



**Faculty of Veterinary Medicine  
Universitas Nusa Cendana**

**PROSIDING**

# **The 1st International Conference on Veterinary And Animal Science (ICon-VAS)**

**The Role of Veterinary Science to Cope with Pandemics**

**The Sub-themes as follows:**

**Veterinary Medicine, Medicine, Public Health, Agriculture,  
Animal Husbandry, Fisheries and Marines,  
Environmental Health, Biomedical**

**2020**



## **Prosiding The 1<sup>st</sup> International Conference on Veterinary and Animal Science (ICon-VAS)**

**Fakultas Kedokteran Hewan Universitas Nusa Cendana**

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## **FOREWORD BY THE DEAN OF FACULTY OF VETERINARY MEDICINE, UNIVERSITAS NUSA CENDANA (UNDANA)**

Good Morning Ladies and Gentlemen

First and foremost, All praise belongs to the Lord for blessing us today to attend this conference, the 1<sup>st</sup> International Conference Veterinary and Animal Sciences (Icon-VAS) 2020.

Ladies and Gentlemen, respectable keynote and invited speakers, presenter and all participants of the conference, all the pleasure is mine to welcome you all to attend to this conference.

Ladies and gentlemen,

We, Faculty of Veterinary Medicine, Universitas Nusa Cendana, held a national conference every year. We have conducted 7 successful national conferences since 2013. Therefore, now we have courage to hold an international conference this year. We really wish to meet all of you in Universitas Nusa Cendana in person, but the Covid-19 pandemic has forced us to conduct the conference virtually. I would like to express my sincere gratitude to all the committee of the 1<sup>st</sup> International Conference Veterinary and Animal Sciences (Icon-VAS) 2020 for their hard work so the conference can still be held during this pandemic.

It is an honor to host the Icon-VAS 2020 as it becomes a media for respectable researchers, experts, scholars, and policy makers to share ideas on emerging issues and important topics in veterinary science and related. With the theme “The Role of Veterinary Science to Cope with Pandemics”, we hope that after this conference, all of us will have advance knowledge to overcome pandemics by working together in a multidisciplinary, interdisciplinary and transdisciplinary manner.

Therefore, I would like to take this occasion to express my appreciation to keynote and invited speakers of the conference, the prominent figures in their field:

1. Prof. Naoaki Misawa (University of Miyazaki, Japan),
2. Dr. drh. Hanna Sidjabat, M.V.Sc (Griffith University, Australia)
3. Prof Michael P. Reichel (Cornell University College of Veterinary Medicine, United States of America)
4. Prof. Dr. drh. I Gusti Ngurah Kade Mahardika (Faculty of Veterinary Medicine, Udayana University of Bali)

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5. Dr. Fred Unger (International Livestock Research Institute Bangkok, Thailand).

My appreciation should also be addressed to all presenters and participants from many universities and research institutions that have shared significant contributions to make this event possible.

Finally, in the name of God, I hereby officially open the 1<sup>st</sup> International Conference Veterinary and Animal Sciences 2020.

Have a wonderful and nice conference,

Thank you,

**Dr. drh. Maxs U.E. Sanam, M.Sc**  
Dean,  
Faculty of Veterinary Medicine  
Universitas Nusa Cendana  
Indonesia

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## **GREETING FROM THE CHAIRMAN OF THE 1<sup>ST</sup> ICON-VAS**

Good morning ladies and gentlemen.

It is my pleasure to welcome all of our presenters and participants to the 1<sup>st</sup> International Conference Veterinary and Animal Sciences (Icon-VAS) 2020. Thanks to the Lord for blessing us so we can hold this International Conference today.

In particular, I would like to extend my gratitude to our keynote speakers: Prof. Naoaki Misawa (Center for Animal Disease Control, University of Miyazaki, Japan), Dr. drh. Hanna Sidjabat, M.V.Sc (Menzies Health Institute, Griffith University, Australia), Prof Michael P. Reichel (Cornell University College of Veterinary Medicine, United States of America) and our invited speakers: Prof. Dr. drh. I Gusti Ngurah Kade Mahardika (Faculty of Veterinary Medicine, Udayana University of Bali) and Dr. Fred Unger (Centre for International Migration and Development at International Livestock Research Institute Bangkok, Thailand).

The Icon-VAS 2020 is the first of many future International Conferences that will be held by the Faculty of Veterinary Medicine, Universitas Nusa cendana, Indonesia. Due to the Pandemic, Corona Outbreak, the 1<sup>st</sup> Icon-VAS 2020, is carried out in a virtual conference.

All the speakers, presenters and participants, the emergence and spread of diseases originated from animals whether zoonotic and or non-zoonotic could become pandemics. Pandemics can have a devastating effect on the lives of people and animals throughout the world. The COVID-19 global pandemic reminds us that infectious disease does not respect borders and possibly also species. Researchers are interested broadly in any animal that the coronavirus could infect, whether or not it causes illness. The African Swine Fever (ASF), a severe viral disease affecting domestic and wild pigs, has responsible for serious production and economic losses of farmers from China and continued into Southeast Asia, including Indonesia in the North Sumatra, Bali and East Nusa Tenggara (NTT).

Pandemics have brought the understanding that research and efforts must be conducted in a multidisciplinary, interdisciplinary and transdisciplinary manner, and it is also well known as One Health. Therefore, the Icon-VAS 2020 raise the topic of “The Role of Veterinary Science to Cope with Pandemics”. It is





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aimed to share knowledge and also strategies in overcoming zoonotic and non-zoonotic pandemic diseases.

I would like to take this opportunity to report some formal information related the Icon-VAS 2020. The numbers of presenter are 13. The committee has already accepted abstracts and distributed them in many topics. The submitted papers are 16. The conference will have 5 plenary sessions and 2 parallel sessions.

Last but not least, Hopefully, the Icon-VAS 2020 will increase research collaborations between institutions involved. I would like to express blessed gratitude to our University management for the support to this conference and also all the committee involved, that without you all, it will not be conducted. Representing the organizer, I proudly welcome all of you to the Icon-VAS 2020. I wish you a successful and enlightening conference.

Thank you.

**Drh. Yeremia Yobelanno Sitompul, M.Sc**

Chairman

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Faculty of Veterinary Medicine

Universitas Nusa Cendana

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## COMMITTEE

### Person in Charge

Dean Faculty of Veterinary Medicine Universitas Nusa Cendana  
(Dr. drh. Maxs U.E. Sanam, M.Sc.)

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Vice Dean for Academic Affair Faculty of Veterinary Medicine Universitas Nusa Cendana  
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Erni F. Kadja, S.Pt.,M.Si  
Achmad A. I. Madjid

### Moderator

Prof. Ir. Frans Umbu Datta, M.AppSc, PhD  
drh. Elisabet Tangkonda, M.Sc  
drh. Larry W. Toha, M.Sc  
drh. Maria AEGA Gelolodo  
drh. Jayusman A Joesoef, M.Si

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## CONFERENCE PROGRAMS

October 20<sup>th</sup>, 2020

Time (Central Indonesia Time/GMT+8)	Description
09.00-09.30	Registration of participants and preparation for the conference
09.30-10.00	<i>Opening the conference by MC</i> Singing Indonesia Raya National Anthem Speech by the Chairman of the committee Speech by Dean of Faculty of Veterinary Medicine, Nusa Cendana University Speech by the Rector of Nusa Cendana University or the representative (officially opening the conference)
10.00-10.20	<i>Keynote Speaker 1</i> <i>Prof. Naoaki Misawa (Direktur Pusat Penyakit Hewan, Universitas Miyazaki, Jepang)</i> <i>“The Role of Vet on Non-Zoonotic Pandemic Diseases”</i>
10.20-10.40	<i>Q&amp; A, moderator: drh. Elisabet Tangkonda, M.Sc</i>
10.40-11.00	<i>Keynote Speaker 2</i> <i>Dr. drh. Hanna Sidjabat, M.V.Sc (Adjunct Research Fellow, Universitas Griffith, Menzies Health Institute Queensland)</i> <i>“The Role of Veterinary Profession in Promoting Human Health”</i>
11.00-11.20	<i>Q&amp; A, moderator: Prof. Ir. Frans U. Datta, MAppSc</i>
11.20-11.40	<i>Keynote Speaker 3</i> <i>Michael P. Reichel (Adjunct Professor pada Department of Population Medicine and Diagnostic Sciences, Cornell University College of Veterinary Medicine)</i> <i>“One Health approach to overcome current and prevent the next pandemics”</i>

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11.40-12.00	<i>Q&amp; A, moderator: Prof. Ir. Frans U.Datta, MAppSc</i>
12.00-12.15	Invited Speaker Prof. Dr. drh. I Gusti Ngurah Kade Mahardika (Guru Besar FKH Udayana) “Updated of African Swine Fever”
12.15-12.30	Invited Speaker Dr. Fred Unger (Senior Scientist of Animal and Human Health, Centre for International Migration and Development at International Livestock Research Institute Bangkok, Thailand) “Animal Source Food Value Chains”
12.30-13.00	<i>Q&amp; A, moderator: drh. Larry Richard Wellem Toha, M.Sc</i>
13.00-13.30	Break Session
13.30-16.00	1 <sup>st</sup> and 2 <sup>nd</sup> parallel session (presentation and discussion), lead by the moderator
16.00	Closing by Dean of Faculty of Veterinary Medicine, Nusa Cendana University

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## SCHEDULES FOR PARALLEL SESSION

Class : Parallel 1  
Class Code : P1  
Moderator : drh. Jayusman A Joesoef, M.Si

No	Time	Presentation Title	First Author
01	13.30 – 13.40	Blood Description of Ongole Beef Cattle of Rote	Yanse Yane Rumlaklak
02	13.40 – 13.50	The Importances of Veterinary Services During Eid-Al-Adha Festivals in Indonesia	Aji Winarso
03	13.50 – 14.00	Some Epidemiological Variables of Septicaemia Epizootica (SE) Disease and Immunity Status Bali Cattle Post Vaccination in Kupang City, East Nusa Tenggara, Indonesia	Maxs U. E. Sanam
04	14.00 – 14.10	Gross Energy Content and Prediction of Digestible Energy and Metabolic Energy of Tamarind Seed Liquid Feed Fermentation of Pig Grower	Redempta Wea
05	14. 10 – 14.20	Birth Weight and Placental Weight of Belgian Malinois Dog - A Case Report	Tri Utami
06	14. 20 – 14.30	Effects of Supplementation of Kacang Asu ( <i>Pueraria phaseoloides</i> ) on Blood Profiles of Kacang Goats Fed Kume ( <i>Sorghum plumosum</i> Var. <i>Timorensis</i> ) Grass Hay	Immanuel Benu

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**Class** : Parallel 2  
**Class Code** : P2  
**Moderator** : drh. Maria A. E. G. A. Gelolodo

No	Time	Presentation Title	First Author
01	13.30 – 13.40	Arbila ( <i>Phaseolus lunatus</i> L) Diversity as Food and Feed in Nekamese Sub-District Based on Morphology of Bean	Bernadete B. Koten
02	13.40 – 13.50	Case Report: Pyometra in a 3 Years Old Dog	Yohanes T. R M. R. Simarmata
03	13.50 – 14.00	A Case Report of Brain Injury in A Cat due to Being Hit by a Vehicle	Yeremia Yobelanno Sitompul
04	14.00 – 14.10	Isolation and Characterization of Dermatophytosis Fungus in Livestock	Dwi Endrawati
05	14. 10 – 14.20	Study Comparative of Skin Histology of Sumba Ongole ( <i>Bos indicus</i> ) and Bali Cattle ( <i>Bos sondaicus</i> )	Vilomena Kusi Toan
06	14. 20 – 14.30	Study of Multidrug Resistance (MDR) <i>E. coli</i> on Pet Dogs in Kupang City	Novalino H.G. Kallau
07	14. 30 – 14.40	The Effect of Bittermelon ( <i>Momordica charantia</i> L) Fruits Infusion Intake on Hepatic Serum Level	Cynthia Dewi Gaina

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## POSTER PRESENTER

No	Presentation Title	First Author
01	Study on The Design and Structure of Traditional Chicken Housing in Kupang Regency	Maxs Urias Ebenheizer Sanam
02	Community Empowerment in Supporting Livestock-Based Agro-Tourism, Nggorang Village, West Manggarai	Annytha Detha
03	The Red Blood Cell Distribution Width in Goats ( <i>Capra hircus</i> ) with Moringa Oleifera Feed Supplementation	Nemay Ndaong
04	Sambiloto And Curcumin Administration as Combination Therapy for The Treatment of Malaria in Berghei Plasmodium Infected Mice	Anita Lidesna Shinta Amat
05	Empowering Farmer Community in Camplong II Village Through Small Scale Farming	Cynthia Dewi Gaina

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**KEYNOTE SPEAKER**

**Prof. Naoaki Misawa**

<b>Name</b>	Naoaki MISAWA, D.V.M., Ph.D.
<b>Gender</b>	Male
<b>Place, Date of Birth</b>	Tokyo Japan, November 2nd, 1957
<b>Address</b>	1-1 Gakuenkibanadai-nishi, Miyazaki 889-2192,
<b>Phone</b>	Japan +81-985-58-7284
<b>Email</b>	<a href="mailto:a0d901u@cc.miyazaki-u.ac.jp">a0d901u@cc.miyazaki-u.ac.jp</a>
<b>Employment history</b>	<p>April/1984- March/1988 Engineer, Miyakonojo Meat Inspection Center, Miyazaki, Japan</p> <p>April/1988 - March/1990 Engineer, Miyazaki Public Health Center, Miyazaki, Japan</p> <p>April /1990 - May/2003 Assistant Professor, Department of Veterinary Science, University of Miyazaki (UOM), Japan</p> <p>April/1996 - March/1998 Research Fellow, School of Medicine, Vanderbilt University, TN, USA</p> <p>June/2003 - May/2009 Associate Professor, Department of Veterinary Science, UOM, Japan</p> <p>June/2009 - March/2013 Professor, Department of Veterinary Science, UOM, Miyazaki, Japan</p> <p>April/2013 - Present Professor &amp; Director, Center for Animal Disease Control. UOM, Japan</p>

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**KEYNOTE SPEAKER**

**Dr. drh. Hanna Sidjabat, M.V.Sc**

**PERSONAL INFORMATION**

Name	: Hanna Evelina Sidjabat
Degrees	: DVM, PGD in Vet Studies, MVSc, PhD, GradCert in Higher Education
Email	: h.sidjabat@griffith.edu.au
ORCID ID	: <a href="https://orcid.org/0000-0001-5625-1317">https://orcid.org/0000-0001-5625-1317</a>
ResearcherID	: G-2675-2010
Scopus Author ID	: 12808650900
Twitter	: @DrHannaSidjabat
Mobile number	: +61 410 388 633

**CURRENT POSITION**

Aug 2020 – now      Casual teaching and marking of **BIO1104, Medical Microbiology and Immunology at the University of Southern Queensland.**

20 Dec 2019 – now      Adjunct Research Fellow, **Menzies Health Institute Queensland, Griffith University**, Gold Coast Campus, Australia.

