد ، محمود الصيفى ، جمال البطاوى

فى هذه الدراسة تم تحضير لقاح من عترة البابينزيا الكواى وقد أجريت تجارب لإختبار  
مدى كفاءته على درجة إصابة الفصيلة الخيلية بمرض الملاريا . وقد قورن هذا اللقاح بآخر  
مستورد من الولايات المتحدة الأمريكية من ناحية تأثير كل منهما على حالة الدم والحالة  
الإكلينيكية للحيوان . ويتضح من نتائج الدراسات المختلفة أن كلاً من اللقاحين ( المحلى  
والمستورد ) يعطى حماية للحيوانات المحصنة بنسبة ٦٦.٦٦٪ على الرغم من أن جرعة التحصين  
من اللقاح المحلى نصف الجرعة المستخدمة من اللقاح المستورد . و من نتائج التجارب  
التي أجريت فى هذا البحث نوصى بإستخدام اللقاح المحلى خصوصاً للخيول القيمة وذلك لقلّة  
التكاليف وتوفيراً لتكاليف الإستيراد وحيث أنه سيكون فى المتناول عند طلب إستخدامه .

**SUMMARY**

In this study a killed vaccine from a locally isolated *Babesia equi* strain was prepared and subjected to different studies. The efficacy of this vaccine was compared with an imported killed *B. equi* vaccine prepared in U.S.A.

The results revealed a slight clinical changes due to vaccination by both local and imported vaccines and challenge by both infected blood & ticks. It was concluded that both vaccines were nearly equal in immunological properties and protecting donkeys, inspite of the component of the local vaccine in half that of the imported one, as confirmed by clinical, haematologica! and seriological investigations.

\* Dept. of Parasitology, Faculty of Vet. Med., Beni-Suef.

\*\* Dept. of Vet. Med., Faculty of Vet. Med., Cairo Univ.

A.A. SALEM, et al.**INTRODUCTION**

Equines play an important role in the economic Nation as it is used for breeding and exportation. In Egypt, equines are infected by several diseases of which babesiosis is one of the important enzootic diseases in several areas. In these areas although horses are premunized against piroplasmosis and act as reservoir of infection, yet this immunity may break down under adverse condition and such animals become clinically infected (THEILER, 1906).

Concerning the work done on the equine premunization against B. equi, SMITH, et al. (1893) suggested the first method for premunized healthy animals against piroplasmosis by injecting them subcutaneously with blood of patent carriers. THEILER (1908) attenuated Babesia without interfering with their immunized properties after four passages in donkey foal for purposes of vaccination. Also NEITZ (1956); MAURER (1962) and SERGENT (1963) reported that, equines can protected for a period ranged between 1 & 5 years by superinfection by B. equi parasites. SINGH, et al. (1981) immunized donkeys against B. equi infection using killed prepared from infected erythrocytes or infected plasma collected during the peak of parasitaemia from a splenectomized donkey and they were found that all vaccinated donkeys were protected.

SALEM, et al. (1986) studied vaccination of donkeys with killed B. equi vaccine resulted in 66.66% immunity as confirmed by challenge using nymphs of Rhipicephalus turanicus ticks 63 days after vaccination. It was found that of great importance to use the vaccines in the control of equine piroplasmosis, therefore, it was necessary to carry out further investigation on this problem.

**MATERIAL and METHODS****I - Animals:**

Fourteen donkeys of about 10-12 months old, were used to study the immune response of Babesia equi vaccines. Seven animals were employed for testing the local prepared vaccine, whereas the other 7 were for testing American B. equi one. All these animals were approved to be B. equi negative.

**II - Vaccines:****1. Preparation:**

- a) Imported Babesia equi vaccine from U.S.A.: A killed vaccine supplied in lyophilized ampoules contained  $1 \times 10^6$  parasitic fraction stored at - 8 C and obtained from protozoology Department U S D A, Beltsville, Myrland, USA.
- b) Locally prepared Babesia equi vaccine, which was prepared as following according to the method described by RISTIC & SIBINOVIC (1964) with a modification of adding special white mineral oil-Copetrole WM2 as adjuvant:

## EQUINE PIROPLASMOSIS

- \* Latent Babesia equi infected donkey of 12 month splenectomized.
- \* Rectal temperature was recorded and blood films were examined daily.
- \* When the parasitaemia attained 30%, the donkey blood was aseptically collected in an equal volume of sterile Alsever's solution.
- \* Three washing of the erythrocytes were applied by centrifugation at 1,500 k.p.m. after successive suspension from the packed erythrocytes was prepared by adding three volumes of packed erythrocytes.
- \* The suspension was twice sonically oscillated in continuous flow ultrasonicator with a current out put of 4.5 ampers and a lysate flow of 50 ml/min.
- \* The oscillated material was sedimented in refrigerated centrifuge at 3,300 r.p.m./30 min.
- \* To each 100 ml of the supernatant lysate, 0.5 of protamine sulphate dissolved in 10 ml of distilled water was added.
- \* The mixture was then left for 2 hours at 4°C and centrifuged at 3,300/30.
- \* Two volumes of phosphate-buffered physiological salt solution PH. 9.0 were added to each volume of precipitate.
- \* Afterwards, the mixture was homogenized with Brock glass grinder and stored for at least 48 hours at -65°C.
- \* The precipitate was then rehomogenized with the same grinder and centrifuged at 12,100 r.p.m./30, the supernatant fluid was called the precipitinogen.
- \* The precipitinogen was mixed in an equal volume of oil adjuvant by syring technique and stored at -65°C in vials containing 4 ml. Each 1 ml contained  $0.6 \times 10^6$  B. equi fraction. Thimersol was added in a concentration of 1:10000 when stored for more than 3 weeks.

## 2. Vaccination of the animals:

The animals used were divided into 2 groups each one contained 7 B. equi free donkeys.

