HORSE ANTI-BOVINE LYMPHOCYTE SERUM.
ITS EFFECTS IN CALVES.

WILLY RUMAWAS

DISSERTATION

BOGOR AGRICULTURAL UNIVERSITY
1979
HORSE ANTI-BOVINE LYMPHOCYTE SERUM.
ITS EFFECTS IN CALVES.

WILLY RUMAWAS

DISERTASI
untuk memenuhi salah satu syarat
dalam memperoleh Gelar Doktor dalam
Ilmu-Ilmu Kedokteran Hewan pada
Institut Pertanian Bogor

Bogor, 17 Maret 1979
Judul disertasi: HORSE ANTI-BOVINE LYMPHOCYTE SERUM. IT EFFECTS IN CALVES.

Nama promovendus: WILLY RUMAWAS

Disertasi dipertahankan pada tanggal 17 Maret 1979 pukul 10.00 bertempat di Aula Departemen Agronomi, Fakultas Pertanian Institut Pertanian Bogor.

Komisi Penasehat:

Prof. A. A. Ressang
D.V.M., Ph.D., M.D.
Promotor Tamu

Prof. Djokowoerjo Sastradipradja
D.V.M., Ph.D.
Ketua

Prof. Andi Hakim Nasoetion
Ir., Ph.D.
Anggota

Prof. R. Soedarjo Sastrohadinoto
D.V.M., Ph.D.
Anggota

Edi Guhardja, Ir., Ph.D.
Dekan Sekolah Pasca Sarjana
Indrawati
Stephanus
Vincentius
Magdalena
ACKNOWLEDGEMENT

The author wishes to express his deep gratitude to Prof. Dr. A.A. Ressang M.D. for his advice, encouragement and guidance throughout the study carried out at the Central Veterinary Institute in Rotterdam, the Netherlands. The success of this project would not have been achieved without his personal involvement.

Special appreciation is given to Prof. Dr. Djokowoerjo Sadtradipradja, Prof. Dr. Ir. Andi Hakim Nasoetion and Prof. Dr. R. Soenarjo Sastrohadinoto for their advice and guidance in the completion of this work.

Sincere acknowledgement is extended to Dr. J.G. Kreeftenberg for supplying the horse anti-bovine lymphocyte serum and the permission to utilize facilities and resources of the National Institute of Public Health in Bilthoven, the Netherlands. His advice and suggestions concerning anti-lymphocyte serum has been indispensable.

The author is deeply indebted to the Government of the Republic of Indonesia and the Government of the Kingdom of the Netherlands for the opportunity to complete this study and for financial support.

The Board of Directors of the Central Veterinary Institute are highly appreciated for their hospitality and the opportunity given to the author to work and complete the experiments at their Institution in Rotterdam.
High appreciation is also expressed to the Rector of Bogor Agricultural University, the Dean of Graduation Study, the Dean of the Faculty of Veterinary Medicine and the Head of the Department of Pathology for their supports and concerns, so that this study became possible.

Appreciation is also extended to Dr Haagsma and his staff at the Central Veterinary Institute in Rotterdam, for performing the tuberculin test on the experimental animals.

Dr Nagel of the National Institute of Public Health in Bilthoven is acknowledge for his permission to use his laboratory facilities for the detection of antibody titer against tetanus toxoid.

Gratitude is extended to the laboratory personnel of the Leukosis Department of the Central Veterinary Institute in Rotterdam. The technical assistance of Mr J. Stentler is highly appreciated.

The author thanks Miss F. Leerling, Mr. H. Ettekoven and Mrs D. Schijf of the National Institute of Public Health in Bilthoven for their technical assistance.

Thanks are also due to the personnel of the Photography Department of the Central Veterinary Institute in Rotterdam for making all the photographs for this study.

Last but not least, the author wishes to extend his sincere appreciation and thanks to his loving wife Indrawati Rwnawas D.V.M. and their childrens, Stephanus, Vincentius and Agdalena Rumawas for their moral support and sacrifices during the time of pursuing this endeavour.
Horse anti-bovine lymphocyte serum.  
Its effects in calves.