**PREVIOUS POSITIONS**

Jul 2018 – 31 Dec 2019      **Honorary Research Fellow** within Infectious Diseases Theme, UQCCR, Australia.  
 Responsibilities: To write manuscripts of the data generated during Dr. Sidjabat’s appointment as a Chief Investigator of Sidjabat Group and Research Officer of Paterson Group and Cervin Group.  
 To supervise one Master student and one visiting PhD student from Colombia.

Aug 2017 – Jul 2018      **Chief Investigator** at the Infection and Immunity Theme, UQCCR, Australia.  
 Responsibilities: Leading a small research group in conducting research in probiotic discovery and development, including development of next generation probiotics. Techniques used in this research workflow is





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- Jan 2016 – Jul 2017 **Research Officer** of Infection and Immunity Theme, UQCCR, Australia.  
Responsibilities: leading the microbiology, whole-genome sequencing and proteomic research of microbiome and probiotic development for chronic rhinosinusitis and otitis media of Prof. Anders Cervin within the Infection and Immunity Theme. Other responsibilities include in supervising research assistant, PhD and Honours students, volunteers.
- 2009 – 2015 **Laboratory Research Leader, Research Officer** of Infection and Immunity Theme, UQCCR, The University of Queensland, Australia.  
Responsibilities: leading research within the Infection and Immunity theme, providing inputs in research design to all laboratory users, managing the laboratory, supervising PhD and Honours students and conducting research.
- 2007 – 2009 **Postdoctoral Research Fellow**  
Division of Infectious Diseases, University of Pittsburgh, Pennsylvania, USA. Responsibilities: conducting research and supervising students.
- 2001 – 2003 **Lecturer in Bacteriology and Immunology** – Faculty of Medicine, Christian University of Indonesia, Jakarta, Indonesia.  
Responsibilities: lecturing and tutoring practical classes.

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**KEYNOTE SPEAKER**

**Michael P. Reichel**

**I. PERSONAL INFORMATION**

Name : Michael Philipp REICHEL  
Degrees : VS, DVPH, DVTM, MVSc, MBA, Dr med vet, PhD  
Address : Dept of Population Medicine & Diagnostic Sciences,  
College of Veterinary Medicine, Cornell University,  
Ithaca, NY, USA  
Email : mpr97@cornell.edu  
Phone :

**II. CURRENT POSITION**

Adjunct Professor at the Dept of Population Medicine & Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA

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**INVITED SPEAKER**

**Prof. Dr. drh. I Gusti Ngurah Kade Mahardika**

I Gusti Ngurah Kade Mahardika  
Jembrana-Bali-Indonesia, in 27<sup>th</sup> October 1963  
Jl. Citarum GL No. 8  
Denpasar 80225  
Bali-Indonesia  
Phone 0061-361-243459; Mobile 08123805727  
Email: gnmahardika@unud.ac.id;

**Employment**

Since March 2014	The Head of Udayana University Center of Excellence of The Indonesian Biodiversity Research Center (IBRC)
Since July 1 <sup>st</sup> 2009	Professor at The University of Udayana Bali
Since 2005	Founded and Head of the Animal Biomedical and Molecular Biology Laboratory, Faculty of Veterinary Medicine Udayana University
1989-now	Lecturer at The Faculty of Veterinary Medicine Udayana University in the subject Virology, Immunology, and Molecular Biology
1999-now	Lecturer in various post-graduate programs at the University of Udayana in the subject Immunology, Molecular Biology, and Bioinformatics

(Scopus Author ID 6503977071; ORCID ID: <http://orcid.org/0000-0001-5525-0793>)

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**INVITED SPEAKER**

**Dr. Fred Unger**

**I. PERSONAL INFORMATION**

Name : International Livestock Research Institute, ILRI |ilri.org  
Degrees : Graduated in Vet Medicine  
Humboldt University Berlin &  
Freie Universität Berlin, International Animal Health  
Address : ILRI, Room 301-302, B1 Building, Van Phuc  
Diplomatic Compound, 298 Kim Ma Street, Ba Dinh  
District, Hanoi, Vietnam  
Email : f.unger@cgiar.org  
Phone : Tel: +84 4 32373995 (ext. 103)| Fax: +84 4 32373996 |  
M: +84 345976093| Skype: fred-deutsch

**II. CURRENT POSITION**

Regional Representative for ILRI East and Southeast Asia, Senior Scientist and  
Veterinary Epidemiologist

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## BLOOD DESCRIPTION OF ONGOLE BEEF CATTLE OF ROTE

**Yanse Yane Rumlaklak**

Program Studi Kesehatan Hewan, Politeknik Pertanian Negeri Kupang  
Jl. Prof. Dr. Herman Johannes, Desa Lasiana, Kecamatan Kelapa Lima, Kota  
Kupang

Corresponding author : yanne\_drh@yahoo.co.id

The Ongole beef cattle of Rote (RO) is one of Indonesian local breeds, which has been patented as a germplasm in Rote Ndao Regency of East Nusa Tenggara Province, Indonesia. As a new local cattle grove, availability of reports on the cattle is lacking. Therefore, a complete blood description is badly required, which would be a reference in maintaining and improving the population of this cattle breed. This study was aimed at describing blood of Ongole beef cattle of Rote. The results will be referred to as a screening test to evaluate health conditions of the cattle in general, immune antibody to combat infectious agents in evaluating physiological states of the animal and diagnostic establishment of one disease. Samples were collected from six heads of Ongole beef cattle of Rote origin especially from Lobalain sub-district. The selected cattle were in health conditions according to the general inspection. Blood parameters consist of blood cell rate, concentration of haemoglobin, haematocrit value, erytroisit index, and leucocyte calculation. The results showed that the average of white blood cells was  $27.7 \times 10^3 / \mu\text{L}$ , red blood cells was  $5.8 \times 10^6 / \mu\text{L}$ , haemoglobin was 12.2g/dl, haematocrit was 21.5%, MCV was 37.7 fl, MCH was 24.0 Pg, MCHC was 61.6g/dl, and platelet was  $250 \times 10^6 / \mu\text{L}$ .

### INTRODUCTION

Rote Ongole beef cattle (ROC) is one of the newest germplasm which was patented as one of the local genetic resources according to Ministry of Agriculture decree number 41/kpts/pk.020/1/2017 date January 20<sup>th</sup>, 2017. Phenotype traits of the cattle were marked by clean white hair coat or that varied with dark red or black stripes. This breed has curved upwards horns, short ears with no hump, small face and head. It has wattle and hump similar to those of Sumba Ongole cattle. As one of a new grove of cows, health factor attracts more attention in raising ROC.

In general, World Health Organization (WHO) defines health condition that covers physical, mental and social welfare. An important physiological body parameter that reflects health condition of animal was blood (Adam et al., 2015). Blood is one parameter of health status of an animal as its components function in regulating animal physiology. The role of blood in general related to transportation of bodily components include nutrients, oxygen, carbon dioxide,



metabolism, hormone and endocrine tissue, heat and body immune (Hoffbrand dan Pettit, 1987). Blood has a more complicated role in maintaining a normal physiological process, therefore optimum animal productivity.

Analysis of blood and its components is a method applied in evaluating health condition of an animal (Hoppe, 2010). Other studies (Duncan and Prasse (2011), Coles (1980), Bush (1991), Nath *et al.* (2014), and Xuan *et al.* (2018), reported that analysis of blood and its components may give some information related to the health status for animal and human being. Furthermore, Jain, 1998 stated that a complete blood analysis in animal can be used as a screening test to judge the general health status, the ability of body to combat infection and to diagnose one disease. The objective of this study was to figure out the blood of ROC since this is novel information. Consequently, data of blood physiology of cattle to diagnose type of diseases refers to results of research on other breeds of cattle fall into both Indonesian local breeds and foreign breeds.

## RESEARCH METODOLOGY

This study was using blood samples of six heads (3 bulls and 3 cows) of clinically healthy ROC origin from Lole Oen Village, Lobalain sub-region, Rote Ndao Region, East Nusa Tenggara Province, Indonesia. Blood sample was collected from jugular vein in the neck. Prior collection, the jugular vein area was disinfected with alcohol 70% then the blood was collected into a 3-ml EDTA tube.

Moreover, blood assay to determine haematology rates of ROC has been conducted in the laboratory of Animal Health, Study Program of Animal Health, Department of Animal Husbandry, State Agricultural Polytechnic of Kupang. A haematology auto analyser (Labomec Inc VH-22) was operated following these steps. Firstly, the tube was turned around following an 8 shape to homogenize the blood sample. Secondly, the auto analyser was prepared, the lid of the tube was opened then placed under sampling nozzle where the tip of the nozzle touch the base of the tube, so that the blood sample was sucked through the nozzle to deliver to the instrument, from which an automatic process takes place in the haematology auto analyser. Lastly, a complete blood assay was analysed by the tools and the results were directly printed out.

## RESULTS AND DISCUSSION

The results, consisting of leucocytes, erythrocyte number, haemoglobin, haematocrit/packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and thrombocyte (platelet), are presented in Table 1.



Table 1. Hemotological parameters in Rote Ongole cattle

No	Test	Results						Average	Nomal range	
		Male (M)			Female (F)				Cattle	Bali cattle
		1	2	3	4	5	6			
1	Leukocyte (10 <sup>3</sup> /μL)	10.6	51.9	48.9	14.9	9	30	27.7	5,1-13,3*	2,3-9,5***
2	erythrocyte (10 <sup>6</sup> /μL)	1.29	4.96	5.31	6.01	8.15	7.99	5.8	4,9-7,5*	6,33 - 8,89**
3	Haemoglobin (g/dl)	5.4	11.7	13	13	15.2	14.3	12.2	5,1-13,3*	9,6 - 10,5**
4	Haematocrit (%)	5.7	20.46	20.86	25.91	27.42	26.71	21.5	21-30*	31,5% - 34,7**
5	MCV (fl)	43	41	39	42	33	33	37.7	36-50*	39-50**
6	MCH (Pg)	42.42	23.59	24.54	21.68	18.74	18.02	24.0	14-19*	11,6 - 15,2**
7	MCHC (g/dl)	96.3	57.2	62.4	50.2	55.7	53.9	61.6	38-43*	29,8 - 33,0**
8	Platelet (10 <sup>3</sup> /μL)	135	188	189	440	191	314	250.7	160-165*	

Source : \*Weiss and Wardrop (2010)  
 \*\*Diparayoga *et al.* (2014)  
 \*\*\*Hartaningsih *et al.* (1983)

Table 1 shows that the normal values as references differ among the breeds. This difference could be due to the geographical position and the environment. This was in line with the site where the samples were collected was from hilly region of central Rote. Swenson (1993); Hoffbrand dan Pettit (1987), argued that haematology could be influenced by many factors such as age, sex, work load, race, nutritional status, lactation, latitude and ambient temperature. Data of early study (Weiss and Wardrop, 2010) revealed that average values of erythrocyte, haemoglobin, haematocrit and MCV are in a normal range but the values for total leucocytes, MCH, MCHC and platelet are right beyond the normal range.

### Leukocyte

Table 1 shows that leucocytes values of ROC was 9x10<sup>3</sup>/μL – 51.9x10<sup>3</sup>/μL. which was higher as compared to that of other cattle. Weiss and Wardrop (2010) reported that leucocytes total was normally range between 5,1 and 13,3x9x10<sup>3</sup>/μL as compared to that of normal leucocytes. The present study recorded two blood samples, namely J1 (10.6x9x10<sup>3</sup>/μL) and B5 (9x9x10<sup>3</sup>/μL ), fall in the normal range while others were higher than the normal range.

The higher leucocytes in the present study might be explained by the nutritional status and environment. The ROC being raised in the dry hilly region might consume less amount and nutritious feed as the green forage availability





depends on rain fall. Aprianti et al. (2006) reported that haematology values could be affected by nutritional status and environment. The dry environment and less forage availability are altogether stimulate body immune as a normal protective response; one of which was the increased of leucocytes as the body's defense cells, known as leukocytosis (Bunga *et al.* 2019). Anderson & Lorraine (2006) further explained that the increased of total leucocytes values indicates a physiological response to protect the animal's body from microorganisms' attack.

## **Erythrocyte, Haemoglobin and Haematocrit**

Total values of erythrocyte, haemoglobin and haematocrit of ROC in the present study were  $5,8 \times 10^6/\mu\text{L}$ , 12.2 g/dl, and 21.5% (Table 1), respectively. These values fall in the normal range (Weiss & Wardrop, 2010). Only one blood sample B5 ( $8.15 \times 10^6/\mu\text{L}$ ) was found to be higher erythrocyte, two samples have higher haemoglobin B5 (15.2 g/dl) and B6 (14.3 g/dl), and three samples of male ROC namely J1, J2 and J3 showed a decrease haematocrit to be 5.7%, 20.5%, and 20.8%, respectively.

Total erythrocyte, haemoglobin concentration and haematocrit value have a positive correlation (Dewi *et al.* 2018). Baldy (2003) argued that the main component of erythrocyte was haemoglobin which transported most oxygen and small amount of carbon dioxide fraction and to maintain normal blood pH. Haematocrit was a percentage of erythrocyte in 100 ml of blood, which was affected by total erythrocyte (Frandsen, 1992; Adam *et al.*, 2015).

The increase of total erythrocyte in B5 cattle could be due to stress resulted from poor nutrition and high ambient temperature. Stress leads to less oxygen supply in the body (hypoxia), which gave result to an increase of erythrocyte value as a body response as found in B5 and B6 samples. This finding was in line with that reported by Bunga *et al.* 2019. Furthermore, Weiss and Wardrop (2010) reported that an increase of erythrocyte production was resulted from tissue hypoxia. Body is trying to fulfil the oxygen demand by way of increasing erythrocyte production so that more oxygen was transported. In this status, kidneys released more erythropoietin to stimulate erythrocyte production as a response on hypoxia in tissue body. The decrease of haematocrit in the three samples J1, J2 and J3 could be due to poor nutrition.

Nutrients are highly influenced erythrocyte total, haemoglobin concentration and haematocrit value. Sufficient nutrients are important factors in processes of both haematopoiesis and erythropoiesis (Adam *et al.* 2015; Bunga *et al.* 2019). Nutrients such as amino acids, iron, vitamin and Cu in feedstuffs are important components to affect total erythrocyte (Frandsen, 1992). Adam *et al.* (2015) explained that minerals and vitamins play an important role in erythropoiesis process. Iron was required in heme synthesis. Copper in the form of ceruplasmin was highly important in releasing iron from tissue into plasma. Vitamin B6 was required as a cofactor in the stage of enzymatic heme synthesis.



Cobalt was very important in the vitamin B12 synthesis in the ruminants (Guyton and Hall, 1997; Adam *et al.* 2015). Vitamin B12 and folic acids deficiency resulted from maturation failure in erythrocyte, which then reduced total erythrocyte in the blood (Guyton and Hall, 1997).