- \* First group subdivided into 4 donkeys (no: 1, 2, 3 & 4) vaccinated by locally prepared vaccine by a dose of 4 ml (each 1 ml contained  $2.4 \times 10^6$  killed B. equi fraction) i.m. in the neck region, and 3 animals (no: 5, 6 & 7) were left as non-vaccinated.
- \* Second group also subdivided into 4 donkeys (no: 8, 9, 10 & 11) vaccinated by U.S.A. obtained vaccine by a dose of 2 ml (each 1 ml contained  $1 \times 10^6$  killed B. equi fraction S/C in the neck region and 3 animals (no: 12, 13 & 14) were left as non-vaccinated.

## III- Challenge of vaccinated and non-vaccinated (control) animals:

The challenge of vaccinated and control donkeys was done 10 weeks after vaccination by 2 methods:

i) By using B. equi infected blood:

Each animal inoculated by 100 ml heparinized blood infected by B. equi obtained from infected donkey showing 30-35% parasitaemia.

ii) And by using B. equi infected Rhipicephalus turanicus ticks

Adults (males & females) of R.turanicus ticks which were bred on B. equi infected donkeys were used. The ticks were applied in ear cage on one ear of each donkey according to the method of ROBY & ANTHONY (1963).

The challenge occurred for 5 animals 3 vaccinated and 2 non-vaccinated in each group. The challenge tacked place using B. equi infected blood for 2 animal (vaccinated 8 non-vaccinated of both 2 groups) and infected Rhipicephalus turanicus ticks for 2 vaccinated and 1 non-vaccinated animals among 1st & 2nd groups. All vaccinated and control animals were investigated for any clinical abnermalities and weekly subjected to the following studies:

- A- Parasitological examination of blood films according to the method described by SHUTT (1962).
- B- Blood pictures including determination of:
- 1- Erythrocytic count According to the method described by JOHN (1977).
  - 2- Haemoglobin content According to the method described by SCHALM (1961).
  - 3- Total and differential leukodytic counts (neutrophils %; Eosinophils %; Basophils %; Lymphocytes % and Monocytes %) according to the method described by JOHN (1977).
- C- Serological studies including:
- 1- Complement fixation test (C F T) according to the method describd by TENTER and FRIEDHOFT (1986).
  - 2- Fossive haemaglutination test (P H A) according to the method described by GOTY (1982).

## RESULTS

The results of parasitological and clinical investigations as displayed in Table (1) revealed that donkeys vaccinated with local B. equi vaccine did not show any changes in the body temperature after vaccination except one animal where it was elevated to 39.3°C for one week post-vaccination, then the temperature returned to normal without any medical interference. Also blood films examination revealed the absence of B. equi for 3 weeks post-vaccination. After challenge with infected blood, the animals did not show any clinical manifestations and the blood remained B. equi free for 8 weeks post-challenge. Otherwise, the animal was kept Babesia free for 18 weeks. By the same manner one animal of those vaccinated and challenged infected Rhipicephalus turanicus ticks does not show any clinical or parasitological manifestations.

## EQUINE PIROPLASMOSIS

On the other hand the other animal which was vaccinated and challenged by the infected ticks showed B. equi in the blood films from 2<sup>nd</sup> week post-challenge. In non-vaccinated and challenged by both infected blood and ticks the Babesia parasite appeared in the blood of first animal in the 2<sup>nd</sup> week post-challenge for 5 weeks then disappeared and in the 1<sup>st</sup> week post-challenge till the end of the experiment.

Similarly, the clinical and blood film examinations of donkeys vaccinated by American strain of B. equi vaccine, the results as shown in table (2) revealed that, the vaccinated animals challenged with both infected blood and ticks showed infected R.B.Cs. within 2 weeks post-challenge with elevation in body temperature.

The results of blood picture studies indicated (table 3) increase in total erythrocytic count, haemoglobin content, total leukocytic count and basophils of all vaccinated animals by locally prepared B. equi vaccine. While those given the American strain showed (table 4) decrease in total leukocytic count.

Concerning the serological studies of vaccinated and challenged donkeys (tables 5 & 6) indicated that the vaccinated animals by local adjuvant B. equi vaccine showed the titer of complement fixation test (C F T) ranged between 1:20 and 1:320 and that of passive haemagglutination test (P H A) ranged between 1:2 and 1:64. Then the titer of C F T declined to disappear to zero after 5 & 6 weeks post-challenge, while the P H A test indicated that the titers remained constant in some vaccinated animals till the end of the experiment and decreased to the following dilution in another. Also the vaccinated animals by American strain of B. equi vaccine showed that the vaccinated animals were negative to C F T & P H A before experimentation and after vaccination both techniques were positive, while the non-vaccinated control animals showed negative results till end of the experiment (21 weeks).

## DISCUSSION

The present investigation was directed to perform further studies for immunizing animals against babesiosis by locally prepared Babesia equi vaccine and compare the efficacy of this vaccine with that of B. equi vaccine imported from U.S.A. on donkeys.

In the study using the local B. equi vaccine resulted in mild reaction at the site of inoculation (slight swelling and hotness) in all vaccinated donkeys, and slight elevation of body temperature (39.3°C) in one animal for one week only. This vaccinal reaction may be attributed to the lower absorbability of the oily portion of the vaccine which acts as foreign material or may be due to delayed cutaneous hypersensitivity of vaccinated animals as reported by SMITH, *et al.* (1979) and PRASAD and BANERJEE (1985). On the other hand the results of using American B. equi vaccine showed no local post-vaccinal reaction at the site of inoculation in donkeys, but elevation of body temperature (39.5°C) during the 1<sup>st</sup> week post-vaccination. The absence of local reaction might be attributed to that this vaccine was reconstituted in distilled water, whereas

post-vaccinal temperature may be due to hypersensitivity of the animal body to the foreign portion of this vaccine.