The experiment was conducted at the Central Veterinary Institute in Rotterdam, the Netherlands, to study the effects of horse anti-bovine lymphocyte serum (ABLS) in calves. ABLS was produced in horses according to the method described by Monaco et al. (1966). Forty, 4 - 6 months old calves of the Dutch Friesian breed were used in these experiments. The calves were divided into four groups of 17, 10, 10 and 3 calves respectively and were used for the various trials. The first group of 17 animals were used to determine the doses of ABLS. It was found that serial injections of 1 ml ABLS per kg body weight per injection was sufficient to decrease the number of lymphocytes without causing toxic effects to the calves. For further trials, each calf received ten subcutaneous injections of 1 ml ABLS per kg body weight per injection at one day intervals. For testing the humoral immune response, ten calves were injected with sheep red blood cells (SRBC), each calf received $75.0 \times 10^6$ cells. Another group of ten calves were injected each with 30 LF tetanus toxoid. The cellular immune responses were determined by delayed hypersensitivity skin tuberculin reaction and skin grafting.

It was found that ABLS rapidly decreased the number of
lymphocytes and that it transiently increased the number of neutrophils. It also caused eosinophilia. The histological alterations in animals that succumbed to high doses of ABLS (7.5 - 10.0 ml per kg body weight per injection) were most severe in the lymph nodes, consisting of a marked reduction in the number of follicles and depletion of lymphocytes in the cortical and para-cortical areas. There was also histio-reticular proliferations, eosinophilic accumulation and fibrosis. The alterations in the spleen were the same as in the lymph nodes, but less severe. Congestion and tubular degenerations were common in the kidneys. In the small and large intestines the submucosal haemorrhages, congestion, oedema and infiltrations of mononuclear cells, neutrophils, eosinophils and plasma cells were prominent. It was concluded that the cause of death of the overdosed animals was due to shock by severe intestinal bleeding and pneumonia by secondary infection.

ABLS suppressed the humoral immune response to SRBC and tetanus toxoid. There was an inhibition of the cellular immune responses, which were evidenced by the suppression of the delayed hypersensitivity skin tuberculin reaction and prolongation (16 - 27 days) of skin graft survival.

ABLS injections into calves caused the calf's lymphocytes to become insensitive to phytohaemagglutinin (PHA) and pokeweed mitogen (PWM) in in vitro stimulation.
It was also observed that ABLS reduced the number of B and T lymphocytes.

It was concluded that ABLS suppressed the humoral and cellular immune responses by disturbing the B and T cell functions of the experimental calves.
CONTENTS

Acknowledgement ............................................................... I
Abstract .................................................................................. III
Contents .................................................................................. Va
List of tables ........................................................................... VI
List of abbreviations .................................................................. XIV

1. Introduction ........................................................................... 1

2. Review of literature ............................................................ 5
   2.1. Production of antilymphocyte serum ......................... 5
   2.1.1. Mechanism of action of antilymphocyte serum ... 9
   2.2. The effects of ALS in vitro ....................................... 11
   2.3. The effects of ALS in vivo ....................................... 13
      2.3.1. Effects on the circulating white cells ......... 13
      2.3.2. Effects of ALS on the tissue histology . 16
      2.3.3. The effects of ALS on the graft survival 19
      2.3.4. Effects of ALS on the humoral antibody response 22
   2.4. The effects of ALS on the responsiveness of lymphocytes to phytohaemagglutinin 23
   2.5. The effect of ALS on the delayed hypersensitivity reaction ......................................................... 24
   2.6. The effect of ALS on the B and T lymphocytes ... 24

3. Materials and Methods ............................................................ 26
   3.1. Preparation of anti-bovine lymphocyte serum ... 26
### 3.2. Determination of cytotoxic and haemolysin titer

### 3.3. Experimental calves

<table>
<thead>
<tr>
<th>Subsection</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3.1. Group I</td>
<td>Determination of the doses of ABLS</td>
</tr>
<tr>
<td>3.3.1.1</td>
<td>Determination of the doses of ABLS</td>
</tr>
<tr>
<td>3.3.1.2</td>
<td>Clinical and postmortem observations</td>
</tr>
<tr>
<td>3.3.2. Group II</td>
<td>Haematological examinations</td>
</tr>
<tr>
<td>3.3.2.1</td>
<td>Haematological examinations</td>
</tr>
<tr>
<td>3.3.2.2</td>
<td>The study of the effect of ABLS on the humoral immune response following parenteral application of sheep red blood cells (SRBC)</td>
</tr>
<tr>
<td>3.3.2.2.1</td>
<td>Preparation, dose and application of SRBC</td>
</tr>
<tr>
<td>3.3.2.2.2</td>
<td>Agglutination test for the determination of antibody to SRBC</td>
</tr>
<tr>
<td>3.3.2.3</td>
<td>Observation of delayed hypersensitivity reaction by tuberculation</td>
</tr>
</tbody>
</table>