## **Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC)**

In general, Table 1 shows that MCV values were normal while MCH and MCHC values increased (Weiss and Wardrop (2010)). Two samples (B5 and B6) of the cows have lower MCH than that of normal. The decrease of MCH occurred as a result of erythrocyte was less than normal (microcytic) which was generally due to iron deficiency, by contrast macrocytic occurred as a result of deficiency of vitamin B12 and folic acid (Stockham and Scott 2008). In the present study, microcytic occurred as the nutrient supply was less than requirement. Table 1 shows an opposite MCH values as compared to MCV values. Normal MCV blood samples increased MCH values, while the increased MCV had a normal MCH. In general, MCH increased in a macrocytosis condition (Nordenson 2002). The increased MCH occurred as a consequent of an increased of erythrocyte numbers with a bigger size than normal, which was due to heparin failure, iron deficiency and anaemia.

All samples in current study have high MCHC values (Table 1). This was known as hyperchromic or the Haemoglobin concentration in the blood sample was higher than normal. This was resulted from haemolysis, rupture of red blood cells and the discharge of haemoglobin into plasma. This condition causes a haemoglobinemia, namely a condition where Haemoglobin was freely available in the blood plasma, so that this Haemoglobin was also counted at the time of haemoglobin concentration was measured, as a result MCHC tends to be higher than normal (Stockham dan Scott 2008). In addition, Siswanto *et al.*, (2014) listed factors that affected this condition including nutrition, environment, disease, and blood storage. The observation at the time of sampling in the site shows that these cattle are healthy, no specific clinical symptoms. Therefore, this condition was assumed to be resulted from nutrient deficiency.

## **Thrombosis/ Platelet**

The average thrombosis value on ROC was  $250 \times 10^3 / \mu\text{L}$  (Table 1). This value was higher than normal (Weiss and Wardrop (2010)). In detail, one sample in the current study had low thrombosis value (J1) and five other increased (J2, J3, B4, B5 and B6). These results can be related to blood cell disorders and thrombocyte production. In this study, thrombocytosis and thrombocytopenia caused by an imbalance of nutrient supply. Similar to erythrocyte, thrombocyte concentration was mostly affected by nutrients. A normal thrombocyte



concentration supports the thrombocyte in fulfilling the main function of thrombocyte in primary haemostasis.

In order to maintain a normal primary haemostasis and thrombosis achieve its function in forming initial platelet plug, then there should be sufficient numbers of thrombocyte available in the blood circulation and the thrombocyte was normally functioning (Swenson, 1993).

## CONCLUSION

It can be concluded that the average value of erythrocyte, haemoglobin, haematocrit and MCV are in normal range, while total leucocytes values, MCH, MCHC and platelet are beyond the normal range.

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## THE IMPORTANCES OF VETERINARY SERVICES DURING EID-AL-ADHA FESTIVALS IN INDONESIA

Aji Winarso<sup>\*</sup>, Novalino H. G. Kallau

Department of Animal Diseases Sciences and Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Nusa Cendana, Kupang, East Nusa Tenggara, Indonesia

\*Corresponding author: [ajiwinarso@staf.undana.ac.id](mailto:ajiwinarso@staf.undana.ac.id)

One of the most important annual festival for Muslims is Eid al-Adha. During the festivals, Muslims slaughter animals specified by sharia and give the meat away to the community. Since in Indonesia the slaughters were majorly done off-abattoir, this festival is remaining of concern in animal welfare and public health issue year by year. This article aims to describe the veterinary services needed by community to improve Eid al-Adha celebration in Indonesia. This article is a case report of veterinary services during Eid al-Adha festival in Matani Insan Unggul Madani Foundation, Kupang, Indonesia. The results showed that veterinary services are important in animal selection, animal transport, animal shelter period, animal slaughter, and meat processing and distribution. Veterinary aids are needed to choose the sacrificed animal since the animal must meet some criteria according to the sharia. Veterinary consults are important during animal transport and animal shelter period to minimize animal stress. Veterinary services are needed during slaughter to confirm health status of the animals by ante-mortem and post-mortem examination, improve the personnel safety, and minimize animal stress during handling and slaughter. Veterinary consults are needed in meat processing and distribution since meat are perishable food. Festival committee has to provide meat of halal (lawful according to sharia), healthy, safe, and intact one.

### INTRODUCTION

One of the most important annual festival for Muslims is Eid al-Adha. During the festivals, Muslims slaughter animals specified by sharia and give the meat away to the community. Since in Indonesia the slaughters were majorly done off-abattoir, this festival is remaining of concern in animal welfare and public health issue year by year (Winarso et al. 2017). The slaughter of sacrificial animals in incidental locations is not equipped with adequate infrastructure as in ruminant slaughterhouses (RPH-R). In addition, human resources who handle animals and meat are often incidental as well (Winarso et al 2018). According to Amanda et al. (2017) the community is less informed about the importance of sanitation in slaughtering sacrificial animals. Therefore, the implementation of animal slaughter outside the slaughterhouse has the potential to cause



environmental contamination by disease agents from slaughtered animals, the spread of foodborne diseases, and a decrease in the quality and hygiene of meat. The government has issued regulation of the Minister of Agriculture Number 114/Permentan/PD.410/9/2014 concerning the slaughter of sacrificial animals (Mentan RI, 2014). The regulation is aimed at ensuring the implementation of good animal slaughter and good production of safe, healthy, intact and halal meat. This article aims to describe the veterinary services needed by community to improve Eid al-Adha celebration in Indonesia.

### METHODS

This article is a case report of veterinary services during Eid al-Adha festival at Matani Insan Unggul Madani Foundation, Kupang, Indonesia in 2020. An observation and interview were conducted during the festival celebration in 2020. The data were then analyzed descriptively using Microsoft Excel.

### RESULTS AND DISCUSSION

The sacrificial committee faces three important issues in the implementation of the Eid al-Adha celebration, namely animals that are slaughtered that meet the sharia requirements, animal welfare implementation, and veterinary public health. In dealing with this issue, the committee needs veterinarian services. This is clearly stated in the regulation of the Minister of Agriculture (Permentan).

Answering the first issue that was presented earlier, about animal requirements, some of animals slaughtered did not meet the sharia requirements (2 of 6 cattle; see Table 1). These animals were a distribution from a national sacrificial committee. It is difficult for veterinarians to provide advice on replacing these sacrificial animals. The owner of the sacrificial animal was not at the slaughter location, they might be from other cities. Communication with the person in charge is also difficult because the animals are delivered by delivery officers.

Table 1. Number of animals slaughtered

Animal species and origin	Quantity (heads)	Number of animals met the sharia requirements (%)	Comments
Bali cattle (local committee)	3	3 (100%)	All of animals are declared healthy and adequate age
Bali cattle (a national organizing committee)	3	1 (33,3%)	1 animal was underage; and 1 animal suffered from lameness (fracture)
Goat (local committee)	4	4 (100%)	All of animals are declared healthy and adequate age



Addressing next issue about animal welfare implementation, researchers provided counseling, invited a halal slaughterer, and aided with clamp pens to knock down cattle. Counseling increased knowledge and is expected to change people attitudes and actions. Knowledge about animal welfare is very important because without knowledge animal welfare is neglected, as it has been stated that the sources of animal suffering are summarized in 4i's, namely ignorance, inexperience, incompetence, and inconsideration (Gregory 1998). Animal welfare during animal slaughter focus on preventing unnecessary animal suffering (Broom & Fraser 2007). Good animal welfare is beneficial for the quality of the meat produced (Collins & Huey 2015). In large volume work, animal welfare can provide energy efficiency because handling stressed animals is more difficult (Haemsworth & Barnett 2000) and increases the risk of injury (Waiblinger et al. 2006). Clamp pens facility could reduce personnel needed to handle the animal and minimize injury risks towards animals or personnel involved.

Meanwhile, hygiene and sanitation aspects showed a quite good implementation in this research (Table 2). Besides aiming at producing safe and healthy meat, also protects the environment from pollution (Aerita et al. 2014). Antemortem examination is important to ensure the animal is healthy before slaughter, while postmortem examination is a follow-up examination to ensure the health of carcass and other organs (Tolistiawaty et al. 2015).

Table 2. Hygiene practices during Eid al-Adha sacrificial slaughter

No	Hygiene practices	Implementation	
		Yes/No	Score
1	An ante-mortem examination was performed	Yes	1
2	A post-mortem examination was performed	Yes	1
3	Excavation is available for waste disposal	Yes	1
4	There is enough clean water and soap for hand washing	Yes	1
5	Clean floors or clean mats for skinning the animals	Yes	1
6	The meat processing plant was separated from the slaughterhouse and offal cleaning	Yes	1
7	Cleanliness of the place and equipment was maintained	Yes	1
8	Meat was placed in a clean container (bucket / basket)	Yes	1
9	The meat was processed on the table	No	0
10	Personnel wearing gloves and masks	No	0
11	Personnel who handle meat did not eat, smoke, or scratch their limbs/hair.	Yes	1
12	Personnels washed their hands before and after handling meat	Yes	1
13	Meat was packaged separately from offal	Yes	1
14	Food grade material / clear plastic for meat packaging	Yes	1
15	Immediate meat distribution	Yes	1
<b>Total Score</b>		<b>13 of 15</b>	
<b>Percentage (%)</b>		<b>86,7%</b>	



Hygiene and sanitation in the slaughter area are well supported by the availability of excavation for waste, clean water, soap, slaughter floors that are easy to clean, and the availability of tarpaulins for skinning. The meat handling area is separate from slaughterhouses and waste handling. A few notes are about adequate gloves and masks availability and proper table for meat processing.

Meat packaging is also separated from offal packaging. Separation of meat from offal reduces the risk of damage to meat (Amanda et al. 2017). The packaging for the meat and offal that will be distributed is also clear plastic. Because there is no refrigeration facility, the meat is immediately circulated to avoid spoilage (Djaafar & Rahayu 2007). This is in accordance with the recommendation of MOA No. 114 / Permentan / PD.410 / 9/2014. The pH test and water binding capacity of beef are still good for up to 8 hours (Setiawan et al. 2017).

## CONCLUSIONS

Veterinary services during Eid al-Adha festival are important in order ascertain animal appropriateness according sharia criteria, ensure animal welfare, and assure food safety. We suggest that national level sacrificial committees involve veterinarians to ensure slaughtered animals meet the requirements before they are distributed to the sacrificial slaughter points.

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**SOME EPIDEMIOLOGICAL VARIABLES OF SEPTICAEMIA  
EPIZOOTICA (SE) DISEASE AND IMMUNITY STATUS BALI CATTLE  
POST VACINATION IN KUPANG CITY, EAST NUSA TENGGARA,  
INDONESIA**

**Maxs U.E.Sanam\*, Tri Utami, Tarsisius C Tophianong**

Lecturer at the Faculty of Veterinary Medicine, Nusa Cendana University,  
Kupang, NTT

\*Corresponding author: maxi\_sanam@yahoo.com

Septicemia epizootica (SE) is a contagious disease affecting ruminants, especially in cattle and buffaloes caused by the bacterium *Pasteurella multocida* serotype B2. This study aims to determine the epidemiological data for SE disease and antibody titers after SE vaccination of Bali cattle in Kupang City in 2018. This research was conducted through interviews of 86 breeders of a total 600 Bali cattles spread across five sub-districts within the region Kupang city, namely the sub-districts of Alak, Maulafa, Oebobo, Kota Raja and Kelapa Lima, and serum examination from 50 Bali cattles serum in Kelapa Lima and Alak sub-districts. Data of epidemiological measures were obtained by filling out a questionnaire including prevalence, morbidity, case fatality rate, and vaccination coverage which were analyzed descriptively, while examination of antibody titer was carried out using the Enzyme Linked Immunosorbent Assay (ELISA). The results showed that the prevalence of SE disease in five sub-districts in the city of Kupang was 9.83%, the mortality rate was 2.83%, the case fatality rate (CFR) was 28.81%, the percentage of vaccination coverage was 93.5%, and the seropositive antibody titer was 92%. Based on the results of the study, it can be concluded that there is a significant correlation between the high percentage of vaccination coverage rates and the high antibody titres of vaccinated cattles. This is consistent with the fact the mortality rate due to SE disease in Bali cattles in Kupang city is also low (2.83%). Septicemia epizootica vaccination for bali cattle in Kupang city creates an adequate immune response and optimal protection.

### INTRODUCTION

Septicaemia epizootica (SE) also known as haemorrhagic seticaemia (HS) or snoring disease in Bahasa Indonesia, is known as an acute and fatal infectious disease that attacks ruminants, especially cattle and buffalo (OIE, 2009; Jaglic *et al.*,2006; Alwis, 1992). The Diagnosis of SE in the field is carried out by observing clinical symptoms, pathological changes, and isolation of bacteria. The SE disease cases are usually reported as livestock deaths within a short period of time. Cattle experience an increase in body temperature, submandibular edema which can spread to the chest area, and respiratory symptoms with snoring or



mucus discharge from the nose. Generally, the animal then experiences lethargy or weakness and death. Buffalo is more sensitive than cows. The course of disease to death in buffaloes is shorter than in cows, the time ranges from less than 24 hours in incidence to 2 - 5 days (Alwis, 1992; Graydon *et al.*, 1993). Young cattle are usually more sensitive than adult livestock (Benkiran and Alwis, 2002). Disease transmission is usually enhanced by stress, animal density, poor management, and seasonality (Carter and Alwis 1989; Alwis and Vipulasiri, 1980).

Septicaemia epizootica disease is almost spread throughout Indonesia causing huge economic losses, due to the high morbidity and mortality rates of livestock. Morbidity and mortality are influenced by various factors and their interactions, including: animal age, endemicity of an area, immunity status of individual livestock, and level of immunity of a herd. The morbidity pattern in endemic and non-endemic areas is usually different (Angi, 2009). The province of East Nusa Tenggara (NTT) is one of the SE endemic areas, except for Lembata district (Agustini *et al.*, 2014; Berek *et al.*, 2015).

The incidence of SE disease in NTT province that has previously been reported, includes: cases of death of 45 cows in West Hauteas Village, North Central Timor Regency (TTU) in early 2014 to April 2014 based on the results of serological examinations and laboratory tests of organ samples carried out by the Balai Besar Veteriner Denpasar, it is known that the cause of livestock death is SE disease, this result is reinforced by the findings of clinical symptoms observed based on the farmer's history before the cattle experience death, including: snoring, edema in the area around the neck, tremors, and standing hair (Agustini *et al.*, 2014). The decline in livestock population due to SE disease in Kupang district occurs every year (Berek *et al.*, 2015). This condition shows that SE disease is still a serious and fatal threat to livestock in NTT province.

