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TRYPANOSOMA EVANSI INFECTION IN BUFFALOES IN NORTH-EAST THAILAND. II. ABORTIONS

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SUMMARY

By exclusion of other possible aetiological agents, strong circumstantial evidence is presented of Trypanosoma evansi *infection being the cause of late gestation abortion and stillbirth in buffaloes.*

INTRODUCTION

A request for investigation of abortions was received in April, 1984 from the National Buffalo Research Station, Surin seven months after the first and one and a half months after the last of a series of abortions. In a breeding herd of 31 animals in late pregnancy 13 cows had aborted and two had had stillbirths, in a breeding herd of 35 animals in mid-term pregnancy three cows had aborted and one had had a stillbirth and in a breeding herd of 41 animals in early pregnancy no cow had aborted. Abortion rates in late, middle and early pregnancy were therefore 48·4, 11·4 and 0% respectively. The same five bulls had served all three breeding herds. Herdsmen stated that dams had shown no signs of illness before or after abortion. Abortions occurred during the best pasture season of the year when animals had received no supplementary feeding.

A second request for investigation of abortions on the same station was received in September, 1984 when six more abortions in late pregnancy had been observed, two of them in September, three in August and one in June had occurred. Again no clinical signs of illness had been seen. In October, 1984 a request for investigation of sick buffaloes and recent abortions was received from farmers in a village in Korat Province 280 km away. Ten farmers presented 12 buffaloes, seven with a history of abortion between three and 19 days prior to the investigation and five with signs of stiffness and with severe conjunctivitis.

The results of these investigations which seem to incriminate *Trypanosoma evansi* as the most likely cause of the abortions are presented.

MATERIALS AND METHODS

Samples were taken from the following animals: 25 cows with history of abortion, 20 cows at different stages of pregnancy and nine bulls at the National Buffalo Research Station, Surin and in a village in Korat Province seven cows with history of abortion and five animals with signs of illness. The types of samples were as follows: blood from the ear vein for the preparation of Giemsa-stained thin blood films; fresh blood from the jugular vein for serum; heparinised blood for the detection of trypanosomes by the method of Woo (1969) and 0·8 ml heparinised blood for inoculation into one mouse. Only 37 of the 66 inoculated mice survived the car journeys of 250 and 280 km to the

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laboratory at 41°C ambient temperature and 75% relative humidity. Additionally vaginal and preputial washings were made from seven of the 25 cows which had aborted and from nine clinically normal breeding bulls at the Research Station.

Giemsa-stained blood films were each examined for five min. Examination of heparinised blood was carried out approximately 15 h after sampling. Mice were inoculated intraperitoneally with 0·8 ml of heparinised blood within 20 min of sampling and wet films made from their tail tips were examined every three days for four weeks.

Trypanosome antibodies were measured by the complement fixation test according to Staak and Lohding (1979). After ultrasonic treatment of parasites from a local *T. evansi* strain propagated in rats and isolated according to Lanham and Godfrey (1970) the supernatant was used for antigen. A titre of 1:5 or more of serially diluted sera was regarded as positive.

Sera from all animals sampled at the National Buffalo Research Station, Surin and from the village in Korat Province were tested for antibodies to brucellosis, leptospirosis, listeriosis and infectious bovine rhinitis using the following methods:

Brucellosis. Serum agglutination slow test and complement fixation test (CFT) according to Alton and Jones (1967). The antigen from the German Federal Health Office, Berlin provided a titre of 1:640 (1,000 i.u.). Test interpretation of a positive serum was 1:40++ and higher in the agglutination test and 1:5 and higher in the CF test.

Leptospirosis. Micro-agglutination test according to Schoenberg, Mueller, Weber, Fingscheidt, Brem, Seeliger and Schaal (1984) with the following *Leptospira interrogans* serovars being used: *hardjo, pomona, tarassovi, grippotyphosa, canicola, saxkoebing, bataviae, copenhagen, autumnalis* and *australis*. The minimum titre considered positive was 1:400.

Listeriosis. Agglutination test according to Seeliger and Finger (1968) with *Listeria monocytogenes* serovars 1/2a and 4b.

IBR/IPV. Enzyme linked immunosorbent assay using "Trachitest-Kit", Flow Laboratories, Meckenheim, Germany.