3.3.3. Group III

<table>
<thead>
<tr>
<th>Subsection</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3.3.1</td>
<td>The study of haematologic response to ABLS</td>
</tr>
<tr>
<td>3.3.3.2</td>
<td>Observation on the cell mediated immune response by skin grafting</td>
</tr>
</tbody>
</table>
3.3.3.2.1. Treatment of the calves
before skin grafting ........ 37
3.3.3.2.2. Skin grafting ............. 38
3.3.3.2.2.a. Preparation of the calves. 38
3.3.3.2.2.b. Preparation of the graft. 38
3.3.3.2.2.d. Preparation of the graft
bed. .......................... 39
3.3.3.2.2.d. Treatment of the grafts... 40
3.3.3.3. The study of the effect of
ABLS on the humoral immune res-
pone following tetanus toxoid
injection .......................... 40
3.3.3.3.1. Origin and dose of tetanus
toxoid .......................... 40
3.3.3.3.2. Determination of antibody
titer against tetanus toxoid
in the blood serum .......... 41
3.3.4. Group IV .......................... 42
3.3.4.1. The study of the influence of
ABLS on the ability of lympho-
cytes to form blast cells follow-
ing stimulation ...................... 42
3.3.4.1.1. Demonstration of blastogenesis
of lymphocytes .................... 43
3.3.4.1.1.a. The collection of lymphocytes
3.3.4.1.1.b. Culture medium and nitrogen
3.3.4.1.1.c. The cell cultures
3.3.4.3. Estimation of the effect of ABLS on the
B and T cells
3.3.4.2.1. Isolation of lymphocytes
3.3.4.2.1.a. Demonstration of T cells
3.3.4.2.1.b. Demonstration of B lymphocytes

4. Results
4.1. Cytotoxic and haemolysin titer
4.2. Effective dose of ABLS
4.3. Clinical signs
4.4. Postmortem observations
4.4.a. Gross lesions
4.4.b. Microscopic findings
4.5. Haematological analysis
4.6. The antibody titer against SRBC
4.7. Delayed hypersensitivity reaction
4.8. Skin graft survival
4.9. The effect of ABLS on the immune response to
tetanus toxoid
4.10. Lymphocyte blastogenesis following stimulation
4.11. The B and T cells percentage following ABLS
administration

Page 43
Page 44
Page 44
Page 45
Page 45
Page 46
Page 47
Page 49
Page 49
Page 50
Page 50
Page 51
Page 51
Page 52
Page 54
Page 56
Page 67
Page 68
Page 72
Page 73
Page 74
5. Discussion .................................................. 76
6. Summary .................................................... 86
7. References .................................................. 92
8. Curriculum vitae ........................................... 104
9. Appendix .................................................... 106
List of tables.

Table 1. Grouping of the experimental calves according to use .................................. 30

Table 2. Number of experimental calves in group 1. Dose and route of application of anti-bovine lymphocyte serum for the estimation of the effective dose ....................... 31

Table 3. Donor-recipient pairs of calves in skin grafting ........................................... 37

Table 4. The cytotoxic and haemolysin titers of ABLS .................................................. 49

Table 5. The reciprocal titer of antibody against SRBC ................................................ 66

Table 6. Tuberculin test ABLS treated and control calves 4 weeks after injection with Mycobacterium microti. Skin thickness in mm before and 72 hours after tuberculin injection .................................................. 67

Table 7. Graft survival time in control and ABLS treated calves ................................. 70

Table 8. Antibody titers against tetanus toxoid in ABLS treated and control calves ......... 71

Table 9. Mitogenic response of lymphocyte stimulation in ABLS treated calves ............ 73

Table 10. B and T cells in ABLS treated calves ............................................................. 75
List of figures.

Figure 1. Raising ABLS in horses ....................... 27

Figure 2. The white cell counts of ABLS treated calves from group II. ABLS was given on days 0, 2, 4, 6, 8, 10, 12, 14, 16 and 18. Each value represents the average of 7 calves .................. 57

Figure 3. The white cell counts of ABLS treated calves from group III. ABLS administrations were as in group II. Each value represents the average of 6 calves ................. 58

Figure 4. The average white cell counts in 4 control calves from group III. Each calf received 4 injections of 1 ml normal horse serum per kg body weight on days 0, 2, 4 and 6 ......................... 59

Figure 5. The average white cell counts in 3 control calves from group II. The animals were untreated ......................... 60