The various preventive actions and control against the spread of SE disease are continuously being carried out. One of the effective to prevent SE disease is vaccination. The vaccines in circulation are oil adjuvant and allum precipitated vaccine (Kartini *et al.*, 2009). In general, the dead vaccine contains *Pasteurella multocida* type B: 2 from local isolates in each country. The Kupang city government, through the Animal Husbandry Division, the Agriculture Service, always provides SE vaccination to cows and buffaloes every year as a preventive measure against the spread of this disease. This study aims to determine epidemiological data which includes prevalence, SE vaccination coverage rate, mortality rate, case fatality rate (CFR) of SE disease in Bali cattle in Kupang City in 2018. The results of this study are useful to get an overview of the magnitude of SE incidence in Kupang city, and become the basis for the analysis of the response to the formation of the immunity of the Bali cattle population after vaccination which is effective to increase protection of Bali cattle against the threat of SE disease.



## METHODOLOGY

**Questionnaires and interviews.** This research was conducted by collecting epidemiological descriptive data through interviews. Interviews were conducted with 86 breeders in 5 (five) districts, namely Alak, Maulafa, Oebobo, Kota Raja, and Kelapa Lima Districts. Filling a questionnaire by the farmer to obtain information about specific clinical signs that lead to cases of SE disease as well as other information, such as: livestock population, livestock health history, vaccination status, and history of livestock mortality.

**Enzym Linked Immunosorbent Assay (ELISA) test.** The research sample consisted of 50 blood serum from Bali cattles that had been vaccinated from two districts, namely Kelapa Lima and Alak sub-districts. The blood sample was taken from the jugular vein as much as 3-4 ml with a venoject. The test method used was the ELISA serological test which was carried out at the Balai Veteriner Lampung Laboratory.

## RESULTS AND DISCUSSION

Septicemia epizootica vaccination has been carried out routinely in the city of Kupang as a preventative measure for SE disease. The government through the Animal Husbandry Division of the Kupang City Agriculture Office provides a SE vaccination program every six months or once a year. The vaccines given to community livestock are inactive SE vaccines marketed from the Center for Veterinaria Farma (Pusvetma), in the form of a liquid preparation, an emulsion of water in oil. Based on questionnaire data and interview results, it is known that the total population of cattle owned by 86 respondent breeders in this study was 600 Balinese cattles. A total of 561 out of 600 cattles owned by breeders were vaccinated against SE by government vaccinator officers in 2018. A total of 20 farmers provided information that 59 of their cattle had experienced illness with clinical symptoms leading to SE disease. The sick cattles were treated by animal health officers, so that 42 cattles were declared cured and 17 others died.

The ELISA test results on 50 Bali cattles blood serum samples taken within 6 months after vaccination from the Kelapa Lima and Alak sub-districts, Kupang City showed 46 of 50 (92%) serum samples were positive for antibody with mean value of antibody titer is 219,5 EU, and 4 out of 50 (8%) samples showed negative SE antibody with mean value of antibody titer is 20,5 EU (Figure 1).

The SE vaccination coverage for cattles in Kupang city in 2018 were 93.5% (561/600), while those were not vaccinated were 6.5% (39/600). Vaccination coverage in Kota Kupang provides a representative level of protective immunity from the two sub-districts, at 92% (46/50) as shown in Figure 1 and Table 2. The high antibody titer indicates that SE vaccination is given to groups of bali cattle in the two sub-districts able to provide an optimal level of immunity. The same thing was also conveyed by Cantona *et al.* (2019) in his



research on Bali cattle in Maulafa and Alak sub-districts in Kupang city also had high antibody titers after SE vaccination. Vaccination has been able to stimulate the formation of antibodies well. According to De Alwis (1999), vaccination coverage of 70% or more can reduce SE disease outbreaks. The high level of protective immunity in livestock in Kupang city can reduce the incidence of disease and livestock mortality due to SE disease. This is indicated by the low prevalence rate of SE in cattle in five sub-districts in Kupang City of 9.83% (59/600), a decrease in livestock mortality by 2.83% (17/600), a case fatality rate of 28.81% (17/59) is shown in Table 1.

Table 1. Data on the number of respondents, the number of sick cows and the death of cows in 5 (five) districts, namely Alak, Maulafa, Oebobo, Kota Raja, and Kelapa Lima Districts in 2018

Information	Bali cattles (N=600)	
	Symptom of SE	Healthy cattles
The number of sick cattles	59	541
The number of dead catlles from the sick cattles group (n = 59)	17	

(source: research data, 2018)

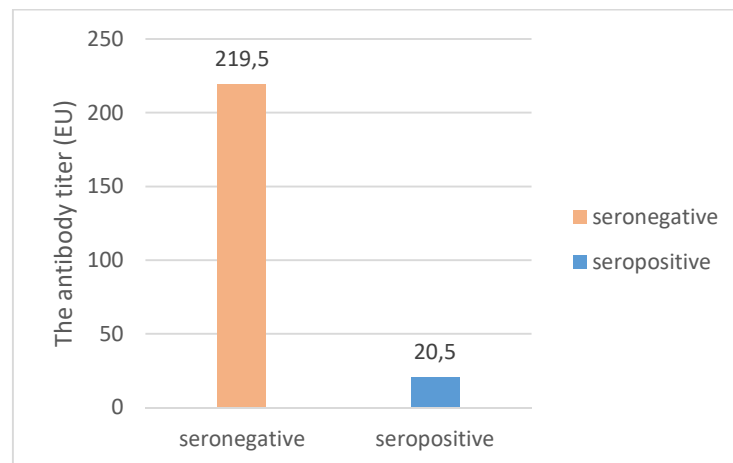


Figure 1. Graph of the results of mean antibody titer testing after SE vaccination in Bali cattles in Kelapa Lima and Alak sub-districts. The antibody titer of 46 samples are seropositive with mean 219,5 EU, while 4 samples are seronegative with mean 20,5 EU (Source: test result from Balai Veteriner Lampung Laboratory, 2018)



## CONCLUSION

Septicemia epizootica vaccination coverage in the city of Kupang in 2018 has a high percentage (93.5%) and has succeeded in stimulating the formation of protective antibodies that are good for livestock groups (92%), so that the incidence rate of disease, morbidity and mortality rates of cattles due to SE disease in the city of Kupang are very low.

## ACKNOWLEDGEMENTS

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SUPPORTED BY





## BIRTH WEIGHT AND PLACENTAL WEIGHT OF BELGIAN MALINOIS DOG - A CASE REPORT

Tri Utami\*, Tarsisius Considus Tophianong

Departement of Veterinary Clinical, Reproduction, Pathology and Nutrition,  
Veterinary Medicine Faculty, Nusa Cendana University, Kupang city, East Nusa  
Tenggara.

Veterinarian practitioners at the Grace Vet Care Joint Veterinary Practices,  
Kupang city, East Nusa Tenggara.

\*Corresponding author: utami.trico@gmail.com

A two years old, 26 kg body weight, brown coat, Belgian Malinois bitch was presented in Grace Vet Care Joint Veterinary Practices at Tuak Daun Merah II, Kupang City, East Nusa Tenggara Province on 16<sup>th</sup> July 2020, with a history of anorexia, distended abdomen, vulvar discharge, restless and occasionally straining. The bitch never been pregnant before, and the first pregnancy was diagnosed on 28<sup>th</sup> June 2020. The confirmation diagnosis was done by ultrasonographic examination using Draminski ultrasound, a transabdominal convex probe, frequency 5.0 MHz, 100 mm scope, 100% zoom. The bitch has given birth to ten puppies, with an average birth weight of 324.0 gr and placental weight of 19.2 gr. The mean birth weight of male neonatal puppies were 348.4 gr and placental weight of 19.4 gr, while the mean birth weight of female neonatal puppies were 301.4 gr and placental weight of 19 gr. The mean duration of birth between puppy was 48.5 minutes, while the long duration of birth from first out to the last puppy was 5 hours 38 minutes.

### INTRODUCTION

Birth weight is an important factor affecting mortality and morbidity during the neonatal period in most mammals, including dogs (Matteo et al., 2020; Debora et al., 2017; Indrebo et al., 2007). Low birth weight is correlated with poor survivability (Kustritz, 2011). Puppies who have low birth weight will experience weakness, feeding difficulty when competing with ideal birth weight puppies, may experience disturbances in thermoregulation and hypoglycemia. Failure to regulate thermoregulation and lack of blood sugar levels due to hypoglycemia will cause puppies to become weak and die (Debora et al., 2017). Normal birth weight for puppies varies by breed, averages of 120 gm for toy breed pups, 250 gm for medium breed pups, 490 gm for large breed pups, and 625 gm for giant breed pups (Kustritz, 2011). Puppies mortality associated with birth weight is 1.4 - 2.1%, especially in large breed dogs with puppies weight  $\leq$  100gm (Indrebø et al., 2007; Mila et al., 2015).





Puppy birth weight is very influenced by maternal, fetal and placental factors. Litter size and body size of the mother also influence the puppies birth weight (Tønnessen et al., 2012; Debora et al., 2017). The litter size and birth weight of puppies will affect the length of time or duration of birth and the incidence of dystocia in bitches (Eneroth, A et al., 1999). Placenta in dogs is a type of zonaria that secretes relaxin to support pregnancy and birth. The placenta in dogs lacks steroidogenetic activity (Debora Groppetti et al., 2017). Removal of the placenta in the dog will occur immediately after removal of each puppies. This paper discusses several things regarding the number of fetuses, sex, birth weight, placental weight, duration of birth between puppies and the length of time of birth from the first to the last puppies.

### CASE HISTORY

On 16<sup>th</sup> July 2020, a bitch was examined at the Grace Vet Care Joint Veterinary Practice (PDHB) Tuak Daun Merah II Kupang City, East Nusa Tenggara Province. The signalement of the patient was a Belgian Malinois dog, 2 years old, brown coated with a body weight of 26 kg. Based on the history, the bitch had never been pregnant and gave birth before. It was mated on 21<sup>st</sup> – 22<sup>nd</sup> May 2020 with a male Belgian Malinois dog, aged 2 years, black coated, weighing 28 kg (Figure 1-A). The first pregnancy examination was carried out on 28<sup>th</sup> June 2020 by ultrasonographic examination using Draminski ultrasound, a transabdominal convex probe, frequency 5.0 MHz, 100 mm scope, 100% zoom (Figure 1-B). The ultrasonography examination was repeated on 5<sup>th</sup> and 7<sup>th</sup> July 2020. Based on the results, it was diagnosed as pregnant with an estimated number of fetuses of 10 puppies.

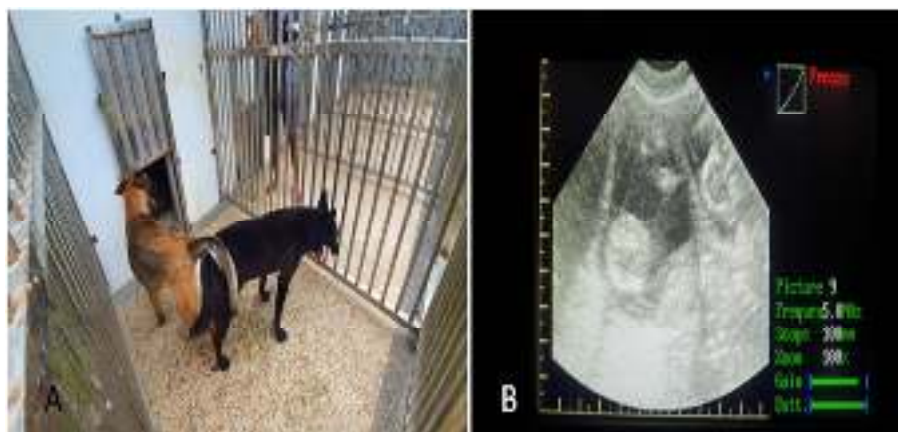


Figure 1. A, The Belgian Malinois dogs were mated on 21<sup>st</sup> and 22<sup>nd</sup> May 2020. B, the appearance of pregnancy sign by ultrasonography transabdominal examination on 28<sup>th</sup> June 2020.



On July 16, 2020, at 5:00 p.m., the bitch showed clinical symptoms of restlessness, scratching the floor, showing the behavior of making a nest from the cloth of the cage, gasping for breath, greenish discharge coming out from the vulva, distended abdomen and anorexia.

### RESULTS AND DISCUSSION

Based on the data in Table 1, the bitch has given birth to ten puppies (Figure 2), with the number of puppies were 5 male puppies and 5 female puppies. The mean birth weight of ten puppies were 324.9 gm and mean placental weight of 19.2 gm. The mean birth weight of male puppies were 348.4 gm and the mean placental weight of 19.4 gm. The mean birth weight of female puppies were 301.5 gm and the mean placental weight of 19 gm. The mean duration of birth between puppy was 48.5 minutes, while the duration of birth from the first to last puppies was 5 hours 38 minutes.

Puppy birth weight is influenced by intrauterine factors which include maternal, fetal and placental factors. Puppy birth weight is also influenced by litter size, sex, breed and body size of the bitch (Fiszdon et al., 2009; Debora et al., 2017). Birth weight is an important factor affecting mortality and morbidity during the neonatal period. Puppies who have low birth weight will experience thermoregulation disorders and hypoglycemia. Hypoglycemia will affect energy intake in the body which will affect the function of organs including the brain (Indrebø et al., 2007; Mila et al., 2015; Debora et al., 2017).

Table 1. Birth data on July 16, 2020

Birth Number	Time (zona wita)	Sex	Birth weight (gm)	Placental weight (gm)	Other Information
1	18.00	Female	292	19	Live, normal
2	18.32	Female	273	18	Live, normal
3	19.11	Female	352	20	Live, normal
4	19.51	Male	408	20	Live, normal
5	20.52	Male	312	19	Live, normal
6	20.59	Female	380	20	Live, normal
7	21.14	Male	318	19	Live, normal
8	21.27	Male	365	20	Live, normal
9	23.31	Female	339	19	Die, hydrocephalus
10	23.38	Female	210	18	Live, normal
<b>Mean</b>			<b>324,9</b>	<b>19,2</b>	



Table 2. The mean of birth weight (gm) and placental weight (gm) of male puppies

Birth number of male puppies	Birth weight (gm)	Placental weight (gm)
4 <sup>th</sup>	408	20
5 <sup>th</sup>	312	19
7 <sup>th</sup>	318	19
8 <sup>th</sup>	365	20
9 <sup>th</sup>	339	19
<b>Mean</b>	<b>348,4</b>	<b>19,4</b>

Table 3. The mean of birth weight (gm) and placental weight (gm) of female puppies

Birth number of female puppies	Birth weight (gm)	Placental weight (gm)
1 <sup>st</sup>	292	19
2 <sup>nd</sup>	273	18
3 <sup>th</sup>	352	20
6 <sup>th</sup>	380	20
10 <sup>th</sup>	210	18
<b>Mean</b>	<b>301,4</b>	<b>19</b>



Figure 2. A, Neonatal puppies. B, The neonatal puppy which die with hydrocephalus.