Vaccinated donkeys were exposed to challenge and the results revealed a slight elevation of body temperature during the 2<sup>nd</sup> & 3<sup>rd</sup> weeks then declined to normal without any treatment. On the other hand, body temperature of control non-vaccinated challenged donkeys reached 39.1°C during the 2<sup>nd</sup> week post-challenge, then declined to normal after administration of Imizol at a dose rate of 2 ml/100 kg.B.W. in 2 doses at 72 hours intervals. This higher temperature of control animals was attributed to parasitaemia as proved by examination of blood films, which could be overcome by treatment. Examinations of stained blood films revealed Babesia organisms in one of vaccinated donkeys after challenge through out the experimental period, this matter was not met in animals challenged by infected ticks which were more virulent. These agreed with that obtained by DALGLIESH (1968), SMITH, et al. (1979), KUTTER and JOHNSON (1980), DEVOS, et al. (1982), KUTTER, et al. (1982), GOODGER, et al. (1985), PIPANO, et al. (1985) and WRIGHT, et al. (1985) who reported a higher body temperature among control non-vaccinated donkeys than among vaccinated animals, and was mostly attributed to parasitaemia. Also, the challenge of the animals which were vaccinated by American B. equi strain produced an elevation in the body temperature ranged 38.9-39.9°C. This fever may be attributed to parasitaemia which was proved by blood film examination.

Haematological investigation of donkeys inoculated by local B. equi vaccine revealed an increase in total erythrocytic count, haemoglobin content, total leukocytic count and basophils of all animals, with 3 out of 4 donkeys increase in neutrophils; and decrease in eosinophils, and lymphocytes in 3 out of 4 animals post-vaccination. These results agreed with those of NETTO and RIBERIO (1955); SALEM, et al. (1986) and TRUMAN and Mc LENEN (1987). On other hand the obtained results were not in full agreement with TIMMS, et al. (1984) who reported only an increase in lymphocytes post-vaccination. While haematological investigation of donkeys inoculated by American B. equi strain revealed an increase of total erythrocytic count, haemoglobin content and monocytes of 2 and of 4 animals post-vaccination. On the other hand there were a decrease in total leukocytic count in all animals, and a decrease in basophils in 3 out of 4 donkeys and also a decrease in lymphocytes in 2 animals.

After challenge of animals vaccinated by locally prepared vaccine, there was a decrease in total erythrocytic count, haemoglobin content, total leukocytic count and basophils of all vaccinated animals with 2 out of 3 donkeys decrease in neutrophils and monocytes, and increase in eosinophils of all animals and the lymphocytes increased in 2 of them. These agreed with that of MALHERB (1956); ROJAS LISCANO (1960); ROBERTS, et al. (1962); CARPIO (1972); ALLEN, et al. (1975); RUDOLPH, et al. (1975); RAMADAN and BAUER (1978); FUTTER, et al. (1980); NAFIE (1980); HOSNEY, et al. (1982) and SALEM, et al. (1986) who reported a decrease in neutrophils of animals post-vaccination and disagreed with ROJAS LISCANO (1960); RANDY and MISHIRA (1977); RAI, et al. (1982) who reported that lymphocytes decreased in animals post-infection.

## EQUINE PIROPLASMOSIS

The challenge of animals that vaccinated by American killed B. equi vaccine resulted in a decrease in total erythrocytic count and eosinophils in 2 out of 3 donkeys, but the basophils decreased in all animals. On the other hand the increased of lymphocytes in all animals post-challenge can be attributed to activity of immune system.

Concerning the serological response for the locally prepared B. equi vaccine by using complement fixation and passive haemagglutination tests. The complement fixation test showed 1:20 - 1:80 positive titer at the 1<sup>st</sup> week and 1:320 at the 7<sup>th</sup> week post-vaccination. This titer decreased to 1:80 at 1<sup>st</sup> week post-challenge and disappeared at 5<sup>th</sup> and 6<sup>th</sup> weeks post-challenge. Also the using of the passive haemagglutination test on donkeys vaccinated by the local vaccine resulted in 1:2-1:8 positive titer at the 2<sup>nd</sup> week then elevated till 1:32-1:64. This titer was declined to 1:16-1:32 in animals during the 1<sup>st</sup> week post-challenge and remained constant till the end of experiment. The obtained results agreed with those obtained by SIBINOVIC, et al. (1969); GOODGER and MAHONEY (1974); MAHONEY, et al. (1979); ABD EL-HADY (1981); SINGH and GAUTAM (1981) and GOTY (1982) who reported that the complement fixation test was sensitive one specially in early infection. Using the C F T & P H A on donkeys vaccinated by American vaccine revealed detectable C F titer (1:10-1:80) after vaccination and (1:20-1:40) after challenge. Also the passive haemagglutination titer was detectable at the 1<sup>st</sup> & 2<sup>nd</sup> week post-vaccination, then declined at the 1<sup>st</sup> week post-challenge.

It was concluded from the results of this study that although vaccine prepared from the locally isolated Babesia organisms has half the dose of that imported one, yet, the two vaccines gave equal immunizing properties.

Therefore, the local prepared vaccine preferred than the imported one due to the higher immunogenicity, the less cost and less time in obtaining such vaccine. It is also advised to use this local vaccine for immunizing of expensive animals to raise their resistance against infection and to maintain babesiosis free animals from the clinical point of view.

## REFERENCES

- Abd El-Hady, S.M.R. (1981): The incidence of Babesia equi in horses and donkeys by the haemagglutination test. Thesis of M.V.Sc. for Faculty of Veterinary Medicine, Cairo University, Egypt.
- Allen, P.C.; Frerichs, W.M. and Holbrook, A.A. (1975): Experimental acute B. caballi infection. I: Red blood cell dynamics. Experimental parasitology 73 (1), 67-77.
- Carpio, J. (1972): First report (in Peru) of equine babesiosis due to Babesia equi. Revista de investigaciones pecuarias, Peru, 1 (2), 177-183.
- Dagliesh, R.J. (1968): Field observation on B. argentina vaccination in Queensland. Aust. Vet. J., 44, 103-104.

