UNIVERSITI PUTRA MALAYSIA

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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SEROEPIDEMIOLOGY, DIAGNOSIS, ISOLATION AND CHARACTERISATION *NEOSPORA CANINUM* AMONG CATTLE IN MALAYSIA

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Chairman : Associate Professor Rehana Abdullah Sani, Ph. D.

Faculty : Veterinary Medicine

Neospora caninum is an apicomplexan protozoan parasite that has worldwide distribution and is known to cause abortion and congenital disease in cattle and dogs. This thesis deals with various aspect of investigations on neosporosis in Malaysian cattle which include seroprevalence in cattle, modes of transmission of *N. caninum* in cattle, effects of *N. caninum* infections in pregnant dairy cattle naturally infected with the parasite, isolation of *N. caninum* and comparison of the standard diagnostic technique, IFAT with ISCOM ELISA.

A cross-sectional study was conducted in dairy and beef cattle in Peninsular Malaysia and Sabah to determine the seroprevalence of *N. caninum* and the association of breeds and farm location to the seroprevalence. In Peninsular Malaysia, a total of 1531sera from Sahiwal-Friesian on three large scale (D1, D2,

D3) and 21 small scale (D4) farms were tested for *N. caninum* antibodies using the IFAT. A total of 1413 sera from beef cattle which comprised Nelore, Droughtmaster and Kedah-Kelantan on four large scale farms (B1-B4) were also tested. In Peninsular Malaysia, the seroprevalence of *N. caninum* in the dairy cattle ranged from 1.9% in D2 farm to 6.7% in D1 farm, while in beef animals the *N. caninum* seroprevalence varied between 2.0% in B4 farm and 5.4% in B2 farm. Among the dairy cattle there was significance between the seroprevalence of *N. caninum* in D2 farm and those of D1 and D3 farms ($p<0.05$). The seroprevalence of *N. caninum* in D1 farm was significantly higher than those in B1, B3 and B4 farms ($p<0.05$). The *N. caninum* seroprevalence in D3 farm was was significantly higher that in B4 farm. In Sabah, a total of 624 sera from Sahiwal cross and pure Friesian cattle on 6 large scale (SD1-SD6) and two small scale (SD7, SD8) farms were tested. A total of 240 sera from beef cattle which were Droughtmaster and Brahman crosses on 3 large scale (SB1-SB3) farms were tested. The seroprevalence was highest in the cattle on SD2 farm (32.5%) and lowest in the cattle on SD7 farm (2.7%). The seroprevalence of *N. canium* of cattle on farms SB1 and SB2 were 12.5% respectively, while in SB3 farm the figure was 2.5%.. There was a significant difference between the seroprevalence of *N. caninum* cattle on D2 farm and cattle on the other farms ($p<0.05$).

In the investigation on modes of transmission, a cross-sectional study was conducted on the relationship between seropoositivity of cows and their calves after colostral intake. Blood samples were collected from 286 dams and their respective calves on D1-D3 farms and from another 233 pairs of beef cattle on B1-B4 farms. The was a strong association between seropositivity of dams and that of their calves ($p<0.001$).

The association of seroprevalence of precolostral calves with their dams was examined by collection of blood samples from 100 dams and their calves before colostral intake. Out of the 100 dams, 13 were seropositive and produced nine (69.2%) congenitally infected calves. There was strong association between presence of antibodies in the dam and congenital infection ($p<0.001$). A total of 83 seronegative heifers were also monitored prospectively from birth for evidence of post-natal infection. No evidence of seroconversion was found in the seronegative heifers.

A study conducted on D1 farm to assess the reproductive performance of eight pregnant seropositive heifers and 10 pregnant seropositive cows till pregnancy was terminated by abortion or parturition. Two heifers repeatedly returned to oestrus. Abortions were seen in four heifers. Two heifers produced weak and underweight calves and one of the calves died 12 hours after birth. The precolostral sera from the two calves had *N. caninum* antibodies. Abortion occurred in one out of the 10 pregnant cows. All the remaining nine cows produced normal calves whose precolostral and postcolostral sera were positive for *Neospora* antibodies. Paired serum samples from the aborted heifers and cow revealed no evidence of active infections due to *Br. abortus*, *Leptospira* spp., *S. dublin*, IBR virus, BVD virus or *T. gondii*. Reproductive disorders were commonly seen in the seropositive heifers when compared to seropositive cows and the difference was found to be significant ($p<0.05$).