The puppies with low birth weight will have difficulty getting colostrum when competing with puppies with ideal birth weight. Difficulty getting access to colostrum will affect the transfer of maternal antibodies, this will make puppies susceptible to infection and growth is delayed. The puppy birth weight will determine the puppy body size which affects the duration of birth and the incidence of dystocia (Chatdarong et al., 2007; Mila et al., 2015; Debora et al., 2017). According to Isabel (2020), the duration of birth between puppies are 20 minutes - 60 minutes can sometimes reach 3 - 6 hours with the length of time that



all puppies are expelled can reach 24 hours while the average birth weight of Belgian Malinois puppies is 240 - 410 gm. Based on Table 1, 2 and 3, the average duration of birth and birth weight of Belgian Malinois puppies are included in the ideal category.

Placenta is one of the products of pregnancy that supports and maintains pregnancy. A placenta weighed an average of 22.5 gm - 32.7 gm, but the weight of the placenta varied greatly among dog breeds. Placental weight correlates with puppies birth weight. The placenta in dogs secretes relaxin which plays a role in the process of pregnancy and birth. While the supply of progesterone in dogs which functions to maintain pregnancy comes from the corpus luteum, because the placenta in dogs does not have steroidogenesis activity. The placenta has the function of exchanging nutrients, gases, as an endocrine gland, barrier (preventing the mixing of fetal and fetal blood), preventing pathogenic bacteria in the mother's blood from entering the fetus's bloodstream and acting as Immune protection. The placenta will be removed in the third stage of the delivery process. Removal of the placenta in the dog will occur immediately after fetal expulsion (Fiszdon et al., 2009; MatteoTesi et al., 2020).

## CONCLUSION

The puppy birth weight and placental weight vary widely between dog breeds. Puppy birth weight is highly influenced by intrauterine factors which include maternal, fetal and placental factors. Puppy birth weight and litter size will affect the duration of birth (second stage of birth: fetal expulsion). Placenta weight is influenced by puppies birth weight. Fetal birth weight is one of the predisposing factors for neonatal mortality and morbidity of puppies.

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**EFFECTS OF SUPPLEMENTATION OF KACANG ASU (*Pueraria phaseoloides*) ON BLOOD PROFILES OF KACANG GOATS FED KUME (*Sorghum plumosum* Var. *Timorense*) GRASS HAY**

**Immanuel Benu<sup>1\*</sup>, I Gusti N. Jelantik<sup>1</sup>, Jalaludin<sup>1</sup>, Tara Tiba Nikolaus<sup>1</sup>, Meity M. Laut<sup>2</sup>, Larry R.W. Toha<sup>2</sup>**

<sup>1</sup>Faculty of Animal Science, University Of Nusa Cendana

<sup>2</sup>Faculty of Veterinary Medicine, University Of Nusa Cendana

\*Corresponding author: immanuelbenu@staf.undana.ac.id

The aims of the present study was to determine the effects of different levels of *Pueraria phaseoloides* supplementation on the haematological and blood biochemistry parameters of male Kacang goats fed Kume grass hay. Twelve male Kacang goats (6-8 months of age; mean body weight = 13.63 kg ± 1.40 kg) were randomly allocated in a 3x4 arrangement to four (4) treatments in an incomplete latin square design. The four diet treatments tested were (P0 = grass hay and concentrate; P10 = P0 + 10% *Pueraria phaseoloides* supplementation; P20 = P0 + 20% *Pueraria phaseoloides* supplementation; P30 = P0 + 30% *Pueraria phaseoloides* supplementation). The experiment was conducted for 76 days (each period consists of 14 days adaptation and 5 days sample collection). Blood samples were drawn from each animal on the last day of the trial and evaluated for RBC's, WBC's and biochemistry profiles. Data were analysed using ANOVA of SPSS. Supplementing grass hay with *Pueraria phaseoloides* had no significant (P>0.05) effect on all haematological and blood biochemistry parameters of male Kacang goats. Therefore, it can be concluded that feeding *Pueraria phaseoloides* at different level of inclusion rates to male Kacang goats had no detrimental effect on haematological and blood biochemistry parameters of male Kacang goats.

### INTRODUCTION

Although Kacang goats have been shown to play an important role in the economics of smallholder farmers in Indonesia, the productivity of this breed is still low as it is hampered by feed availability and quality during the dry season. During this period, the animals are often energy and particularly protein deficient. Hence, protein supplementation is necessary important if goat production expected to increase. However, protein supplement is often expensive and unaffordable for smallholder farmers. Therefore, the use of alternative protein feeds, including legumes is essentially important to fulfil the protein requirement to the animals during dry season and at the same time reducing feeding cost in production system.



Kacang asu (*Pueraria phaseoloides*) is a one of the promising legumes (Paz et al., 2016) available to improve animal production due to its high nutritional value, high palatability and ability to incorporate nitrogen into the soil. The leaves of *Pueraria phaseoloides* are rich in protein, which makes this legume an excellent protein supplement to ruminants (Akingbade et al., 2015). This legume has been utilized as protein supplement for ruminant animals, because it is palatable and provide good amount of protein (Gulizia and Downs, 2019).

Information regarding levels of supplementation of *Pueraria phaseoloides* in goats fed tropical grass basal diet is still limited particularly its effect on blood parameters. Blood parameters are often used to address the animal's health, nutritional and physiological status (Nogalski et al., 2012 ); (Overton et al., 2017) (Mohammed et al., 2016) as well as pathological status (Šoch et al., 2011). In addition, (Tao et al., 2018) indicated that blood serum parameters represent the utilisation of nutrient. Therefore, this study was designed to evaluate the effect of levels of *Pueraria phaseoloides* supplementation on blood metabolites of Kacang goats consuming grass hay basal diet.

## MATERIALS AND METHODS

### *Experimental Site, Animals and Management*

The experiment was carried out at the dry land laboratory (-10.1761°S, 123.3793°E) of Nusa Cendana University Kupang, west Timor, Indonesia. Twelve male Kacang goats (6-8 months of age; mean body weight = 13.63 kg ± 1.40 kg) were used in this study. Throughout the experiment, the animals were individually penned in metabolic crates (1 m x 1.5 m) within a covered goat facility house. The animals were given their feed treatments (Table 1) at two times (0800 hours and 1700 hours) each day in approximately equal amounts to ensure *ad libitum* intakes. The amount of hay given to individual animals was calculated from the actual intake of the previous day + 20% extra hay (w : w). Water was available to the animals *ad libitum* in 5-L buckets. Hay refusals were recorded once daily at 0800 hours before morning feeding.

### *Experimental design*

The experimental design was conducted following a replicated Latin Square Design. Three sets of four goats were formed and in each set four treatments were randomly allocated to the animals in each period. The study lasted for four periods where each experimental period consisted of 10 days preliminary feeding period and 5 days of sample collection. The dietary treatments consisted of grass hay and concentrate (P<sub>0</sub>, control); and supplementation with 10% (P<sub>10</sub>), 20% (P<sub>20</sub>), and 30% (P<sub>30</sub>) *Pueraria phaseoloide*. The natural grass hay used in this study was Kume grass (*Sorghum plumosum var. Timorensis*) which cut at vegetative stage.



*Pueraria phaseoloides* meal preparation

Fresh *Pueraria phaseoloides* was harvested from eastern Flores during May and June and air dried under the sun for hay. The *Pueraria phaseoloides* hay were then transported to Kupang and milled using a milling machine with 1 mm sieve to obtained *Pueraria phaseoloides* meal which is then used for supplementation.

Table 1. Feed ingredients of the basal diets

Feed ingredients	Treatments			
	P <sub>0</sub>	P <sub>10</sub>	P <sub>20</sub>	P <sub>30</sub>
Kume grass hay	70	70	70	70
Corn meal	15	15	15	15
Rice bran	9	9	9	9
Fishmeal	5	5	5	5
Mineral	1	1	1	1
Total	100	100	100	100

Table 2. Chemical composition of experimental diets

Chemical composition, % DM	<i>Pueraria phaseoloides</i>	Kume grass hay	Corn meal	Rice bran	Fishmeal	Mineral
DM	89.905	94,205	86.524	89.274	81.952	97.114
OM	80.000	88,493	83.505	75.929	61.193	49.060
CP	18.801	7,091	7.816	11.820	58.947	0.000
Ash	9.905	5,712	3.019	13.345	20.759	48.054
CF	31.135	30,622	5.646	19.911	2.532	28.280
CHO	65.544	83,246	67.942	56.896	8.645	47.506
NFE	34.409	52,623	62.296	36.985	11.177	19.226
EE	5.654	3,157	7.747	7.213	10.891	1.555

*Blood collection and Analysis*

Blood samples from each goat were collected on the last day of each trial period, 4 hours after morning meal, from the jugular vein into two vacutainer tubes (3 ml contained EDTA and 5 ml without EDTA). The samples were immediately placed on ice and brought to the laboratory for further analysis. Red blood cell (RBC) and white blood cell (WBC) were measured with the aid of Neubauer counting chamber (haemocytometer). Packed Cell Volume (PCV) were measured with the haematocrit test. Blood haemoglobin (Hb) were measured with heamoglonometer. Blood samples were also analysed for blood biochemistry following these methods: biuret reaction test to measure the total protein in serum, enzyme hexokinase test to measure plasma glucose, Blood Urea Nitrogen (BUN)





test to measure blood urea, and kinetic IFCC test to measure alanine transaminase (ALT) and aspartate aminotransferase (AST).

### STATISTICAL ANALYSIS

Data obtained in this study were statistically analysed using GLM procedure adapted by IMB SPSS Statistic for windows, version 21 (IBM Corp., Armonk, N.Y., USA) for user's guide with ANOVA. Duncan's multiple range tests within SPSS program was conducted to examine the degree of significance among means.

### RESULTS AND DISCUSSION

The concentrations of metabolites in the blood are commonly reflected nutrient absorption as well as their metabolism and utilization in the animal's body. Blood glucose concentration is an indication of carbohydrate digestion, absorption and metabolism. Meanwhile, blood plasma concentration of total protein and urea are depending upon protein digestion, absorption and metabolism. As presented in Table 3, there were no significant differences between the supplemented group and control group in the blood metabolites concentration. Glucose concentration in the present experiment was expected to increase with increasing levels of *Pueraria phaseoloides* supplementation. When rumen fermentation is limited by nitrogen availability in the rumen, supplementation of protein rich feed commonly improve rumen fermentation (Bach et al., 2005). Higher rate of rumen fermentation will stimulate higher propionate concentration (Rodríguez et al., 2007). In addition, higher rate of rumen fermentation stimulate higher microbial protein synthesis which will bring about increasing amino acids absorption (Bach et al., 2005). Since propionate and amino acids are the important precursors for glucose synthesis in the liver (Forbes, 2007), supplementation of *Pueraria phaseoloides* therefore is expected to increase blood glucose concentration.

Factor responsible for the failure of increasing levels of *Pueraria phaseoloides* supplementation in increasing blood glucose concentration in the present experiment may be related to the degradable protein from concentrate in the basal diet (about 30%) which provides sufficient amino acids for microbial synthesis and therefore the blood glucose kept decrease. Other reason for lower blood glucose concentration is probably due to the role of liver which is to control the uptake of nutrients from digestive tract and to detect the rates of metabolites uptake such as glucose and amino acids to keep it more stable levels for the general circulation (Forbes, 2007). The concentrations of blood glucose in the blood serum are governed by the nutritional regime and hormones (Mohammed et al., 2016) Supplementing goats with increasing level of *Pueraria phasoeloides* in the diet had no different for blood glucose ( $P = 0.701$ ).



Blood plasma protein is an indicator of protein status (Vazzana et al., 2014). In the present study, blood plasma protein was not improved by increasing the *Pueraria phasoeloides* level in the diet ( $P = 0.514$ ).

Blood urea is correlated with nitrogenous substances uptake in the feed and their utilization (Jelínek et al., 1996). There was no difference between treatments for blood urea ( $P = 0.622$ ) observed in this study. This result is also unexpected since increasing level of *Pueraria phasoeloides* supplementation increase protein content of the diets. (Mohammed et al., 2016) stated that lower concentration of blood urea indicate low protein in the diet or hepatic chronic disease, in contrast higher blood urea concentration probably due to renal failure and body dehydration. The possible reason for the absence of increasing BUN concentration with increasing level of *Pueraria phasoeloides* supplementation is that ammonia produced from protein degradation in the rumen is efficiently used for microbial protein synthesis. This is possible since in the present experiment, about 30% of the basal diet was consisted of highly degradable concentrate. This amount of concentrate may provide sufficient energy and carbon skeleton for microbial synthesis thereby ammonia absorbed from the rumen wall was kept low.

AST is an enzyme which found in the liver, cardiac muscle, skeletal muscles, lungs, kidneys, pancreas, brain, red blood cells, and leukocytes (Omidi et al., 2018) which is play a crucial role in amino acid metabolism (Kiran et al., 2012). In this study, supplementing goats with increasing inclusion rates of *Pueraria phasoe* in the diet had no significant differences between treatments for AST ( $P = 0.469$ ). A similar result was reported by (Akingbade et al., 2015) where blood protein of goats was unaffected by the increasing *Pueraria phasoeloides* inclusion rates in the diet. On the other hand, ALT is an enzyme mostly found in the liver and often used to detect liver injury (Kiran et al., 2012). There was no differences among supplemented group and control for ALT ( $P = 0.998$ ).

Tabel 3. Biochemical status of male Kacang goat fed Kume grass hay and supplemented with different levels of *Pueraria phasoeloides*

Parameters	Dietary levels				SEM	P-Values
	P0	P10	P20	P30		
Total protein (mg/dl)	6.767	6.733	6.983	6.933	0.138	0.514
Glucose (mg/dl)	91.708	93.260	89.158	92.262	2.529	0.701
Urea (mg/dl)	50.620	48.167	48.290	50.555	1.758	0.622
AST (U/L)	22.300	30.067	28.517	29.067	3.753	0.469
ALT (U/L)	103.067	103.483	102.067	101.733	7.851	0.998

AST = aspartate aminotransferase; ALT = alanine aminotransferase

The data of haematological blood of Kacang goats are as presented in Table 4. The results of these parameters did not demonstrate any significant differences between the supplemented group and control. The haematological



parameters (Packed Cell Volum (PCV), Red Blood Cell (RBC), Haemoglobin (Hb), White Blood Cell (WBC)) of the animals of all treatments were in the normal physiological range for goats (Weiss and Wardrop, 2010). The values of the PCV (P = 0.117) and RBC (P = 0.615) were similar across the supplemented group and were within the normal reference for healthy goats. Similarly, supplementing goats with increasing levels of *Pueraria phasoeloides* in the diet had no significant differences between treatment on Hb (P = 0.117) and WBC (P = 0.318) and were within the normal reference for healthy goats. In the present study, the animals were all healthy and therefore this could probably be the reason for the similarity values of haematological parameters between the supplemented group and control.