- De vos, A.J.; Combrink, M.P. and Ressenger, R. (1982): B.begemina vaccine: composition of the efficacy and safety of australian and south african strains under experimental conditions in south africa. Onderstepoort J. Vet. Res., 49, 155-158.
- Futter, G.J.; Belonje, P.C. and Berg, A. Ven Den (1980): Studies of feline babesiosis, 3. Haematologica findings. Journal South African Vet. Assoc., 51 (4), 271-280.
- Goodger, B.V. and Mahoney, D.F. (1974): Evaluation by the passive haemagglutination test for diagnosis of B.argentina infection in cattle. Aust. Vet. J., 50 (6), 246-249.
- Goodger, B.V.; Wright, I.G.; Waltisbuhl, D.J. and Mirre, G.B. (1985): B.bovis, successful vaccination against homologous Challenge in splenectomized calves using a fraction of haemagglutination antigen.
- Goty, F. (1982): Suitability of ELISA, IFAT, IHA and CFT for detecting B.equi infection. Inaugural Dissertation, Ludwig-Maximilians. Universitate, Munchen, 39 pp.
- Hosney, Z.; Hafez, M.A. and Edries, M. (1982): On equine babesiosis: Significance of blood cytological examination of horses and donkeys. Assiut Vet. Med. J., 9 (17), 34-37.
- John, B. Mail (1977): Laboratory Medicine haematology. 5th Ed. C.V. Mosby Co.
- Kutter, K.L. and Johnson, L.W. (1980): Immunization of cattle with a B.bigemina antigen in Am. Freund's complete adjuvant. J. Vet. Res., 41 (4), 536-538.
- Kutter, K.K.; Levy, M.C. and Ristic, M. (1982): Efficacy of non-viable culture-derived B.bovis vaccine. Am. J. Vet. Res., 43 (2), 281-284.
- Mahoney, D.F.; Wright, I.G. and Goodger, B.V. (1979): Immunity in cattle to B.bovis after single infection with parasites of various origin. Aust. Vet. J., 55(1), 10-12.
- Malherbe, W.D. (1956): The manifestation and diagnosis of Babesia infections. Ann. N.Y. Acad. Sci., 64, 128-146.
- Nafie, T.S.A. (1980): Effect of blood parasitic infestation - Equine piroplasmosis on the picture of some trace elements in equines. Thesis, M.V.Sc., Faculty of Vet. Med., Assiut Univ., Egypt.
- Neitz, N.O. (1956): Classification, Transmission and Biology of piroplasma of Domestic Animals. Ann. New York Acad. Sci., 64, 56-111.
- Netto, A.R. and Riberio, I.F. (1955): Changes in the haemoglobin, plasma protein and haematocrit Values during preimmunization of cattle against piroplasmosis and anaplasmosis. Rev., Fac. Med. Vet., S. paulo, 5, 317-334.
- Pandey, N.N. and Mishra, S.S. (1977): Studies on the haematological changes and blood glucose level in B.begemina in indigenous cow calves. Indian Vet. J., 54 (11), 880-883.
- Pipano, E.; Krigel, Y.; Markovics, A.; Rubinstein, E. and Frank, M. (1985): Mitigation of the response of friesian calves to live B.bovis vaccine by treatment with long acting oxytetracycline. The Vet. Rec., 10, 413-414.
- Prasad, K.D. and Banerjee, D.P. (1985): Immune response in cattle vaccination with killed and irradiated B.bigemina vaccines. Indian J. of Vet. Med., 5 (2), 88-92.



## EQUINE PIROPLASMOSIS

- Rai, R.B.; Dhirendra, S. and Singh, N.P. (1982): Clinico-pathological studies on bovine babesiosis. *Haryana Veterinarian*, 21 (1), 11-16.
- Ramadan, P. and Bauer, M. (1978): Blood picture in babesiosis in dog. *Veterinarski Archive*, 48 (5), 251-256.
- Ristic, M. and Sibinovic, S. (1964): Equine babesiosis: Diagnosis by precipitation in Gel and a one step fluorescent antibody inhibition test. *Am. J. Vet. Res.*, 25, 1519-1526.
- Roberts, E.D.; Morehouse, L.G.; Gainer, S.H. and Danial, H.A. (1962): Equine piroplasmosis. *J. Am. Vet. Med. Assoc.*, 141, 1323-1329.
- Roby, T.O. and Anthony, D.W. (1963): Transmission of Equine piroplasmosis by *Dermacentor nitens* (Neuman). *J. Am. Vet. Med. Assoc.*, 142 (7), 768-769.
- Rojas Liscano, D.R. (1960): Changes in the blood picture during premunization of cattle against protozoal disease. *Rev. Med. Vet. Parasit.*, Maracay, 18, 193-194.
- Rudolph, W.; Corea, J.; Zurita, L. and Manely, W. (1975): Equine piroplasmosis: leukocytic response to *B.equii* (Laveran, 1910) infection in Chile. *British Vet. J.*, 131 (5), 601-609.
- Salem, A.A.; El-Battawy, G.; El-Seify, M.A.; Selim, M.K. and Hosney, Z. (1986): Preliminary investigation on haematological and biochemical picture of blood of premunized donkeys against *B.equii* *Assiut Vet. Med. J.*, 16 (31), 147-157.
- Schalm, O.W. (1961): *Veterinary Haematology*. 1st Ed. Lea and Febiger, Philadelphia, United State of America.
- Sergent, E. (1963): Latent infection and premunition. Some definitions of microbiology and immunology. Granham, Pierce, and Roitt *Immunity to protozoa*, 39-47. (Black well scientific publications, Oxford, 1963).
- Shutt, P.G. (1962): Special article: the staining of malaria parasites. *Transactions of the Royal Society of Tropical Medicine & Hygiene*, 60 (3), 412-419.
- Sibinovic, S.; Sibinovic, H. and Ristic, M. (1969): Equine babesiosis: Diagnosis by bentonite agglutination and passive haemagglutination tests. *Am. J. Vet. Res.*, 30 (5), 691-695.
- Singh, B. and Gautam, O.P. (1981): A comparison of serological tests for the diagnosis of equine babesiosis. *Indian J. of Vet. Med.*, 1 (1), 44-50.
- Singh, B.; Gautam, O.P. and Banerjee, D.P. (1981): Immunization of donkeys against *B.equii* infection using killed vaccine. *Veterinary parasitology*, 8 (2), 133-136.
- Smith, F.; Kilbron, F.L. and Schroeder, W.F. (1893): Additional observation in Texas cattle fever. *Bureau of animal Industry, U.S. Dep. of Agric., Bull.* 3, 67-72.
- Smith, R.D.; Carpenter, J.; Cabera, A.; Gravel, S.M.; Erp, E.E.; Osorno, M. and Ristic, M. (1979): Bovine babesiosis: vaccination against tick-borne challenge exposure with culture-derived *B.bovis* immunogens. *Am. Vet. Res.*, 40 (2), 1678-1682.
- Tenter, A.M. and Friedhoff, K.T. (1986): Serodiagnosis of experimental and natural *B.equii* and *B.caballi* infections. *Vet. parasitology*, 20 (1/3), 49-60.
- Theiler, A. (1906): Further notes on piroplasmosis of the horse, mule and donkey. *Rep. Gov. Vet. Bact. Transv.*, pp. 94-106.