In an attempt on isolation, brain homogenate from a congenitally infected calf born to a seropositive heifer was inoculated intraperitoneally into 10 BALB/c mice. Four out of the 10 mice died during the observation period and brains from the remaining 6 were homogenised and cultured onto Vero cell lines. Tachyzoites resembled those of *N. caninum* were detected in the cell cultures 14 days after the inoculation of brain homogenate from one of the six mice. Serology, electron microscopy and molecular studies revealed no difference between this new Malaysian bovine isolate and reference *N. caninum* isolate Nc-1, confirming that it was *N. caninum*. The isolate was designated Nc-MalB1

A total of 661 dairy and 375 beef cattle sera from Peninsular Malaysia and Sabah were used in the comparison IFAT with ISCOM ELISA in the detection of *N. caninum* antibodies. Based on the cut-offs recommended by the manufacturers, there was reasonably good agreement between the two tests as indicated by high kappas' values of 0.80 in dairy and 0.82 in beef cattle.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia, sebagai memenuhi keperluan untuk ijazah Doktor Falsafah.

SEROEPIDEMIOLOGI, DIAGNOSA, PENGASINGAN DAN PENCIRIAN *NEOSPORA CANINUM* PADA LEMBU DI MALAYSIA

Oleh

CHEAH TONG SOON

September 2004

Pengerusi : Profesor Madya Rehana Abdullah Sani, Ph.D.

Fakulti : Perubatan Veterinar

Neospora caninum adalah parasit apicomplexan yang terdapat diseluruh dunia dan di ketahui sebagai penyebab keguguran dan penyakit kongenital pada lembu dan anjing. Tesis ini melibatkan beberapa penyiasatan berkaitan neoprosis pada lembu di Malaysia meliputi seroprevalen pada lembu, cara jangkitan penyakit, kesan jangkitan *N. caninum* pada lembu bunting, pengasingan *N. caninum* dan perbandingan IFAT dan ISCOM ELISA dalam mengesan antibodi .

Satu ‘cross-sectional study’ telah dijalankan ke atas lembu tenusu dan lembu pedaging di Semenanjung Malaysia dan Sabah untuk menentukan seroprevelensi *N. caninum* dan perkaitan di antara baka dan lokasi ladang dengan seroprevalensi. Di Semenanjung Malaysia, sejumlah 1531 serum dari lembu tenusu kacukan Sahiwal-Friesian di tiga buah ladang berskala besar (D1, D2, D3) dan 21 ladang kecil diuji

bagi mengesan kehadiran antibodi terhadap *N. caninum* menggunakan IFAT. Sejumlah 1413 serum (n=1413) dari lembu pedaging yang terdiri dari Nelore, Droughtmaster dan Kedah-Kelantan dalam empat ladang berskala besar (B1-B4) juga diuji menggunakan kaedah yang sama. Di Semenajung Malaysia, seroprevalensi lembu tenusu kacukan Sahiwal Friesian (SFX) adalah antara 1.9% di ladang D2 hingga 6.7% di ladang D1. Terdapat perbezaaan seroprevalen yang signifikan di kalangan lembu tenusu di ladang D2 berbanding lembu di ladang D1 dan D3 ($p<0.05$). Pada lembu pedaging, seroprevalen pada Droughmaster (DM) adalah tertinggi (5.4%) dan seroprevalen pada baka indigenous Malaysia, lembu Kedah Kelantan di ladang B4 adalah terendah (2%) dan perbezaan ini adalah signifikan ($p<0.05$). Diantara lembu tenusu dan lembu pedaging, seroprevalen lembu pedaging baka Nelore dan KK adalah rendah dan signifikan berbanding lembu tenusu di ladang D1 dan D3 ($p<0.05$).

Di Sabah, sejumlah 624 serum dari 6 ladang tenusu berskala besar (SD1-SD6) dan 2 ladang kecil (SD7,SD8) baka SFX dan Friesian telah diuji. Seroprevalen pada ternakan di ladang SD2 (32.5%) adalah tertinggi dan terendah pada lembu diladang SD7 (2.7%). Seroprevalen pada lembu di ladang SD2 adalah tertinggi berbanding lembu tenusu dan pedaging di Sabah dan Semenanjung Malaysia ($p<0.001$). Sejumlah 240 serum lembu pedaging di 3 ladang berskala besar (SB1-SB3) telah diuji dan ternakan berbaka kacukan seperti DM, Brahman (Br). Kacukan Br, Bali (B) dan kacukan B. Seroprevalen lembu di ladang SB1 dan SB2 (12.5%) adalah tinggi dan signifikan berbanding lembu di SB3 (2.5%). Seroprevalen lembu di ladang SB1 dan SB2 juga tinggi dan signifikan berbanding lembu tenusu dan lembu

pedaging di Semenanjung Malaysia ($p<0.05$). Perkaitan diantara seropositif yang lebih tinggi dengan lembu SFX yang dibeli pada ladang D1 and D2 mempunyai signifikasi yang tinggi ($p<0.001$).