Bacteriological examination of preputial and vaginal washings made from animals on the second visit to Surin Station was initiated 15 h after sampling using nutrient, blood, brucella and MacConkey agar. Material was also subjected to dark field microscopy.

RESULTS

Sera from all 66 animals sampled were negative for brucellosis, listeriosis and infectious bovine rhinotracheitis. One animal showed a leptospirosis titre of 1:1,600 and three had titres of 1:400. Bacteriological results revealed *Streptococcus* spp. two, *Enterobacter* spp. three, *Escherichia coli* six, *Moraxella* spp. four, *Aeromonas salmonicida* three and *Pseudomonas aeroginosa* three. No vibrio or leptospira were detected by dark field microscopy. Parasitological and serological detection rates of *T. evansi* infection are shown in Table I.

Parasitological detection rates in cows with histories of abortion at Surin Station and in the village in Korat Province were 40% and 86% respectively and very high in comparison with the detection rate of 5% in pregnant cows at Surin. The parasitological detection rate of 86% and the serological mean titre of 1:104 in cows with a recent history of abortion in Korat Province were much higher than those of 40% and 1:36 in cows with an older history of abortion at Surin

TABLE I

Results of parasitological and complement fixation for T. evansi *infection*

History	No. of animals	*Parasitology* Blood smear	Woo test	Mouse test	Total +ve animals	Detection rate	*Complement fixation titres* <5	5	10	20	40	80	160	320	Mean titre	Positive titres
Cows which aborted, Surin	25	0/25	1/25	9/15	10	40%	5	3	0	6	5	1	5	0	36	80%
Pregnant cows, Surin	20	0/20	0/20	1/13	1	5%	6	0	1	6	3	3	1	0	36	70%
Breeding bulls, Surin	9	0/9	0/9	2/2	2	22%	2	0	1	2	3	0	1	0	34	78%
Cows which aborted, Korat	7	2/7	3/7	4/4	6	86%	0	0	0	1	0	3	2	1	104	100%
Animals with signs of illness	5	1/5	1/5	3/3	3	60%	1	0	1	1	1	1	0	0	30	80%
Total	66	1/66	5/66	20/37	22		14	3	3	16	12	8	9	1		
Mean						33·3%									39	79·7%

Station. Five of the 25 cows which had aborted at Surin Station were serologically *T. evansi* negative yet one of these animals was parasitologically positive. Parasitological detection rates in breeding bulls at Surin Station and in sick animals in the village in Korat Province were 22% and 60% respectively. Serological detection rates were high in all groups ranging from 70% to 100%. Mean parasitological and serological detection rates and mean CF titres in animals of all groups were 33·3% and 79·7% and 1:39 respectively. Parasitological detection was highest by the mouse inoculation test. Since 29 (44%) of the inoculated mice had died during transport it is assumed that the infection rates, particularly in the animals at Surin Station where many inoculated mice had died, were higher than the above detection rates.

DISCUSSION

During field studies which were published recently by Löhr, Pholpark, Srikitjakarn, Thaboran, Bettermann and Staak (1985) only two cases of abortion were observed in association with *T. evansi* infection and a causal link was not suspected. Therefore when first investigating the outbreak of abortions at the National Buffalo Research Station, Surin any possible cause such as thermal, nutritional or toxic substances or infectious origin was considered. The history very soon excluded the first three so investigations concentrated on infectious diseases. After comparing abortion rates, stages of gestation in which abortions had occurred and general history of the outbreak with the parameters listed by Morrow (1980) for aetiological agents associated with abortion in cattle, investigations were narrowed to a few infectious causes: brucellosis, leptospirosis, listeriosis and infectious bovine rhinitis. The inclusion of trypanosomiasis in the investigation was at this stage incidental and was due to a recent case of *T. evansi* infection on the station.

The serological results excluded all the above infections from the aetiology of the abortions except leptospirosis and trypanosomiasis. Among 19 animals with history of abortions or stillbirths, four showed low leptospira titres and 15 mainly high *T. evansi* titres with nine of these 19 animals being parasitologically positive. It could be argued that *T. evansi* infection was immunosuppressive to antibody production against infectious diseases and animals may have aborted following leptospirosis or any other infection although they had only low or no antibody titres. This argument could be supported by the observation of highly reduced antibody production to *Leptospira biflexa* in calves infected with *Trypanosoma congolense* (Rurangirwa, Tabel, Losos and Tizard, 1979). However, transplacental transmission of trypanosomes in ruminants and subsequent abortions have been demonstrated by a number of authors as reviewed by Ogwu and Nuru (1981) and in the buffalo Paikne and Dhake (1972) reported the abortion of a fully developed calf which was positive for *T. evansi*. These authors attributed the abortion to *T. evansi* infection.