A.A. SALEM, et al.

- Theiler, A. (1908): Continuation of experiments for inoculation against piroplasmosis. Rep. Gov. Vet. Bact. Transv., pp. 13-23.
- Timms, P.; Stewart, N.P.; Rodwell, B.J. and Barry, D.N. (1984): Immune responses of cattle following vaccination with living and non-living B.bovis antigens. Vet. parasitology, 16 (3/4), 243-251.
- Trueman, K.F. and Mc Lennan, M.W. (1987): Bovine abortion due to prenatal B.bovis infection. Aust. Vet. J., 64 (2), 63.
- Wright, I.G.; Mirre, G.B.; Rode-Bramanis, K.; Chamberlain, M.; Goodger, B.V. and Waltisbuhl, D.J. (1985): Protective vaccination against virulent B.bovis with a low-molecular-weight antigen. Infection and Immunity, 48 (1), 109-113.

Table (1)  
Results of clinical and blood film examinations of donkeys vaccinated by oil adjuvant Babesia equi locally prepared vaccine

parameters of animal	vaccinated donkeys								Non-vaccinated control donkeys						
	challenged by infected							Non challenged control	challenged by infected				Non challenged		
	blood		ticks			4	blood		ticks		7				
	1	2	3	4	5		6	7							
weeks	Temp. °C	Bl.f.	Temp. °C	Bl.f.	Temp. °C	Bl.f.	Temp. °C	Bl.f.	Temp. °C	Bl.f.	Temp. °C	Bl.f.			
pre-vaccination	1	37.2	-ve	37.4	-ve	37.6	-ve	37.6	-ve	37.8	-ve	37.5	-ve	37.4	-ve
	2	37.4	-ve	37.2	-ve	37.2	-ve	37.6	-ve	37.8	-ve	37.5	-ve	37.5	-ve
	3	37.6	-ve	37.6	-ve	37.4	-ve	37.2	-ve	37.6	-ve	37.2	-ve	37.6	-ve
post-vaccination	1	38.0	-ve	38.0	-ve	39.3	-ve	37.0	-ve	37.9	-ve	37.0	-ve	37.5	-ve
	2	38.7	-ve	38.1	-ve	38.3	-ve	37.3	-ve	38.1	-ve	37.8	-ve	38.2	-ve
	3	38.7	-ve	37.5	-ve	38.3	-ve	38.3	-ve	38.5	-ve	38.5	-ve	38.2	-ve
	4	38.2	-ve	37.8	-ve	37.6	-ve	37.0	-ve	38.0	-ve	38.5	-ve	37.0	-ve
	5	38.2	-ve	37.2	-ve	37.5	-ve	37.7	-ve	38.2	-ve	38.0	-ve	37.5	-ve
	6	37.6	-ve	37.5	-ve	37.2	-ve	37.5	-ve	37.4	-ve	37.8	-ve	37.5	-ve
	7	37.8	-ve	37.6	-ve	37.7	-ve	37.3	-ve	37.9	-ve	38.5	-ve	37.0	-ve
	8	37.5	-ve	37.5	-ve	37.6	-ve	38.2	-ve	37.8	-ve	38.5	-ve	37.8	-ve
	9	37.7	-ve	37.0	-ve	37.5	-ve	38.4	-ve	37.8	-ve	37.4	-ve	37.2	-ve
	10	37.6	-ve	37.5	-ve	37.5	-ve	38.4	-ve	37.8	-ve	38.5	-ve	37.8	-ve
post-challenge	1	37.2	-ve	37.4	-ve	37.0	-ve	37.9	-ve	38.9	-ve	38.1	+ve	37.5	-ve
	2	38.6	-ve	38.5	+ve	38.5	-ve	38.4	-ve	39.1	+ve	39.1	+ve	37.5	-ve
	3	37.8	-ve	37.5	+ve	37.5	-ve	38.2	-ve	38.5	+ve	38.7	+ve	37.5	-ve
	4	37.6	-ve	37.7	+ve	37.5	-ve	38.0	-ve	38.7	+ve	38.0	+ve	37.0	-ve
	5	37.0	-ve	37.0	+ve	37.5	-ve	38.3	-ve	38.5	+ve	38.4	+ve	37.1	-ve
	6	37.7	-ve	37.8	+ve	37.5	-ve	38.0	-ve	38.3	+ve	38.3	+ve	37.2	-ve
	7	37.6	-ve	38.2	+ve	38.1	-ve	38.0	-ve	38.3	-ve	37.9	+ve	37.5	-ve
	8	37.7	-ve	38.2	+ve	38.0	-ve	38.1	-ve	38.2	-ve	38.1	+ve	37.5	-ve

\* Temp. = Temperature Bl.f. = Blood films

M.B. All vaccinated donkeys showed mild local reaction only at the site of injection including mild swelling & hotness