Dalam kajian mengenal pasti cara jangkitan parasit tersebut, satu 'cross-sectional study' telah dijalankan atas perkaitan di antara status seropositif lembu betina dengan anak lembu masing-masing selepas pengambilan colostral. Sampel darah telah diambil dari 286 lembu betina dan anak lembu dalam ladang D1-D3 serta dari 233 pasang lembu daging dalam ladang B1-B4. Terdapat perkaitan rapat di antara status seropositif lembu betina dan anak lembu ($p<0.001$). Perkaitan di antara seroprevalensi anak lembu yang belum mengambil colostral dengan lembu betina diuji melalui pengumpulan sampel darah daripada 100 lembu betina dan anak mereka sebelum pengambilan colostral. Daripada 100 lembu betina, 13 adalah seropositif dan melahirkan 9 (69.2%) anak lembu yang menpunyai jangkitan congenital. Terdapat perkaitan yang kuat diantara kehadiran antibodi pada ibu lembu dan dan jangkitan kongenital ($p<0.001$). Sejumlah 83 lembu dara yang seronegatif diperhatikan selepas kelahiran untuk menbukitkan jangkitan postnatal. Tiada tanda – tanda serokonversi pada lembu dara yang seronegatif

Satu kajian telah dijalankan dalam ladang D1 bagi mengkaji tahap kesuburan 8 seropositif lembu dara yang mengandung pertama kali dan 10 seropositif lembu mengandung. Keguguran dilihat pada empat lembu dara bunting dan dua darinya melahirkan anak yang lemah dengan berat badan yang rendah dan seekor anak tersebut mati 12 jam selepas dilahirkan. Serum yang diambil dari dua anak lembu

sebelum mereka menghisap kolostrum mengandungi antibody terhadap *N. caninum*. Keguguran berlaku pada satu dari 10 lembu bunting. Kesemua sembilan lembu melahirkan anak lembu yang normal dengan antibodi dikesan pada darah sebelum mereka menghisap kolostrum. Serum berpasangan daripada lembu gugur menunjukkan tiada tanda jangkitan aktif disebabkan oleh *Br. Abortus, Leptospira* spp. *S.* Dublin, virus IBR virus, virus BVD atau *T. gondii*. Namun begitu, masalah pembiakan kerap dilihat pada lembu dara berseropositif berbanding lembu betina yang telah menghasilkan anak dan perbezaan ini adalah signifikan ($p<0.05$).

Dalam percubaan pengasingan *N.caninum,* homogenate otak daripada anak lembu berseropostif pada serum yang diambil sebelum pengambilan kolostrum, telah disuntik ke 10 BALB/c mencit secara intraperitoneal. Empat sdari 10 mencit mati dalamasa pemerhatian dan otak daripada 6 mencit yang hidup telah dihomogenasi dan dikulturkan ke dalam tisu didik vero. Tachyzoites menyerupai *N.caninum* telah dikesan di tisu didik 14 hari selepas inokulasi otak pada satu daripada enam mencit tersebut. Kajian serologi, pemerhatian elektron mikroskop dan kajian molekular menunjukkan tiada perbezaan di antara isolat baru diasingkan di Malaysia dan isolat rujukan *N. caninum* Nc-1. Isolate tersebut dikenali sebagai Nc-Ma1B1

Penyelidikan terakhir membandingkan antara IFAT dengan ISCOM ELISA bagi mengesan jangkitan *N.caninum* pada lembu. Sejumlah 661 serum lembu tenusu dan 375 lembu pedaging dari Semenanjung Malaysia dan Sabah telah digunakan bagi perbandingan. Berdasarkan ‘cut –off point’ vendor, terdapat persetujuan yang

munasabah di antara kedua – dua ujian tersebut yang di tunjukkan dengan nilai kappa yang tinggi iaitu 0.80 pada lembu tenusu dan 0.82 pada lembu pedaging.

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I certify that an Examination Committee met on 3rd September 2004 to conduct the final examination of Cheah Tong Soon on his Doctor of Philosophy thesis entitled "Seroepidemiology, Diagnosis, Isolation and Characterisation of *Neospora caninum* among Cattle in Malaysia" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recomends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fullfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

_____

CHEAH TONG SOON

Date :

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