รายงานวิจัย

เรื่อง

การศึกษาเปรียบเทียบหาความชุกของโรคทริปปาโนโซม ในโคนมของจังหวัดเชียงใหม่และลำพูน โดยวิธีทางปรสิตวิทยาและวิธีการ์ดแอกกลูติเนชัน

Comparative Study on Prevalence of Trypanosomosis of Dairy Cattle in Chiang Mai and Lam Phun Provinces Diagnosed by Hematocrit Centrifugation method and Card Agglutination technique

โดย

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งานนี้ได้รับทุนสนับสนุนจากงบประมาณเงินรายได้

คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

ประจำปี 2546

Research Report

on

Comparative Study on Prevalence of Trypanosomosis of Dairy Cattle in Chiang Mai and Lam Phun Provinces Diagnosed by Hematocrit Centrifugation method and Card Agglutination test

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Granted by Faculty of Veterinary Medicine,
Chiang Mai University 2003

Acknowledgements

The authors are extremely grateful to Prof. Dr. Dr. h.c.mult. Franz Hoerchner and Dr. Peter–Henning Clausen, Institute for Parasitology and Tropical Veterinary Medicine, Free University, Berlin, Germany for their guidance, encouragement, and facilities for Card Agglutination Technique. Our appreciation extends to Dr. Witaya Suriyasathapron for his help in statistical work. We are also thankful to all selected farm owners for their cooperation. We are highly indebted to Faculty of Veterinary Medicine, Chiang Mai University for the financial grant for this research.

Authors

October, 2004

การศึกษาเปรียบเทียบหาความชุกของโรคทริปปาโนโซมในโคนม ของจังหวัดเชียงใหม่และลำพูน โดยวิธีฮีมาโตคริตเซนติฟูเกชัน และวิธีการ์ดแอกกลูติเนชัน

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บทคัดย่อ

ทำการศึกษาฟาร์มโคนมที่เลี้ยงแบบผูกยืนโรงตลอดจำนวน 46 ฟาร์มในจังหวัดเชียงใหม่และลำพูน โดยมีจำนวนโคนมรวมทั้งสิ้น 743 ตัว เพื่อศึกษาหาความชุกของโรคทริปปาโนโซมในช่วงเดือนตุลาคม 2546 ถึงเดือนกุมภาพันธ์ 2547 และเปรียบเทียบความชุกที่ได้จากการตรวจโดยวิธีฮีมาโตคริตเซนติฟูเกชันและความชุกของโรคที่ได้จากการตรวจโดยวิธีการ์ดแอกกลูติเนชัน ผลการศึกษาพบว่า ความชุกของโรคทริปปาโนโซมโดยวิธีฮีมาโตคริตเซนติฟูเกชัน เท่ากับ 1.21 เปอร์เซ็นต์ และความชุกของโรคโดยวิธีการ์ดแอกกลูติเนชันเท่ากับ 42.35 เปอร์เซ็นต์ จากการวิเคราะห์หาความสัมพันธ์ของวิธีการตรวจวินิจฉัยโรคทริปปาโนโซมโดยวิธีฮีมาโตคริตเซนติฟูเกชันและวิธีการ์ดแอกกลูติเนชัน พบว่า ทั้งสองวิธีมีความสอดคล้องกันทางสถิติเพียงเล็กน้อย (kappa = 0.05)

คำสำคัญ : ความชุก โรคทริปปาโนโซม โคนม เชียงใหม่ ลำพูน วิธีฮีมาโตคริตเซนติฟูเกชัน วิธีการ์ดแอกกลูติเนชัน

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Comparative Study on Prevalence of Trypanosomosis of Dairy Cattle in Chiang Mai and Lam Phun Provinces Diagnosed by Hematocrit Centrifugation method and Card Agglutination test

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Abstract

The forty six zero-grazing dairy farms with 743 dairy cattle in Chiang Mai and Lam Phun provinces were selected in order to determine the prevalence of trypanosomosis during October 2003 to February 2004. Besides, Comparison the prevalence between the disease diagnosed by Hematocrit Centrifugation method and Card Agglutination test was statistically estimated. The results indicated that the parasitological prevalence was 1.21%; on the other hand, the seroprevalence of trypanosomosis was 42.35%. Statistical expected agreement by chance was calculated and the result showed that there was slight agreement between Hematocrit Centrifugation method and Card Agglutination test (kappa = 0.05).