Table (2)  
Results of clinical and blood film examinations of donkeys vaccinated  
by killed american Babesia equi vaccine

parameters of animal	weeks	vaccinated donkeys								Non-vaccinated control donkeys					
		challenged by infected							Non challenged control	challenged by infected				Non challenged	
		blood		ticks			11	blood		ticks		14			
		8	9	10	11	12		13	14						
Temp. °C		Bl. f.		Temp. °C		Bl. f.		Temp. °C		Bl. f.		Temp. °C		Bl. f.	
pre- vaccination	1	37.7	-ve	38.7	-ve	37.7	-ve	37.6	-ve	37.8	-ve	37.5	-ve	37.4	-ve
	2	37.8	-ve	37.8	-ve	38.3	-ve	37.6	-ve	37.8	-ve	37.5	-ve	37.5	-ve
	3	37.9	-ve	37.0	-ve	37.0	-ve	37.2	-ve	37.6	-ve	37.2	-ve	37.6	-ve
post- vaccination	1	37.2	-ve	37.0	-ve	39.5	-ve	37.0	-ve	37.9	-ve	37.0	-ve	37.5	-ve
	2	37.9	-ve	37.8	-ve	38.9	-ve	37.3	-ve	38.1	-ve	37.8	-ve	38.2	-ve
	3	37.7	-ve	37.4	-ve	38.7	-ve	38.3	-ve	38.5	-ve	38.5	-ve	38.2	-ve
	4	38.0	-ve	37.0	-ve	38.5	-ve	37.0	-ve	38.0	-ve	38.5	-ve	37.0	-ve
	5	38.2	-ve	38.2	-ve	38.5	-ve	37.7	-ve	38.2	-ve	38.0	-ve	37.5	-ve
	6	38.0	-ve	37.6	-ve	38.5	-ve	37.5	-ve	37.4	-ve	37.8	-ve	37.5	-ve
	7	38.4	-ve	37.9	-ve	38.0	-ve	37.3	-ve	37.9	-ve	38.5	-ve	37.0	-ve
	8	37.8	-ve	37.9	-ve	38.3	-ve	38.2	-ve	37.8	-ve	38.8	-ve	37.8	-ve
	9	38.3	-ve	38.4	-ve	38.7	-ve	38.4	-ve	37.8	-ve	37.4	-ve	37.2	-ve
	10	38.4	-ve	38.4	-ve	38.4	-ve	38.4	-ve	37.8	-ve	38.5	-ve	37.8	-ve
post- challenge	1	38.3	-ve	38.0	-ve	38.0	-ve	37.9	-ve	38.9	-ve	38.1	+ve	37.5	-ve
	2	39.9	+ve	39.5	+ve	38.8	+ve	38.4	-ve	39.1	+ve	39.1	+ve	37.5	-ve
	3	38.8	+ve	39.0	+ve	37.8	+ve	38.2	-ve	38.7	+ve	38.5	+ve	37.5	-ve
	4	38.7	+ve	38.8	+ve	38.5	+ve	38.0	-ve	38.7	+ve	38.0	+ve	37.0	-ve
	5	38.0	-ve	38.8	+ve	38.3	-ve	38.3	-ve	38.5	+ve	38.4	+ve	37.1	-ve
	6	37.9	-ve	38.1	+ve	38.4	-ve	38.0	-ve	38.3	+ve	38.3	+ve	37.2	-ve
	7	37.9	-ve	37.2	+ve	38.4	-ve	38.0	-ve	38.3	-ve	37.9	+ve	37.5	-ve
	8	37.6	-ve	38.2	+ve	38.5	-ve	38.1	-ve	38.2	-ve	38.1	+ve	37.5	-ve

° Temp. = Temperature

Bl. f. = Blood films

N.B. All vaccinated donkeys showed no local reaction at the site of injection

A.A. SALEM, et al.

Table (3)

Results of blood picture in donkeys vaccinated by oil adjuvant *Babesia equi* vaccine

parameters	Animals	Blood picture	Total erythrocytic count	Haemoglobin content		Total leukocytic Count	Differential leukocytic count					
				g%	%		Neutrophils %	Eosinophils %	basophils %	Lymphocytes %	Mono-cytes %	
3 weeks pre-vaccination		1	04.647	58.000	09.417	10.383	35.667	03.000	00.000	56.670	01.667	
			00.262	00.471	00.068	00.496	01.440	00.817	00.000	02.326	00.272	
			2	05.093	63.000	10.083	16.867	41.000	06.333	00.333	50.000	01.007
				00.201	00.471	00.118	02.478	02.450	01.407	00.272	03.259	00.519
			3	04.633	65.330	10.250	12.367	31.667	06.667	00.000	58.200	03.667
				00.486	01.440	01.087	02.284	01.963	02.126	00.000	03.300	00.296
			4	04.360	55.000	09.000	12.067	35.670	01.330	04.670	56.670	01.670
00.171				00.566	03.070	00.270	00.270	02.600	00.270			
5	04.640	64.000	10.350	17.950	39.670	00.330	03.670	53.670	01.330			
	00.209	01.700	00.250	01.059	03.600	00.270	00.720	03.660	00.270			
6	06.350	69.670	11.170	19.217	41.330	01.330	01.000	56.000	01.330			
	00.307	00.544	00.140	02.784	06.330	00.270	00.470	06.400	00.270			
7	04.983	68.657	10.917	14.933	33.667	04.667	00.000	56.330	02.000			
	00.321	00.544	00.136	01.104	05.504	02.288	00.000	08.450	00.817			
10 weeks post-vaccination	vaccinated donkeys	1	04.861	63.800	10.250	14.595	40.600	02.300	00.500	53.300	01.700	
			00.250	01.190	00.190	01.154	02.822	00.247	00.255	02.864	00.302	
			2	05.112	65.700	10.575	16.870	33.700	04.500	01.200	27.200	02.300
				00.247	00.011	00.146	01.918	02.436	00.861	00.571	02.378	00.247
			3	05.087	66.800	10.700	14.065	40.300	02.700	00.800	53.900	02.200
				00.145	01.690	00.252	01.012	04.579	00.376	00.465	02.211	00.310
			4	05.276	57.300	09.325	11.530	27.700	06.600	04.600	51.300	01.600
00.174	00.820	00.142		00.442	03.067	01.401	00.697	00.155	01.200			
5	05.746	57.300	09.275	13.190	30.500	03.300	00.800	64.900	00.500			
	00.197	01.386	00.208	00.054	02.160	00.722	00.341	02.674	00.150			
6	06.158	63.200	10.200	13.555	37.800	03.500	01.500	56.000	00.800			
	00.273	02.230	00.346	00.771	02.630	01.171	00.765	02.047	00.276			
7	05.489	77.100	12.375	17.400	35.000	03.800	00.500	60.700	01.900			
	00.141	02.750	00.181	00.490	01.490	00.660	00.301	02.404	00.411			
8 weeks post-challenge	challenged by infected	blood	1	04.260	63.875	10.531	12.819	43.375	04.500	00.125	50.125	02.125
				00.134	00.389	00.082	00.655	02.292	00.771	00.117	03.370	00.328
				2	04.304	65.250	10.496	14.844	27.375	06.250	00.125	62.875
	00.143	00.125	00.112		00.996	02.622	00.117	00.117	03.457	00.328		
	3	04.825	66.625	11.156	09.763	37.750	05.750	00.000	55.500	01.375		
		00.120	01.520	00.238	00.440	02.737	01.284	00.000	03.226	00.346		
	4	05.740	62.375	10.630	12.731	37.500	04.750	01.375	54.875	01.375		
00.357		00.900	00.299	00.774	01.871	01.867	00.856	02.501	00.351			
5	04.640	48.750	07.844	11.563	38.750	02.625	00.375	55.125	03.125			
	00.424	02.320	00.359	01.114	02.920	00.466	00.351	02.706	00.372			
6	05.843	65.875	09.219	12.688	33.750	03.375	00.875	61.000	01.000			
	00.274	01.482	00.200	00.702	02.530	00.529	00.482	02.574	00.354			
7	05.158	78.375	12.500	11.494	32.000	02.875	00.000	62.500	01.000			
	00.200	01.286	00.214	00.551	01.358	00.375	00.000	01.194	00.306			