Tabel 4. Haematological parameters goats fed Kume grass hay and supplemented with different levels of *Pueraria phasoeloides*

Parameters	Dietary levels				SEM	P-Values
	P0	P10	P20	P30		
PCV (%)	31.932	32.143	33.960	32.505	0.614	0.117
RBC (x 10 <sup>6</sup> /μl)	9.460	9.610	10.073	9.440	0.377	0.615
Hb (g/dl)	10.645	10.715	11.320	10.835	0.204	0.117
WBC (10 <sup>3</sup> /μl)	10.777	10.220	10.078	10.575	0.287	0.318

### CONCLUSION

Therefore, it can be concluded that feeding *Pueraria phaseoloides* at different level of inclusion rates did not improve the haematological and blood biochemistry parameters of male Kacang goats.

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**ARBILA (*Phaseolus lunatus* L) DIVERSITY AS FOOD AND FEED IN NEKAMESE SUB-DISTRICT BASED ON MORPHOLOGY OF BEAN**

**Bernadete B. Koten<sup>1\*</sup>, Redempta Wea<sup>1</sup>, Melkianus D. Randu<sup>1</sup>, Twenfonsel Dami Dato<sup>2</sup>, Allan Prima Titong<sup>3</sup>**

<sup>1</sup> Animal Husbandry Departement Kupang State Agricultural Polytechnic

<sup>2</sup> Faculty of Animal Science Nusa Cendana University

<sup>3</sup> Faculty of Veterinary Medicine Nusa Cendana University.

\*Corresponding author: bernadete\_koten@yahoo.com

This study aims to identify the diversity of arbila (*Phaseolus lunatus* L.) as food and feed source based on bean morphology. This study was conducted in 5 months in Nekamese Sub-district, Kupang. The study was conducted with survey and sample (beans) collection. Materials used were *Phaseolus lunatus* L. beans, digital scale, and calipers. Variable observed were color patterns, weigh per 100 beans, beans length, width, and thickness. Data were documented and analyzed descriptively. The result showed that in Nekamese Sub-regency, *Phaseolus lunatus* L. could be grouped by the variables into 20 different groups. Beans weight ranges from 25.5 – 128.57 g/100 beans, bean length ranges from 0.90 – 1.94 cm, bean width ranges form 0.56 – 1.40 cm and bean thickness ranges from 0.23 – 0.62 cm. It was concluded that based on bean morphology, there are 20 different kinds of *Phaseolus lunatus* L. in Nekamese Sub-district that differs in color patern, weight, length, width, and thickness.

## INTRODUCTION

Nekamese Sub-regencyis, a part of Kupang, East Nusa Tenggara, located at 50-1400 m above sea. Cattle population in this area continue to increase over the years. In 2017, the population reaches 2.312 individuals and 9.145 in the year 2020 ( Indonesian Central Bureau of Statistics (ICBS), 2020). This potential have must be supported with the availablility of quality livestock feed. East Nusa Tenggara The geographic location of NTT causes this region to have a dry season for 8 months (April - December) and a rainy season which lasts only for 4 months (January - March) (ICBS, 2019). Forage crops will experience drought and even death at the end of the dry season for crops that are not resistant to drought. It is necessary to introduce the potential of local NTT forage plants including local legumes, especially in Nekamese District, which are high production and resistant to drought and are able to survive on marginal land.

*Phaseolus lunatus* L, as one of native legumes, and has the potential to provide quality feed for cattle in Nekamese Sub-district. This legume has a high adaptability to the environment and produces well wether in lowlands or highlands, is resistant to drought, and able to grow in almost all types of soil.



Koten *et al.*, (2012) reported that the vegetative part of *P. lunatus* L legumes contained 11.67% dry matter (DM), 13.48% ash, 21.21% crude protein (CP), 3.79% crude fat (CF), and 24, 21% crude fiber (CFb). The bean pods contain 18.80% CP, 0.6% CF, 4% ash, 17.5% CFb, and 59.1% nitrogen-free extract (NFE). The beans contain even higher nutrients, namely 27.2% CP, 0.9% CF, 5.5% ash, 5.2% CFb, and 61.2% NFE (Tarruco-Uco, 2009). This legume are commonly used as listock feed, forage and beans.

It was reported that the color of *Phaseolus lunatus* L beans varies, namely Vivid purplish red (Koten, 2012), dark brown (Koten and Wea, 2014), black (Koten *et al.*, 2016), white (Koten *et al.*, 2017), light brown (Koten *et al.*, 2018) and brown with black stripes (Koten *et al.*, 2019). In general, people in East Nusa Tenggara group these beans into 2 groups based on the beans morphology, those that can be consumed by livestock and those that can not, because of their poison. Based on the interview with the locals, *P. lunatus* L beans which is poisonous can still be consumed by livestock if it is repeatedly boiled. Beside that, the darker the color of the beans, the more it is poisonous for humans. Currently, *P. lunatus* L species can only be classified based on bean morphology. Characterization of the collected *P. lunatus* L beans is important to obtain agronomic morphological characters so that *P. lunatus* L species phenotypes can be distinguished quickly and easily.

Until now, information on the diversity of *Phaseolus lunatus* L. species in Nekamese Sub- district, Kupang Regency, which is characterized by the morphology of their seed, is not yet available. This study aims to evaluate the level of arbila diversity as measured by the morphological diversity seed of beans in Nekamese Sub-district, Kupang Regency.

## MATERIALS AND METHOD

### Time and Location

This research was conducted in Nekamese Sub-district, Kupang Regency and in the Laboratory of Nutrition and Livestock Feed, Kupang State Agricultural Polytechnic. This research was carried out from from May to September 2020.

### Materials

The material used in this research was seed of *Phaseolus lunatus* L beans. The tools used were Camry brand digital scale with a capacity of 5 kg with a sensitivity of 1 g for weighing seed of beans, a tray as a container, a glass jar with a capacity of 2 kg as a storage container for *P. lunatus* L beans, a caliper to measure the diameter of the seed, newspaper envelopes and oven for drying.



## Methods

The research procedure were as follows:

1. *P. lunatus* L seed collection. *P. lunatus* L seed are collected from sample area.
2. Collected seed of beans were identified according to the observed variables.
3. Identified seed of beans were then grouped into their respective groups and documented.

## Observed variables

The variables observed were:

- a. Category in food or feed. This category is determined based on the results of interviews with respondents.
- b. Seed color. The determination of the color of the beans was carried out by observing the color and their coloring patterns, based on the indicators from the Royal Horticultural Society color chart, which had been prepared.
- c. Seed length. The length of the seed of beans was measured by using a caliper on the longest part of the seed (cm).
- d. Seed width. The width of the seed of beans was measured by using a caliper on the widest part of the seed (cm).
- e. Seed thickness. The thickness of the beans was measured using a caliper on the largest part of the seed (cm).
- f. The weight of 100 air-dried seeds. From the grouped bean groups, 100 seed of beans were selected randomly each and than weighed (gram). This weighing process was carried out with 4 repetitions.
- g. Weight of 100 oven-dried beans. The beans were then put into a 55°C oven for 3 days, then weighed (grams). This weighing process was carried out with 4 repetitions.
- h. Beans category. 100 beans were taken randomly and then weighed and grouped into light, medium and heavy sizes.

## Research methods

This research is a survey (exploration) research with sampling using accidental sampling technique. Samples were collected from Oben, Oemasi, Usapi Sonbai and Taloetan villages.

## Data analysis

The data obtained in the study were documented, tabulated and analyzed in a simple method.





**RESULT AND DISCUSSION**

Nekamese Sub-regency, Kupang Regency consists of 11 villages with an altitude of 40-1700 m above sea with a weather of 25 - 34°C and humidity of 40-85%. In general, people in East Nusa Tenggara group *P. lunatus* L into 2 groups based on the beans, namely those that can be consumed and those that can not be consumed by livestock because poisonous. The results of the interview showed that *P. lunatus* L which is poisonous can still be consumed by livestock if it is repeatedly boiled. Beside that, the darker the color of the beans, the higher the toxicity for humans.

Tabel 1. Arbila diversity based on seed morphology in Nekamese Sub District

Code of seed	Food /Feed	Color **	Size (cm)			Weight of 100 seeds (g)		Category based on weight*
			Length	Width	Thickness	Dry seeds	Oven-dried	
N1	Food	Black with brown spots	1.95	1.40	0.46	128.57	101.43	Big lima
N2	Food	black with white stripe (like zebra color)	1.79	0.90	0.32	58.5	39.00	Kidney
N3	Food	Yellowish White	1.76	1.07	0.41	123.50	103.29	Big lima
N4	Food	Black	1.31	0.76	0.36	48.00	40.00	Kidney
N5	Food	White with black spots	1.27	0.71	0.32	51.00	31.00	Kidney
N6	Feed	Light brown with thin black stripes	1.22	0.78	0.24	33.75	29.00	Kidney
N7	Food	Red with white stripe (like zebra color)	1.20	0.63	0.50	50.00	42.00	Kidney
N8	Feed	Black with brown spots	1.18	0.70	0.27	36.00	25.00	Kidney
N9	Feed	Red with light brown spots	1.17	0.73	0.26	28.57	11.43	Sieva
N10	Feed	Light brown with thick black stripes	1.17	0.77	0.23	34.25	26.00	Kidney



N11	Feed	Deep purplish red	1.15	0.70	0.23	31.50	24.00	Kidney
N12	Feed	Light brown with black spot	1.13	0.70	0.27	32.50	23.00	Kidney
N13	Feed	Dark brown with thin black stripes	1.12	0.69	0.25	34.25	28.00	Kidney
N14	food	Greenish brown	1.12	0.78	0.51	41.25	32.00	Potato
N15	Food	Dark brown white with a black stripe near the hilum	1.10	0.77	0.42	40.00	25.00	Potato
N16	Food	Vivid purplish red	0.94	0.56	0.32	34.00	23.00	Sieva
N17	Feed	Dark brown	0.93	0.66	0.27	28.50	20.00	Sieva
N18	Feed	Reddish black	0.93	0.65	0.34	25.50	18.00	Sieva
N19	Feed		0.90	0.78	0.62	49.00	39.00	Potato

Based on the exploration results, there are 19 different accessions of *P. lunatus* L in the Nekamese sub-regency. This shows the high diversity in the accessions of phaseolus lunatus that were found. The number of *Phaseolus lunatus* accessions that is more diverse than that found by Purwanti (2014) in East Java, namely 16 types. Genetic diversity between individuals or populations can be estimated using morphological markers.



Picture 1. The appearance of the kinds arbila in Nekamese Sub District

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**Food or livestock feed category.** There are 9 *P. lunatus* accessions (47.37%) that are categorized as food, namely those that can be eaten directly without further processing, and 10 accessions (52.63%) that require further processing or as feed.

**Color.** *P. lunatus* beans colors are categorized based on a single color and a combination of 2 or more colors. The single color consists of black, white, pink, and a combination of 2 or more colors. Based on indicators from the Royal Horticultural Society color chart, the colors of the arila are shown in Table 1. Based on the color of the seeds displayed, the color of this *Phaseolus lunatus* is in accordance with that described by IBGR (1982), which has a one-color pattern (black, white, maroon) and a mixed color pattern with a white, light brown, red and black background. However, there are two color patterns that have not been reported by IBGR (1982), namely a zebra-like color pattern on a black and red background.

**Seed Thickest.** The thickest bean diameter was found in N13 beans with a diameter of 0.62 cm, and the smallest diameter is N15 beans with a diameter of 0.23 cm. 3 accessions (15.79%) are within the diameter range of 0.50 - 0.62 cm, 8 accessions (42.11%) diameters are within 0.27 - 0.46 cm and 8 accessions (42.11%) diameters are within the range of 0.23-0.27 cm.

**Seed length.** The longest arila beans are accession N11 beans (1.95 cm) and the shortest is in the accession N13 (0.90 cm). There were 3 accessions (15.79%) with a length of 1.76 - 1.95 cm, namely accessions N11, N10 and N9. There were 12 accessions (63.16%) with bean lengths ranging from 1.10 to 1.31 cm, and 4 accessions (21.05%) with lengths ranging from 0.90 to 0.94 cm. These results show a longer *Phaseolus* beans than that reported by Purwanti (2014) which ranged from 0.99 to 2.38 cm, which is divided into groups sieva (small), potato (intermediate) and inca (big) (Purwanti, 2014).

**Seed width.** The widest bean width was found in accessions N11 with a width of 1.40 cm. 6 accessions (31.58%) were categorized as wide with sizes range from 0.78 to 1.40 cm, namely N11, N9, N10, N13, N19 and N1. 9 accessions were categorized as medium with a width of 0.69 - 0.77 cm, namely N14, N12, N7, N8, N17, N2, N5, N3, and N16. 4 accessions were categorized as narrow with width range 0.56 - 0.66 cm. It turns out that the width of the *Phaseolus lunatus* found was lower than that reported by Purwanti (2014), namely 0.90 - 1.51 cm, and wider than *Phaseolus* at Benin Republic (Africa) reported by Loko *et al.* (2018) i.e 0.30 – 0.90 cm.

**100 dried seeds weight.** Arila (*Phaseolus lunatus* L) seeds dry weight ranges from 25.50 – 128.57 g/100 seeds.

**100 oven-dried seeds weight.** Accessions N9 had the heaviest beans, (103.29 g / 100 beans). 2 accessions or 10.53% (N9 and N11) weight more than 100 g / 100 beans, 11 accessions or 57.89% weighing 25 - 42 g / 100 beans and 6 accessions or 31.58% weight 11.43 - 24 g / 100 beans. The weight of 100



*Phaseolus* beans in Nekamese Sub-regency, Kupang Regency is higher than that reported by Purwanti (2014) which ranges from 9.50 - 36.10 g. The weight of 100 *P.lunatus* beans found here is relatively the same as 100 beans of *Arachis hypogea*, (44.3 g) as reported by Zulchi and Puad (2017).

**Seed of Bean Category.** Of the 19 accessions, 6 accessions were categorized as light with weights range of 11.43 - 24 g / 100 beans, 11 accessions were categorized as medium with weights ranging from 25 - 40 g / 100 beans and 2 accessions (N9 and N11) were categorized as heavy with weight sizes were on accessions with a weight of 101, 43 - 103.29 g / 100 beans.

Of the 19 accessions, N9 beans were long, wide and heavy, while N12, N14 and N16 were medium-sized, and N4, N5 and N6 were beans with short, narrow and light categories. This category is slightly different from reported by Zulki and Puad (2017) regarding the category of peanut seeds based on weight of 100 seeds by Descriptors for groundnut IBPGR, namely small (<40 g), intermediate (40 – 55 g), and (> 55g). Many factors affect plant productivity such as genetics, cultivation techniques, environment, climate, harvest and post-harvest (Purbajanti, 2013).

## CONCLUSION

It was concluded that based on bean morphology, there are 19 different kinds of *Phaseolus lunatus* L. in Nekamese Sub-district that differs in color pattern, weight, length, width, and thickness.