Keywords : Prevalence, Trypanosomosis, Dairy Cattle, Chiang Mai, Lam Phun, Hematocrit Centrifugation method, Card Agglutination technique

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Introduction

Trypanosoma evansi, a protozoan blood parasite, is the causative agent of surra or trypanosomosis in Thailand. It infects a wide range of mammalian animals, including ruminants in the subtropical and tropical regions. In the north and north-east of Thailand, where the dairy production industry has been expanding significantly during the last decades, the incidence as well as the prevalence of *T. evansi* infections in dairy cattle herds increased rapidly causing severe economic losses (Indrakamhang, 1998).

Surra is transmitted by blood sucking flies, mainly by *Tabanidae*. Clinical signs observed in cattle during the acute stage of infection are characterized by anemia, intermittent pyrexia, progressive loss of condition and weakness (Luckins, 1998; Indrakamhang, 1998; Lang, 2001). Chronic infections are characterized by herd sterility and abortion of fetuses particular in the first 4^{th} to 5^{th} month of gestation (Trisanarom, et al.,1987; Sarataphan, et al., 1989), severe anemia and milk and weight loss (Timsad, et al, 1985; Loehr, et al, 1986; Luckins, 1998).

It was pointed out that diagnosis of the disease is a primary step in the proper control strategy (ILRAD, 1992). An Accurate epidemiological assessment of trypanosomosis is important in all stages of control program in order to describe the local disease impacts and the success of the control measures. In situation where case finding and treatment is part of the control strategiey, accurate diagnostic techniques are required to achieve an effective application of chemotherapeutic drugs. The main difficulty of diagnosing trypanosomal infection is the frequent scarcity of parasites in the host. The severity of infection is not necessarily related to parasitaemia and it may be difficult or impossible to find trypanosomes in the blood of a chronically infected animal even when it is about to die. (Killick-Kendrick, 1968)

In most hoses *T. evansi* can induce mild clinical or subclinical carrier state infections with low parasitaemia in which it is diddicult to demonstrate the parasites. Concentration methods such as Hematocrit centrifugation, Dark-

ground/phase-contrast buffy coat technique, or haemolysis techniques, become necessary. Animal inoculation is also used to reveal subclinical infections in domestic animals. However, mouse inoculation is not 100% sensitive. (Monzon, et al., 1990)

Though detection of parasite in an animal is the most specific diagnostic test available, its practical value is limited by lack of diagnostic sensitivity. Therefore, indirect techniques for the detection of trypanosomal antibodies or antigens in the body fluids of the host contribute substantially to the diagnosis of trypanosomosis. Card agglutination technique (CATT) is the alternative diagnostic technique recommended by OIE that is more likely to classify correctly truly infected animals.

In Thailand, trypanocidal drugs are used to control *T. evansi* infections in cattle and buffaloes. Diminazene aceturate (Berenil®, Intervet) is the only trypanocidal drug available on the Thai market (Indrakamhang, 1998). It is applied as a therapeutic drug when clinical signs are observed and severe economic losses occur. No other strategic control or prophylactic measures are applied in Thailand.

The objective of this study is to comparison the prevalence of Trypanosomosis of dairy cattle in the early cold season in ChiangMai and LamPhun provinces by different diagnostic methods including parasitological method and Card Agglutination technique.

Materials and Methods

Study area

The study was conducted in ChiangMai and LamPhun provinces which belong to the Upper North region of Thailand. These provinces are characterized by a high number of dairy herds and most farms are managed in zero-grazing unit. Besides, Chiang Mai University has been regularly visiting these areas to measure animal health and productivity data. There were six districts distributing in ChiangMai and LamPhun that were selected belonging to the

highest ranking of dairy population, including SanKamPhang, MaeOn, SanSai, and SanPaTong districts of ChiangMai province and BanThi and BanHong of LamPhun province.

Study population

The 46 dairy herds managed in zero-grazing units from which total approximately 280 farms were selected by stratified random sampling. The 743 dairy cattle were also selected by stratified random sampling from these 46 farms were collected whole blood and sera for parasitological and serological diagnostic methods during October 2003 to February 2004. Only 477 sera stratified selected from these 743 samples were tested by serological method.

Study design

Cross sectional study (CSS) to investigate the prevalence of trypanosomosis was performed. Different diagnostic methods such as concentration technique as Hematocrit centrifugation and indirect method as Card Agglutination technique (CATT) were used.