Figure 6. The average thrombocyte counts of 4 control and 7 ABLS treated calves from group II. ABLS administrations were on days 0, 2, 4, 6, 8, 10, 12, 14, 16 and 18. Each value represents the average of 7 calves 61
VIII

Figure 7. The average thrombocyte counts of 4 control and 6 ABLS treated calves from group III. ABLS administrations were as in group II. Each value represents the average of 7 calves .......................... 62

Figure 8. The average haematocrite values of 3 control and 7 ABLS treated calves ............ 63

Figure 9. The percentage of differential counts of calves treated with 1 ml ABLS per kg body weight on days 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 from group II. Each column represents the average of 7 calves. The column on the extreme left is the pretreatment value. The column designated $X$ represents the average of 3 control calves .......... 64

Figure 10. The percentage of differential counts of calves treated with 1 ml ABLS per kg body weight on days 0, 2, 4, 6, 8, 10, 12, 14, 16 and 18 from group III. Each column represents the average of 6 calves. The column on the extreme left represents the pretreatment value. The column designated $X$ represents the average of 4 controls... 65
Figure 11. Removal of skin graft the size of a microscope slide

Figure 12. The donor's wound was sutured

Figure 13. The sutured wound was covered with wound paste, sterile gauze and plaster tape...

Figure 14. Graft beds after they have been prepared and the bleeding has been stopped

Figure 15. The grafts in position

Figure 16. The grafts were sutured

Figure 17. The sutured grafts were covered with wound paste, sterile gauze and Hansooper tape

Figure 18. The covered grafts (Fig. 17) were dressed with plaster tape

Figure 19. White skin grafts of ABLS-treated calf ten days post-operative. The right graft is the control autograft, the left is the allograft. Both show no sign of injection.
Figure 20. Two grafts three weeks after operation.
The left autograft shows signs of desquamation of the epidermal layer but no signs of rejection. White discoloration of surrounding hairs was caused by the application of wound paste. The right graft is an allograft showing signs of rejection.

Figure 21. Section of a lymph node of a calf that died of an overdose of ABLS (7.5 - 10 ml/kg body weight) showing an enlarged follicle with nuclear debris, fibrin deposition and a few surviving germinal cells. Magnification 350x

Figure 22. Section of a lymph node of a calf sacrificed 4 days after receiving 18 ABLS injections. An enlarged follicle with depletion of follicular cells surrounded by a small rim of perifollicular cells. It is expressing immunological exhaustion. Magnification 350x
Figure 23. The paracortical area of a lymph node of a calf that died of an overdose of ABLS. Cell depletion and proliferation of reticular cells and fibroblasts. Magnification 350x ............... 110

Figure 24. A greater magnification of the upper left area of figure 23. Plasma cells, eosinophils and a few lymphocytes are seen among the proliferated reticular cells and fibroblasts. Magnification 600x ........... 110

Figure 25. Section of a lymph node of a calf that died of an overdose of ABLS. Extensive cellular depletion in the paracortical area. Magnification 60x ................. 111

Figure 26. Section of a lymph node of a calf killed 4 days after receiving 18 injections of 1 ml ABLS per kg body weight. Extensive cellular depletion in the cortical area. Islands of surviving follicles with surrounding cells are present. Magnification 60x ......................... 111
Figure 27. Section of a lymph node of a calf sacrificed 3 days after receiving 18 injections of 1 ml ABLS per kg body weight. Extensive cellular depletion in cortical and paradortical area (right) which shows sinusoidal congestion. Magnification 60x

Figure 28. Section of a lymph node of a calf sacrificed 4 days after receiving 18 injections of 1 ml ABLS per kg body weight. Cortical area with starry sky appearance. Magnification 150x

Figure 29. Section of a lymph node of a calf mentioned in figure 27. Medullary area shows widening of sinuses with proliferation and desquamation of sinusoidal cells ("sinus catarrh"). Remarkable infiltration of eosinophils in the medullary cords. Magnification 150 x

Figure 30. Section of a lymph node of a calf that died of an overdose of ABLS. Medullary area with congested lymph vessels and scarcity of cells. Magnification 150x...
Figure 31. Section of the large intestine. Loss of Lieberkühn’s glands in areas with extensive haemorrhagic necrosis.
Magnification 60x ......................... 114

Figure 32. Section of the kidney of a calf sacrificed 4 days after receiving 18 injections of ABLS. Two glomeruli showing engorgement of glomerular capillaries with erythrocytes.
Magnification 350x ......................... 114