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SUPPORTED BY





## CASE REPORT: PYOMETRA IN A 3 YEARS OLD DOG

Yohanes T.R.M.R. Simarmata<sup>1\*</sup>, Allan P. Titong<sup>2</sup>, Ni Made Restiati<sup>3</sup>

<sup>1</sup>Laboratorium Penyakit Dalam Hewan Besar, Fisiologi, Farmakologi, Biokimia  
Fakultas Kedokteran Hewan Universitas Nusa Cendana

<sup>2</sup>Fakultas Kedokteran Hewan Universitas Nusa Cendana

<sup>3</sup>Bali Veterinary Clinic, Bali

\*Corresponding author: drh.joe.saragih@gmail.com

The case handled was the pyometra case in a 3 year old beagle. The patient was subjected to a physical examination and anamnesa supported by supporting examinations using a Complete Blood Count, and imaging using ultrasound and Xray. Based on the results of physical and follow-up examination, it was concluded that the patient had pyometra. This diagnosis was supported by blood tests that showed a persistent increase in WBC despite being treated using antibiotics during the observation period. In addition, an ultrasound examination showed a buildup of fluid in the form of pus in the uterus. Treatment was carried out by ovariohysterectomy surgery supported by antibiotic therapy. The patient's recovery was going well.

### INTRODUCTION

Pyometra is a case of reproductive disorders found in dogs and cats. This case is often described as a case of bacterial infection of the uterus. These cases were classified into two groups, namely open and closed based on the opening and closing of the cervix or in other words the presence or absence of mucus discharge from the vagina. In the case of closed pyometra, bacteria and pus accumulate in the uterus, causing more severe disturbances than open pyometra.

Risk factors for pyometra include age, nullparity, race, and exogenous administration of either progesterone or estrogen. The incidence of pyometra is more common in dogs than in cats. This is because female cats require copulatory stimulation for ovulation and progesterone production from the corpus luteum. Pyometra can also develop following postpartum ovariohysterectomy or metritis.

One of the cases found during the internship activities at the Bali Veterinary Clinic was a pyometra case. This report describes the implementation of examination, treatment, and prognosis as well as a discussion of the case.

### DIAGONSE AND TREATMENT

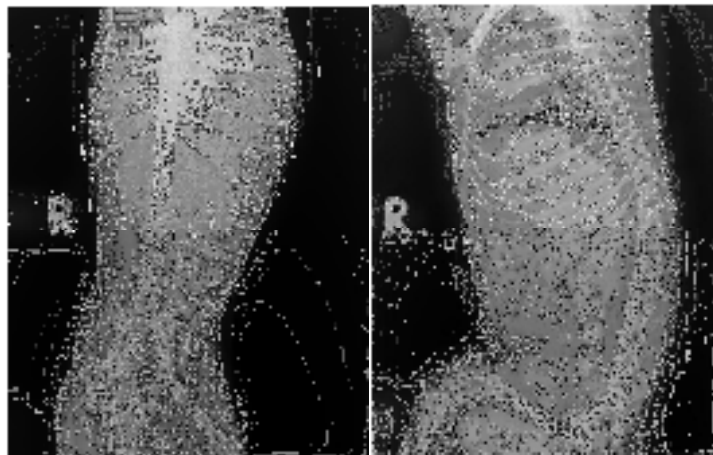
#### Patient data and history

The patient is a beagle named Lulu. The patient is 3 years old and weighs 10.6 kg. The patient was brought to the clinic complaining of loss of appetite and laying down. The patient was known to have not been sterilized.



### Clinical examination

Based on observation, in general, the patient appeared to show signs of laying down. In addition, the patient looked calm and responsive to stimuli. Examination of vital signs showed normal respiratory rate and pulse rate. Measurement of rectal temperature shows the patient was in a fever condition. In addition, examination of the mucosa and skin turgor showed the patient is in a state of dehydration. Physically, the patient did not show certain symptoms that refer to a particular case. Based on these results, further examination was carried out, namely through blood tests and imaging using x-rays and ultrasonography and blood tests through a complete blood count. The patient was then decided to be hospitalized so that further examinations could be carried out while observing the progress of the case, while further testing was carried out to determine the diagnosis of the case.



Picture 1. X ray result

### Blood test (CBC)

Blood tests were carried out twice within three days in determining this case. The results of the examination can be seen in Table 1. The results of the tests vary in the two test results. The picture that remains persistent on the two results is an increase in WBC and a decrease in RBC. Based on the results of CBC examination It was known that patients have an increase in circulating white blood cells (WBC). This increase in white blood cells indicates several things, including that there was an increase in the production of WBC as an immune reaction to infection. In addition, patients was also known to have increased neutrophils.





Tabel 1. Blood test result

Poin	Normal range	1st test result	2nd result
WBC (x 10 <sup>3</sup> /uL)	6-17	26.7	25
LYM (%)	12-30	3.6	47
MID (%)	2-9	3.9	18
GRA (%)	60-83	92.5	33.8
RBC (x 10 <sup>6</sup> /uL)	5.5-8.5	5	5.3
HGB (g/dL)	12-18	11.9	12.6
MCHC (g/dL)	30-38	38	39
MCH (pg)	20-25	23	23.6
MCV (fL)	62-72	55	54.8
RDW-CV (%)	11-15.5	15	14.4
RDW-SD	35-56	43	42.5
HCT (fL)	37-55	27	29.2
PLT (x10 <sup>3</sup> /uL)	200-500	313	258
MPV (fL)	7-12.9	6.9	7.6
PDW (fL)	10-18	6.6	8
PCT (%)	0.1-0.5	0.2	0.1
P-LCR (%)	13-43	14.7	20.1

### Imaging using ultrasound

A further examination that was also performed is an ultrasound examination. The results of the examination showed a buildup of fluid exudate in the uterus.



Picture 2. USG result.

### Determination of the diagnosis

Based on the results of physical examination and follow-up, it was concluded that the patient had pyometra. This diagnosis was supported by blood tests that showed a persistent increase in WBC despite antibiotic therapy during



the follow-up period. In addition, an ultrasound examination shows a buildup of fluid in the form of pus in the uterus.

## DISCUSSION

Memon (2019) argues that the factors associated with the incidence of pyometra include prolonged administration of progesterone and estrogen as well as infections that occur post-insemination or post-copulation. Progesterone increases endometrial development and glandular secretion and reduces myometrial activity. Bacteria originating from normal vaginal flora or subclinical infection of the urinary tract are the most likely causes of uterine contamination. *Escherichia coli* is the bacteria most often isolated from pyometra cases, however, staphylococcus, streptococcus, *Pseudomonas*, *Proteus* spp and several other bacteria are also potential and have been found in samples.

Pyometra often occurs during the diestrus. During this time progesterone stimulates endometrial growth and the activity of glandular secretion. Progesterone also suppresses contractibility of the myometrium and maintains cervical closure. Accumulation of the above factors then creates a suitable environment for bacterial growth. Furthermore, an increase in progesterone in the diestrus decreases immune function by decreasing the chemotaxis and phagocytic ability of neutrophils and increasing the chance of adhesion of bacteria to the endometrium. In this pathogenesis, estrogen also has a role where it increases the ability of progesterone production from the endometrium and secretions from glands that play a role in the diestrus period (Ververidis HN, 2004).

The pyometra case found this time is a pyometra case with a closed cervix. In the case of closed pyometra there was no discharge from the vagina (Memon, 2019). Furthermore, clinical symptoms that are often found in pyometra patients are lethargy and dehydration. Based on the clinical symptoms seen in the patient, it is difficult to determine the cause of the visible disorder. In order to be properly diagnosed, further tests were carried out.

The first tests performed in this case were blood tests and imaging using x-rays. Examination using x-ray did not show any conclusive symptoms in the patient. The examination did not show significant changes in the digestive, respiratory, and cardiovascular systems. In the use of x-ray examination in cases of pyometra, often seen a shift of the intestinum towards the cranial. This occurs due to the enlargement of the uterus as a result of fluid accumulation in the uterine lumen. This symptom is often seen, especially in the case of pyometra with a closed cervix. In this case, such symptoms cannot be observed. The uterus has not been enlarged significantly enough to propel the intestines and is visible on x-ray imaging of the abdominal area.

Ultrasound examination showed the accumulation of fluid in the uterus. The uterus appears to be enlarged with the lumen filled with fluid exudate. On



ultrasound, the lumen of the uterus is semi-opaque. This happens because the fluid that accumulates in the uterus is not a transudate, but has a thick consistency and is filled with dead cells. The solutes in this liquid then provide a reflection image on the ultrasound examination.

Blood tests show the patient has an increase in white blood cells. This indicates an infection in the patient's body. In addition, the increase in leukocytes was more pronounced in patients with closed pyometra, this is due to the accumulation of pus in the uterus in cases of closed pyometra (Mojzisoova et al., 2000). The WBC differential shows a neutrophil that increases at the first examination and decreases at the second examination. Neutrophilia indicates that the infection is, most likely, a bacterial infection. By knowing the results of the final diagnosis, namely pyometra, the results of the second examination which show neutropenia can be interpreted as a result of endotoxin released by bacteria due to endotoxin from infecting bacteria (Romagnoli, 2002). In addition, these symptoms can also be caused by migration of neutrophils to the uterine tissue. The determination of the maturity level of the circulating neutrophils was not carried out. However, based on observations on the samples obtained, it was seen that some of the neutrophil cells were not segmented. Increased left shift neutrophils are common in cases of pyometra. This is because pyometra, as a case of severe bacterial infection, stimulates the bone tissue to release more neutrophil cells into the circulation, including immature white blood cells. (Fransson et al., 1997).

During the observation period, based on clinical symptoms, the patient was suspected of having an infection with possible sepsis. On this basis, the patient was treated with a trial (trial) using a broad spectrum of antibiotics to treat cases of infection that occurred. After being treated for three days, the results of the CBC examination showed the patient had a high WBC count. These results, coupled with the history that the patient was not sterilized, confirms the suspicion that the case that happened was a case of pyometra. With this suspicion, an ultrasound examination was performed to observe changes that occurred in the uterus. After being observed, it was seen that the uterus had accumulated fluid.

After removal of the ovary, the fluid that has accumulated in the uterus is aspirated and observed under a microscope. The collected fluid looks dark red with a thick consistency. In this fluid, many red blood cells and white blood cells are observed. The dominant white blood cells are seen on the This observation is a neutrophil. These results again confirm that the case that happened was a case of bacterial infection of the uterus. Simultaneously with the infection in the uterine tissue, neutrophils, through chemotaxis, will move to the infected tissue.

Metronidazole antibiotic has been known as an antibiotic that is often used in cases of female reproductive tract infections (Stephens and Slee, 1998). This antibiotic has a spectrum for gram-negative and positive bacteria, but is limited to anaerobic bacteria. This antibiotic has properties that are easily absorbed in the



intestine and once absorbed will produce high drug concentrations in plasma and peripheral tissues (Nagel and Aronoff, 2015). The successful use of metronidazole in pyometra cases is due to several factors, namely the rapid and comprehensive distribution throughout the body, the low ability to bind to proteins so that it is effective in purulent areas and low bacteriocidal concentrations (Goldman, 1980).

### CONCLUSION

Diagnosis of pyometra cases is carried out based on history, physical examination and follow-up examinations. Supporting examinations that can be done is such as a Complete Blood Count examination, as well as imaging using ultrasound and X-rays. Treatment can be done using drug therapy or by surgery. Surgical handling is done by lifting reproductive tract of females. Administration of antibiotics is intended to kill pathogenic bacteria that cause infection in the uterus.

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Metronidazole for the treatment of bovine pyometra L R STEPHENS and K J SLEE Department of Agriculture and Rural Affairs, Regional Veterinary Laboratory, P.O. Box 483, Bairnsdale Victoria 3875  
Jerod L. Nagel, David M. Aronoff, in Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases (Eighth Edition), 2015

SUPPORTED BY





## A CASE REPORT OF TRAUMATIC BRAIN INJURY IN A CAT DUE TO BEING HIT BY A VEHICLE

Yeremia Yobelanno Sitompul<sup>1</sup>, Haswita Fitri Amalia<sup>2\*</sup>

<sup>1</sup>Department of Veterinary Clinical, Reproduction, Pathology and Nutrition,  
Faculty of Veterinary Medicine, Universitas Nusa Cendana, Kupang, East Nusa  
Tenggara, Indonesia

<sup>2</sup>Dok Has Pet&Vet, Bangka Belitung, Indonesia

\*Corresponding author: haswita.fitriamalia@gmail.com

A five-month-old male domestic cat was brought to Dok Has Pet&Vet, Bangka Belitung, after being hit by a vehicle. The clinical signs were slightly dehydrated (5%), lethargic, epistaxis, miosis, bruises in the upper lip, but no sign of bone fracture. Based on Modified Glasgow Coma Scale (MGCS), the cat was classified as moderate. A diagnosis of traumatic brain injury (TBI) was made. Firstly, fluid therapy of Normal Saline 0,9%, followed by Furosemide (1mg/kg, IM), was given to stabilize the cat. Afterwards, the cat were treated by Mannitol 20% infusion (1g/kg, IV) administered slowly for 20 minutes, dexamethasone (2mg/kg, SC) with ranitidine (2mg/kg, SC) as a premedication and Amoxicillin (15mg/kg, IM). The treatment was done for 3 days. Oxygen therapy was given for two days, started from the second day after the cat showed a sign of dyspnea. On the third day, the cat showed a good progress of healing. From day 4 to day 10, the treatment was continued by giving gabapentin (15mg/kg), prednisone (1mg/kg), and amoxicillin (10mg/kg) orally and topical gentamycin for the wound in the upper lip. No sign of any brain injury was observed after ten days of the treatment and the cat was fully recovered.

### INTRODUCTION

Traumatic brain injury (TBI) in small animals may happen due to vehicle accidents, crush injuries, gunshot, attacks from other animals, falls, and intentional attacks from humans (Dewey and Fletcher, 2008; Sande and West, 2010; DiFazio and Fletcher, 2013). In Indonesia, not only stray dogs and cats, pet dogs and cats usually can move freely outside their house. This may increase the risk of vehicle accidents that leads to traumatic brain injury.

Moreover, traumatic brain injury is a challenging case for veterinary practitioners. Traumatic brain injury can be divided into primary and secondary brain injury (Dos Santos *et al.*, 2018). Primary brain injury is the physical disruption of intracranial structures that happens immediately at the time of the impact. Secondary brain injury may occurs from minutes to days after the trauma incident because there are physical and biochemical changes that cause intracranial hypertension (Fletcher and Syring, 2009). Since primary injury is



mostly irreversible and beyond control of the veterinarian, the professional should have knowledges about the treatment of secondary brain injury (O'Connor *et al.*, 2011).

Unfortunately, despite needing advance and expensive laboratory tests, such as Computed Tomography (CT) or Magnetic resonance imaging (MRI), the appropriate treatment is still open to debate in veterinary medicine (Evans and Fernandez, 2019). Reliable retrospective and prospective data are still lacking, so clinical recommendations usually come from experimental studies, human head trauma studies, as well as on the personal experiences (Dewey, 2000). Even though there is no published case report of TBI in Indonesia, it is believed that the case often happens in reality. Therefore, this first case report of TBI in Indonesia was made to share an experience in TBI management in a cat due to a vehicle accident.