Methods

1. Prepare the questionnaire, equipment
2. Collection the blood from the cattle from tail vein 8 cc. per cattle
3. Diagnosis by Hematocrit centrifugation and Card Agglutination technique (CATT) (Van Meirvenne and Magnus, 1992)
4. The primary field isolates are deep frozen in -196°C for further research.
5. Statistical evaluation the prevalence and agreement between concentration technique and CATT were calculated.

$$\text{Prevalence} = \frac{\text{No. of positive samples}}{\text{Total samples}} \times 100$$

$$\text{Kappa value} = \frac{\text{observed agreement} - \text{expected agreement}}{100\% - \text{expected agreement}}$$

Results

Parasitological prevalence was estimated based on Hematocrit centrifugation and seroprevalence was estimated based on Card Agglutination test result. Detection of trypanosomes by parasitological technique indicated overall 1.21 % prevalence as present in table 1. 1.33% and 1.21% of prevalence by Hematocrit centrifugation were indicated in ChiangMai and LamPhun province, respectively. The overall seroprevalence diagnosed by Card Agglutination test was 42.35%. Provincial prevalence of Trypanosomosis was 4.28% and 56.67% in ChiangMai and LamPhun provinces, respectively.

Kappa (k) value was calculated as 0.05 that indicated slight agreement between Hematocrit centrifugation method and Card Agglutination test. It mean that there was slightly relationship between these two tests.

Table 1 Estimates of prevalence and seroprevalence of trypanosomosis in the study population distributing in provinces and districts of each province

Province	District	Parasitological method		%	Serodiagnostic method		%
		No.	positive		No.	Positive	
ChiangMai	SanKamPhang	139	0	0	94	34	36.17
	MaeOn	167	6	3.59	111	57	51.35
	SanSai	167	0	0	74	18	24.32
	SanPaTong	204	3	1.47	138	59	21.01
	Total	**677**	**9**	**1.33**	**417**	**168**	**40.28**
LamPhun	BanThi	51	0	0	50	29	58.00
	BanHong	15	0	0	10	5	50.00
	Total	**66**	**0**	**0**	**60**	**34**	**56.67**
Total		**743**	**9**	**1.21**	**477**	**202**	**42.35**

Discussion

The aim of this study was to estimate the prevalence of trypanosomosis in dairy cattle of ChaingMai and LamPhun provinces and to compare the prevalence between prevalence of disease diagnosed by parasitlogical method as Hematocrit centrifugation and seroprevalence of disease using Card Agglutination test. The sampling frame consisted of 46 dairy farms from which

280 farms were selected by stratified random sampling. From the selected farms, the 743 dairy cattle were also selected by stratified random sampling from these 46 farms were collected whole blood and sera for parasitological and serological diagnostic methods during October 2003 to February 2004. Only 477 sera stratified selected from total 743 samples were tested by serological method as Card agglutination test.

There is obviously a discrepancy between the parasitological prevalence (1.21%) and the seroprevalence (42.35%). The low parasitological prevalence can be attributed to the low analytical sensitivity of Hematocrit centrifugation; 6.25 x 103 trypanosomes per milliliter of blood (Paris, et al., 1982).

The agreement between the parasitological detection as Hematocrit centrifugation test and serological method as Card Agglutination test was calculated by kappa test. This test measures the agreement of test results beyond chance agreement. The overall agreement between Hematocrit centrifugation test and Card agglutination test was quite low as indicated by low kappa coefficients (k=0.05). So that, there was slight relashionship between these two tests.

Though detection of parasite in an animal is the most specific diagnostic test available, its practical value is limited by lack of diagnostic sensitivity. Therefore, indirect techniques for the detection of trypanosomal antibodies or antigens in the body fluids of the host contribute substantially to the diagnosis of trypanosomosis. Card agglutination technique (CATT) is an alternative diagnostic technique recommended by OIE that is more likely to classify correctly truly infected animals. Besides, in the situation where there is overt disease, CATT tests can be used to target individual animals for treatment with trypanocidal drugs. For declaring disease-free status, serial testing – ELISA followed by re-testing of suspect samples by CATT is recommended.

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Appendages

Card Agglutination Test for Trypanosomiasis (CATT/T. evansi)

(Van Meirvenne and Magnus, 1992)

Principle

CATT/T.evansi is an experimental direct agglutination test for detection of antibodies in serum or plasma of infected animals. The antigen consists of blood stream form of trypanosomes RoTat 1.2, a variable surface antigen type common to all T.evansi stocks examined hitherto. The organisms have been fixed, stained and freeze-dried. They are agglutinated by antibodies to RoTat 1.2 variable antigen itself but also by antibodies to some invariable surface antigen componetns. The test is done on a plastic card. Resuspended antigen is mixed with diluted serum or plasma and agitated for 5 minutes. Blue clumping indicates a positive result. It should be noted that the test is not strickly species-specific. This may complicate the interpretation of positive results in areas where still other species of salivarian trypanosomes occur.