Table (4)

Results of blood picture in donkeys vaccinated by American *Babesia equi* vaccine

parameters of Animals		Blood picture	Total erythr-		Haemoglobin content		Total leukocytic Count	Differential leukocytic count				
			ocyte Count	gm	%	Neutrophils %		Eosino-phils %	Baso-phils %	Lympho-cytes %	Mono-cytes %	
3 weeks pre-vaccination		8	05.977 00.485†	65.000 00.943†	10.420 00.180†	10.350 01.616†	33.330 10.640†	02.330 00.980†	01.670 00.720	61.330 01.960†	01.000 00.020†	
		9	05.497 00.248†	65.970 01.440†	10.500 00.240†	15.717 01.731†	39.000 07.850†	02.670 00.720†	00.330 00.070	53.000 04.640†	01.670 00.270†	
		10	05.753 00.214†	60.000 01.886†	09.670 00.300†	15.383 01.516†	39.000 02.160†	02.330 00.720†	02.000 00.470	55.000 01.700†	01.330 00.270†	
		11	04.360 00.171†	55.000	09.000	12.067 00.566†	35.670 03.070†	01.330 00.270†	04.670 00.270†	56.670 02.600†	01.670 00.270†	
		12	04.640 00.209†	64.000 01.700†	10.330 00.250†	17.950 01.059†	39.670 03.600†	00.330 00.270†	03.670 00.720	53.670 03.660†	01.330 00.270†	
		13	06.350 00.307†	69.670 00.544†	11.170 00.140†	19.217 02.784†	41.330 06.330†	01.330 00.270†	01.000 00.470	56.000 06.480†	01.330 00.270†	
		14	04.983 00.321†	68.667 00.544†	10.917 00.136†	14.933 01.184†	33.667 05.504†	04.667 02.288†	00.000 00.000	56.330 08.450†	02.000 00.817†	
10 weeks post-vaccination	vaccinated donkeys	8	05.459 00.190†	57.100 00.842†	09.225 00.139†	10.540 00.209†	34.100 01.769†	02.500 00.292†	01.200 00.506	60.800 01.561†	01.400 00.323†	
		9	06.189 00.290†	66.600 01.340†	10.375 00.504†	13.145 00.713†	37.400 01.935†	02.400 00.696†	01.400 00.429	56.900 02.071†	00.900 00.411†	
		10	05.162 00.159†	56.800 00.360†	09.200 00.069†	14.110 01.348†	43.400 02.475†	02.900 00.877†	00.900 00.411	50.900 02.381†	02.000 00.520†	
	Non-vaccinated donkeys	11	05.276 00.174†	57.500 00.828†	09.325 00.142†	11.530 00.442†	27.700 03.087†	06.600 01.401†	02.600 00.897	51.900 08.155†	01.600 01.200†	
		12	05.746 00.197†	57.300 01.386†	09.275 00.208†	13.190 00.854†	30.500 02.160†	03.300 00.722†	00.800 00.341	64.900 02.674†	00.500 00.158†	
		13	06.158 00.273†	63.200 02.230†	10.200 00.0346	13.555 00.771†	37.800 02.630†	03.500 01.177†	01.500 00.765	56.600 02.847†	00.800 00.276†	
		14	05.489 00.141†	77.100 02.758†	12.375 00.181†	17.400 00.490†	35.000 01.490†	03.800 00.660†	00.500 00.381	60.700 02.404†	01.900 00.411†	
8 weeks post-challenge	challenged by infected ticks	8	04.191 00.208†	47.375 00.450†	06.221 00.122†	10.100 00.530†	34.000 01.912†	01.500 00.345†	00.125 00.117	62.500 00.031†	01.625 00.706†	
		9	05.368 00.208†	62.250 03.381†	09.563 00.537†	12.825 00.959†	35.625 01.503†	03.000 00.840†	01.125 00.598	58.125 01.736†	02.125 00.351†	
		10	05.306 00.266†	59.500 01.392†	09.563 00.621†	17.763 01.624†	42.250 04.643†	01.125 00.449†	00.625 00.466	52.375 04.500†	02.375 00.351†	
	Non-challenged control	11	05.740 00.357†	62.375 00.900†	10.630 00.299†	12.731 00.774†	37.500 01.871†	04.750 01.867†	01.375 00.856	54.875 02.540†	01.375 00.351†	
		12	04.640 00.424†	48.750 02.320†	07.844 00.359†	11.563 00.359†	38.750 02.920†	02.625 00.466†	00.375 00.351	55.125 02.786†	03.125 00.372†	
		13	05.843 00.274†	65.875 01.482†	09.219 00.200†	12.688 00.702†	33.750 02.530†	03.375 00.529†	00.875 00.482	61.000 02.574†	01.000 00.354†	
		14	05.158 00.200†	78.375 01.286†	12.500 00.214†	11.494 00.551†	32.000 01.358†	02.875 00.375†	00.000 00.000	62.500 01.104†	01.000 00.306†	