After the second visit to the National Buffalo Research Station serology, bacteriology and parasitology could link only trypanosomiasis to the possible cause of the abortions. Six of the seven animals with a very recent history of abortion in Korat Province were parasitologically *T. evansi* positive and none was serologically positive to any of the tests except *T. evansi*. High serological *T. evansi* detection rates and high antibody titres indicating fairly recent infections (Pholpark, Löhr, Siriwan, Khoonphasee, Thaboran, Pholpark, Bettermann and

Staak, unpub.) were found in animals which had aborted and in animals of early, mid-term and late stages of pregnancy, i.e. *T. evansi* infection occurred in all stages but abortions only in the late stage of pregnancy. Wells (1981) and Löhr *et al.* (1985) thought that stress is an important factor causing severe clinical symptoms. It would seem that the stress of late pregnancy may be a main contributory factor to *T. evansi* causing abortion.

The high parasitological and serological detection rates and the low presence of clinical trypanosomiasis could be indicative of an enzootic situation with little reason to associate *T. evansi* with the cause of these abortions. It is therefore of interest to compare the parasitological and serological detection rates and the mean serological titres of this study with those of a survey carried out in most provinces of north-east Thailand three years previously (Löhr *et al.*, 1985). In the survey parasitological and serological detection rates were much higher during the rainy season than during the dry season. The parasitological and serological detection rates and the mean titres during the dry and rainy seasons were 3·7%, 15·4% and 1:30 and 17·1%, 23·6% and 1:30 respectively and were considerably lower than those of 33·3%, 79·7% and 1:39 in this study i.e. the abortions at the Buffalo Research Station and in the village in Korat Province were concurrent with epizootic outbreaks of *T. evansi* infection.

From the findings of this study which are supported by the above-mentioned literature there is strong circumstantial evidence of *T. evansi* infection being a frequent cause of abortion in buffaloes at a late stage of gestation. In the past this Centre has rarely been able to identify the cause of abortion in buffaloes. Future investigations may reveal *T. evansi* as being the most frequent cause and may also show that abortion is the most significant clinical sign of *T. evansi* infection in buffaloes in North-east Thailand.

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INFECTIONS A *T. EVANSI* CHEZ LES BUFFLES DU NORD-EST DE LA THAILANDE. II. AVORTEMENTS

Résumé—Par exclusion de rôle étiologique possible d'autres agents, de fortes preuves indirectes sont présentées qui indiquent que l'infection par *Trypanosoma evansi* est la cause d'avortements en fin de gestation et de mortinatalités chez 32 bufflesses.

INFECCION POR *TRYPANOSOMA EVANSI* EN BUFALOS DEL NORORIENTE DE TAILANDIA. II. ABORTOS.

Resumen—Se presentan evidencias circunstanciales de infecciones por *Trypanosoma evansi*, como causa de abortos y momificaciones al final de la gestación en 32 búfalos.

BOOK REVIEW

Animal Health in Australia. Volume 6. **Bacterial and Fungal Diseases of Pigs,** J. R. Buddle. Australian Government Publishing Service, Canberra. 1985. 247 pp. ISBN 0 644 02967 6.

This is another excellent publication in the animal health series promoted by the Australian Agricultural Health and Quarantine Service.

Specific bacterial diseases are discussed under headings of clinical features, pathogenesis and lesions, epidemiology, diagnosis and control. Other useful sections cover the following disease complexes: abscesses, arthritis, farrowing-fever (mastitis-metritis-agalactia) and other reproductive disorders. The appendices include tables for the differential diagnosis of clinical syndromes, a procedure for cleaning pens, useful antibacterial drugs and vaccines and principles for the prevention and control of enteric and respiratory diseases.

The book is easy to read and well indexed. It shows uniformity, the advantage of having a single author. References to published work are quoted frequently and concisely. Although many of the specific diseases are covered in a way similar to other textbooks, the sections on disease complexes and the appendices are novel. The appendices in particular will be extremely useful to student, farmer and veterinarian alike because of their practical nature.

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