Reagents and accessory materials

Antigen

Bloodstream form trypanosomes of T.evansi RoTat 1.2, fixed with formaldehyde, stained with Coomassie blue, freeze-dried and stored under nitrogen. Stability of the dry product at 5-10°c for more than 1 year and at 45°c still usuable after 1 month.

Buffer

Phosphate buffered saline, pH7.2 was used. And preservation with sodium azide 0.1% was done. This solution uses for reconstritution of antigen and controls and for dilution of test samples.

Positive control

Freeze-dried goat antiserum, stored under nitrogen is the positive control which preserve with sodium azide 0.1%

Negative control

Freeze-dried bovine serum albumin solution, stored under nitrogen is the negative control which preserve with sodium azide 0.1%.

Accessories

Syringe for transfer of buffer, droppers for delivery of antigen and controls (± 45 µl per drop), plastic cards with 10 test circles, stirring rods, and card rotator 12V/220V are necessary.

Preparation of reagents

Antigen (50 tests per vial)

1. Open the vial
2. Add 2.5 ml of buffer
3. Put on a dropper
4. Shake for a few seconds so as to obtain a homogeneous suspenion
5. Shake again before use and first check reactivity with controls

Notes;

- preferentially use freshly reconstituted antigen
- keep the reagent as cool as possible
- Stability of the wet antigen (use control for follow-up) at 37°c for up to 24 hours, below 10°c for up to 1 week, but do not freeze!

Positive and Negative control (0.5 ml per vial)

1. Open the vial
2. Add 0.5 ml of buffer
3. Put on a dropper
4. Shake and keep as cool as possible

Test sample

1. Use clean serum or plasma
2. For the screening test;
   - Prepare a 1/8 dilution in buffer (the optimal dilution depends on the host species)
3. For titration;
   - prepare a few further twofold dilutions

Execution of the test

1. Put 1 drop (about 45 µl) of well suspended antigen per circles as the following detail;
   - Shake the antigen

- Turn the dropping vial upside down
- Hold it in a vertical position
- Gradually squeeze the dropper and, without releasing pressure, load all the circles of a card in one run
- Let drops fall freely without touching the card

2. Add test samples as the following detail;
   - 25 µl diluted serum or plasma
   - one drop for positive and negative control

3. Mix the reagents;
- With a clean stirring rod for mixing and spreading out
- Staying about 1 mm from the edge of the circle
- Wipe off the stirring rod

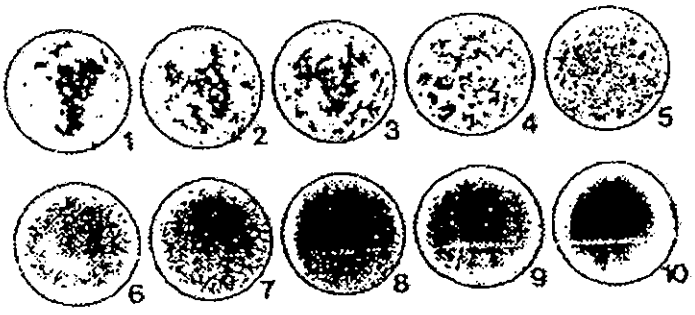
4. Agitate for 5 minutes;
- On a special rotator (eccentric 12-16 mm. Circle, 60-70 rpm)
- Hold the card between both hands
- Tilt it slowly in a circular way so as to obtain rotation of the reaction mixtures

Reading the results

Reading the results immediately before removing the card from the turntable. Evaluate the presence of blue granules and score as follows;

Matching figures	score	
1-3	+++	strongly positive
4-5	++	strongly positive
6-7	+	positive
8	±	weakly positive
9-10	0	negative

Note; the positive control should give a +, the negative control a 0 result



Interpretation

At the present stage, CATT/T.evansi should be considered as an experimental test required further evaluation of sensitivity and specificity. Results should be confronted with clinical, parasitological and other serological data. The optimal screening dilution (in general 1/4 or 1/8) may vary from one host species to another.

The test may be positive in case of infection with salivarian trypanosome species other that T.evansi. In cured animals the test probably remains positive for a long time.