Table (5)  
Results of coeplement fixation (CFT) and passive haemagglutination (PHA) tests in donkeys vaccinated by the local oil adjuvant *Babesia equi* vaccine

parameters of animal	vaccinated donkeys								Non-vaccated control donkeys					
	challenged by infected						Non challenged		challenged by infected				Non challenged	
	blood		ticks						blood		ticks			
	4		5		6		7		8		9		10	
weeks	CFT	PHA	CFT	PHA	CFT	PHA	CFT	PHA	CFT	PHA	CFT	PHA	CFT	PHA
pre- vaccination	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-
post- vaccination	1	1:80	-	1:20	-	1:40	-	1:20	1:16	-	-	-	-	-
	2	1:80	1:2	1:40	1:8	1:40	1:2	1:20	1:32	-	-	-	-	-
	3	1:80	1:2	1:160	1:8	1:160	1:2	1:20	1:32	-	-	-	-	-
	4	1:80	1:2	1:320	1:16	1:160	1:2	1:20	1:32	-	-	-	-	-
	5	1:80	1:8	1:160	1:16	1:320	1:2	1:20	1:32	-	-	-	-	-
	6	1:80	1:8	1:320	1:16	1:320	1:8	1:40	1:64	-	-	-	-	-
	7	1:80	1:8	1:320	1:16	1:320	1:32	1:40	1:64	-	-	-	-	-
	8	1:80	1:8	1:160	1:32	1:160	1:32	1:40	1:64	-	-	-	-	-
	9	1:160	1:16	1:160	1:32	1:160	1:64	1:40	1:64	-	-	-	-	-
	10	1:160	1:32	1:160	1:64	1:160	1:64	1:40	1:32	-	-	-	-	-
post- challenge	1	1:80	1:16	1:8	1:32	1:80	1:32	1:40	1:32	1:20	1:16	1:20	-	-
	2	1:40	1:16	1:4	1:32	1:40	1:32	1:40	1:32	1:20	1:32	1:20	-	-
	3	1:40	1:16	1:2	1:32	1:40	1:32	1:20	1:32	1:20	1:32	1:40	1:8	-
	4	1:20	1:16	1:2	1:32	1:40	1:32	1:20	1:32	1:20	1:32	1:40	1:32	-
	5	-	1:16	-	1:32	1:20	1:32	1:10	1:16	1:40	1:32	1:40	1:32	-
	6	-	1:16	-	1:32	-	1:32	1:10	1:8	1:40	1:32	1:40	1:32	-
	7	-	1:16	-	1:32	-	1:16	1:10	1:8	1:40	1:32	1:40	1:32	-
	8	-	1:16	-	1:32	-	1:16	1:10	1:8	1:40	1:32	1:40	1:32	-

Table (6)  
Results of complement fixation (CFT) and passive haemagglutination (PHA) tests in donkeys vaccinated by killed American *Babesia equi* vaccine

parameters of animal	vaccinated donkeys								Non-vaccated control donkeys					
	challenged by infected						Non challenged		challenged by infected				Non Challenged	
	blood		ticks						blood		ticks			
	8		9		10		11		12		13		14	
weeks	CFT	PHA	CFT	PHA	CFT	PHA	CFT	PHA	CFT	PHA	CFT	PHA	CFT	PHA
pre- vaccination	1	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-
post- vaccination	1	-	1:16	1:10	-	1:10	-	1:20	1:16	-	-	-	-	-
	2	1:10	1:32	1:10	1:64	1:10	1:64	1:20	1:32	-	-	-	-	-
	3	1:20	1:32	1:10	1:64	1:10	1:64	1:20	1:32	-	-	-	-	-
	4	1:20	1:32	1:10	1:64	1:10	1:64	1:20	1:32	-	-	-	-	-
	5	1:20	1:32	1:10	1:64	1:10	1:64	1:40	1:32	-	-	-	-	-
	6	1:20	1:32	1:10	1:64	1:10	1:64	1:40	1:64	-	-	-	-	-
	7	1:20	1:64	1:10	1:64	1:10	1:64	1:40	1:64	-	-	-	-	-
	8	1:40	1:128	1:20	1:64	1:20	1:64	1:40	1:64	-	-	-	-	-
	9	1:40	1:128	1:40	1:64	1:20	1:128	1:40	1:64	-	-	-	-	-
	10	1:40	1:64	1:80	1:128	1:20	1:128	1:40	1:32	-	-	-	-	-
post- challenge	1	1:20	1:32	1:40	1:64	1:10	1:64	1:40	1:32	1:20	1:16	1:20	-	-
	2	1:20	1:32	1:20	1:64	1:10	1:64	1:40	1:32	1:20	1:32	1:20	-	-
	3	1:20	1:32	1:20	1:64	1:10	1:64	1:40	1:32	1:20	1:32	1:40	1:8	-
	4	1:20	1:32	1:20	1:64	1:10	1:64	1:20	1:32	1:20	1:32	1:40	1:32	-
	5	1:20	1:16	1:20	1:64	1:10	1:64	1:20	1:16	1:40	1:32	1:40	1:32	-
	6	1:20	1:16	1:20	1:64	1:10	1:64	1:10	1:8	1:40	1:32	1:40	1:32	-
	7	1:10	1:16	1:20	1:64	1:10	1:32	1:10	1:8	1:40	1:32	1:40	1:32	-
	8	1:10	1:16	1:10	1:32	1:10	1:16	1:10	1:8	1:40	1:32	1:40	1:32	-