## CASE HISTORY

A five-month-old male domestic cat was presented to Dok Has Pet&Vet, Bangka Belitung with an information that the cat was hit by a vehicle. Its weight and body temperature were 1,3 kg and 37,5<sup>0</sup>C. Based on physical examination, the cat was slightly dehydrated (5%), lethargic, epistaxis, miosis, wounded in the upper lip. It showed no sign of bone fracture. Based on Modified Glasgow Coma Scale (MGCS), the cat was classified as moderate. A diagnosis of traumatic brain injury was made according to the clinical sign only since the veterinary medical facilitation in Bangka Belitung is still lacking. The initial treatment was fluid therapy of Normal Saline 0,9%, followed by Furosemide (1mg/kg, IM), to stabilize the cat. Afterwards, the cat was treated by Mannitol 20% infusion (1g/kg, IV) administered slowly for 20 minutes, dexamethasone (2mg/kg, SC) with ranitidine (2mg/kg, SC) as a premedication and Amoxicillin (15mg/kg, IM). During the initial treatment, the cat's head was positioned 30-degree angle to the body. The treatment was done for 3 days. Oxygen therapy was given for two days, started from the second day after the cat showed a sign of dyspnea. On the third day, the cat showed a good progress of healing. From day 4 to day 10, the treatment was continued by giving gabapentin (15mg/kg), prednisone (1mg/kg), and amoxicillin (10mg/kg) orally and topical gentamycin for the wound in the upper lip.



Figure 1. The first day after the accident, the cat was lethargic and had wounded upper lip.



Figure 2. The tenth day after the accident, the cat was fully recovered.

### RESULTS AND DISCUSSIONS

Through physical examination, the cat was indicated having traumatic brain injury. A modified version of the Glasgow Coma Scale (MGCS), which incorporate into three domains: (1) level of consciousness, (2) posture and limb motor function, (3) brainstem reflexes, has been used to assess the current neurologic signs. Each domain has a score of 1-6 and the total score for all domains is from 3 to 18 (Platt *et al.*, 2001). The total score in this cat was 12, classified as moderate. The diagnosis was traumatic brain injury and it was made without laboratory examination because of the lack of veterinary medical facilitation in Bangka Belitung. Advanced imaging should be valuable, however, except the cost and the availability, in an emergency case like TBI, anaesthetic risk should be considered. Sande and West (2010) suggested that the advanced imaging is preferably done when the patient does not respond to or become worse responding to initial medical therapy.

The major concern of this injury is to prevent an increase in intracranial pressure (ICP). Elevated ICP causes the disruption of blood flow, including the reduction of cerebral blood flow (CBF), and lead to ischaemia and hypoxia in the brain (Lubillo *et al.*, 2009; Cecil *et al.*, 2011). A severe elevated ICP is indicated when a patient shows Cushing reflex, which presents as an increased mean arterial





pressure (MAP) and bradycardia to tackle hypercapnia in the brain (Dunn, 2002; Laffey and Kavanagh, 2002; Portella, et al. 2005; Stocchetti et al. 2005; Guyton and Hall, 2006). Firstly, normal saline 0.9%, an isotonic crystalloid, was given as initial treatment to improve tissue perfusion, including cerebral perfusion pressure (CPP) (Syring, 2005). Furosemide was subsequently administered to the cat in this case because it is believed that it can work synergistically with mannitol by decreasing hypertension and cerebrospinal fluid (CSF) production (Roberts *et al.*, 1987). The position of the cat's head at 30-degree angle to the body is considered to be simple but effective to help facilitating greater arterial supply and venous drainage in the brain (Dos Santos *et al.*, 2018; Opperman, 2014).

In this case, the first choice of treatment to decrease or prevent elevated ICP was mannitol. Mannitol is an osmotic diuretic that can reduce ICP by promoting the drainage of extravascular fluid (cerebral oedema) into the intravascular fluid. Moreover, mannitol also induces the vasoconstriction reflex. The administration of mannitol by intravenous infusion will reduce blood viscosity so the CBF will be maintained. The reduction of CSF production to prevent extra accumulation of fluid in the brain can be achieved by the use of mannitol (Sande and West, 2010; DiFazio and Fletcher, 2013).

Supplemental oxygen were given when the dyspnoea occurred. Maintaining the partial pressure of oxygen in arterial blood to normal (80 mm Hg) is necessary (Dos Santos *et al.*, 2018). The dyspnoea was a sign that the cat had hypoxia. It disappeared after the cat was treated via face mask oxygenation. The additional drugs were corticosteroids (dexamethasone and prednisone) as analgesics, ranitidine as a gastrointestinal protectant, gabapentin as an anticonvulsant therapy, and antibiotics (amoxicillin and topical gentamycin). They were used as precautionary measures to prevent unpleasant condition, such as pain, stress ulceration, seizure, and secondary bacterial infections.

Appropriate treatment for TBI is still arguable (Sande and West, 2010; DiFazio and Fletcher, 2013, Dos Santos *et al.*, 2018). Some drugs used in this case were controversial, such as furosemide and corticosteroids. Several research claimed that the use of mannitol alone has the same effect on brain water compared to the use of mannitol plus furosemide (Todd *et al.*, 2006). Corticosteroids have adverse effect, including hyperglycaemia, immunosuppression, delayed wound healing, and gastric ulceration (Syring, 2005). Moreover, there is an increase of human mortality in the use of corticosteroid in TBI treatment (Roberts *et al.*, 2004; Edwards *et al.*, 2005). Thus, those drugs are not recommended. Oxygenation is highly proposed in patient with TBI. While hyperoxygenation is considerably safe for patients with TBI, oxygen was not supplemented immediately in this case due to an avoidance of the adverse effect of hyperoxygenation, such as changes in non-damaged tissues, cerebral hyperoxic vasoconstriction, inhibition of metabolic enzymes and formation of free oxygen radicals (Floyd *et al.*, 2003; Magnoni *et al.*, 2003; McLeod *et al.*, 2003).



Despite all the arguments above, TBI treatment should be immediate and aggressive. Early identification and treatment have higher chance to recover. Small animals are considered to have exceptional ability to compensate for the deficiency of cerebral tissue, hence rash prognostic conclusions should not be made on the initial presentation of a dog or cat with TBI (Sorjonen *et al.*, 1991; Sande and West, 2010). In this case, no sign of any brain injury was observed after ten days of the treatment and the cat was fully recovered.

Even though the benefit of hypertonic saline in reducing ICP is being studied, the availability of Mannitol infusion in veterinary clinics should be considered. Another reason is that in Indonesia, not a few people allow their pets to move freely outside their house, so it increases the chance of vehicle accidents causing TBI in pets. Unfortunately, mannitol infusion for animal use only is still rare in Indonesia and the mannitol used here was bought from a human hospital.

## CONCLUSIONS

Treatment management of traumatic brain injury is not limited to reducing pain, stopping bleeding and healing wounds in the head. It should focus on restoring cerebral perfusion pressure by maintaining blood flow and, if needed, oxygenation (Garosi and Adamantos, 2011; Dos Santos *et al.*, 2018). As it is reported that the increase of intracranial pressure becomes the most frequent cause of disability and death in animals (Dos Santos *et al.*, 2018), the knowledge and availability of treatment to avoid or decrease elevated intracranial pressure is necessary for veterinary practitioners. The use of mannitol is recommended in preventing or reducing the elevated intracranial pressure.

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## ISOLATION AND CHARACTERIZATION OF DERMATOPHYTES FUNGUS IN LIVESTOCK

**Dwi Endrawati<sup>\*</sup>, Ani Kusumaningtyas, Riza Zainuddin Ahmad**

Laboratory of Mycology, Indonesian Research Center for Veterinary Sciences,  
Bogor

\*Corresponding author: d.endrawati@gmail.com

Dermatophytosis is a mycotic disease that attacks the keratin substrate in human and animal skin, nails and hair. The cause of dermatophytosis is molds from the genus *Trichophyton*, *Microsporum* and *Epidermophyton*. Generally, dermatophytosis is chronic, although there are no reports stating that dermatophytosis can cause death, it can cause losses due to the decreased quality of the livestock. This study aims to characterize the fungus causing dermatophytosis in livestock in Kunak (Kawasan Usaha Peternakan) Bogor, Cisarua Bogor and Sukabumi. Sampling was carried out on 15 cattle suspected of dermatophytosis by scraping the skin lesions. From 15 test samples, obtained one *Trichophyton mentagrophytes* isolate. The identification of fungi that cause dermatophytosis is done conventionally and molecularly. Based on the results of conventional examination, it was found that the macroscopic formation of *T. mentagrophytes* with white colonies, cream-colored colony base, velvety and dense texture, hard when scratched with ose. Amplification of the ITS and chitin synthase genes resulting 650 bp and 350 bp PCR products. Digestion of ITS fragments using the restriction enzyme *DdeI* resulting amplicon pieces with lengths: 600, 500, 400, 250, 100 bp, and *HinfI* enzymes: 450 bp and 200 bp. Based on these results, the characteristics of *T. mentagrophytes* in this study can be used as molecular markers for *T. mentagrophytes* isolated from Bogor.

### INTRODUCTION

Fungal infections can attack humans and animals skin, on the surface of the skin as well as to the deeper layers of the epidermis. One of the skin disease commonly known as ringworm or tinea, is an infection of the skin due to dermatophyte fungi, called dermatophytosis. Dermatophytosis is a zoonotic disease that can occur in humans and animals, caused by dermatophyte fungi.

Dermatophytes are filamentous fungi that live by utilizing a keratin substrate in the epidermis of skin, hair and nails (Alshawa et al. 2012). Anamorphically, dermatophytes are divided into 3 genera, *Epidermophyton*, *Microsporum*, and *Trichophyton* which can cause superficial infections in humans and animals (Behzadi et al. 2014).

Dermatophytosis in animals is mostly found in pets, livestock and wildlife (Moriello. 2014; Pal. 2017; Melo et al. 2018). In livestock, dermatophytosis is



often caused by *Trichophyton mentagrophytes* and *T. verrucosum* (Dalis et al. 2018; Pal 2017). Pal (2017) in his research found a case of dermatophytosis in livestock with clinical symptoms of alopecia in the neck and abdomen area caused by *T. verrucosum*. Dermatophyte can be transmitted from animals to humans (zoonosis), almost 20-50% of human skin infections originate from dermatophytes found in pets and can also be transmitted to other animals (*Microsporum canis* and *T. mentagrophytes*) (Scott et al. 2001; Weese and Fulford. 2010). Dermatophytosis affects the health of the skin of the host and the quality of the skin products of animal origin and can be transmitted easily (Ahmad 2005; Ahmad and Gholib 2016). Day et al. 2012 reported that more than 50-70% prevalence of human mycotic infection cases originate from animal hosts or especially domestic animals.

Examination and diagnosis confirmation of patients with suspected dermatophytosis can be carried out by conventional and molecular methods. Conventional examinations include: the use of Wood's-lamp, native examination of skin scraping samples using KOH 10-20%, growing samples on agar media. Molecular examination can be carried out using molecular PCR techniques. Molecular examination can save diagnostic time when compared with conventional examinations. In addition to using PCR, researchers also use restriction enzymes on the isolates found and compared with positive control isolate from ATCC (American Type Culture Collection) and BCC (BBlitvet Culture Collection), this is intended to see the similarities or differences in the molecular markers of isolates found with positive control isolates.

## MATERIAL AND METHODS

### *Sample Isolates*

Skin scraping samples came from 15 livestock suspected of dermatophytosis from Sukabumi, Kunak (Koperasi Usaha Peternakan) Bogor and Cisarua Bogor. The sample is stored in a sterile place.

### *Positive Control Isolates*

The positive control isolates used were *Trichophyton interdigitale* from ATCC and *T. mentagrophytes* from BCC (F0217). Isolates were cultured again on Sabouraud Dextrose Agar (SDA, BD Difco™, USA) medium added with 0.05% Chloramphenicol (Sigma) antibiotics, incubated at 37 °C for 14 days (Ainsworth and Austwick 1973).

### *Conventional Identification*

Conventional identification was carried out by native examination using 10-20% KOH. Some of the samples were cultured on SDA media supplemented with the antibiotics *Chloramphenicol* (Sigma) 0.05% and *Cycloheximide* (Sigma)



0.5%. The medium containing the specimens was incubated at 37 °C for 7–14 days (Ainsworth and Austwick 1973).

Colony observation is to identify morphology of suspected dermatophytes both macroscopically and microscopically with reference to Al-Doory (1980).

### *Molecellar Identification*

#### *DNA Extraction*

DNA extraction was carried out in accordance with White et al. (1990) with several adjustments. Extraction materials were dermatophyte mycelium from control isolate and fungal mycelium from field samples cultured on SDA media supplemented with antibiotics and incubated for 7 days. The purity and percentage of Dermatophyte DNA content were measured using a NanoDrop Spectrophotometer at a wavelength of 260/280 λ.

#### *PCR*

Dermatophyte fungal DNA amplification process was carried out using primary pairs as mentioned in Table 1.

Table 1. Sequence of primer used in the research

Primer	Sequence
ITS	1 (F) 5'-TCCGTAGGTGAACCTGCGG-3'
	4 (R) 5'-TCCTCCGCTTATTGATATGC-3'
CHS 1	(F) 5'-GAAGAAGATTGTCGTTTGCATCGTCTC-3'
	(R) 5'-CTCGAGGTCAAAGCACGCCAGAG-3'

ITS 1 and ITS4 amplify the internal transcribed spacer (ITS) region on ribosomal DNA. According to Tartor et al. (2016) and Zhang et al. (2011), the amplicon length target for dermatophyte fungi is ~ 600– ~ 750 bp. The Chitin Synthase 1 (CHS1) gene with an amplicon length of 288 bp was used to determine the presence of a dermatophyte-specific sequence of fungi (Putty et al. 2018). The PCR process for ITS and CHS 1 primers used a thermocycler (Eppendorf, Germany) with a platinum green hot start master mix reagent. The reaction begins with pre-denaturation at 95 °C for five minutes, 35 cycles of DNA amplification at 95 °C for three seconds, annealing at 56 °C for one minute, extension at 72 °C for three seconds and final extension at 72 °C. for five minutes. PCR results were examined using agarose gel and dye electrophoresis techniques (Invitrogen™ SYBR™ safe stain, USA). The results of electrophoresis were visualized using an ultraviolet (UV) transiluminator.

#### *Sequencing*

ITS PCR products that were positive for dermatophytes were then sequenced. The sequenced DNA sequences were read with Bioedit® software and



synchronized using the DNA Local Alignment Tool (BLAST) program with the reference source sequence in the data at Gene Bank ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)).

### *Restriction Fragment Length Polymorphism*

The restriction enzymes used in the RFLP process are DdeI and HinfI. The RFLP process used 10 µl of PCR product (from ITS) which was dermatophyte positive, the composition of the mixed solution and the test protocol followed the rules recommended by the enzyme manufacturer (ThermoFisher Scientific®, USA). The results of the RFLP were electrophoresed using 1.5% agarose gel and safe SYBR dye (Invitrogen®) with a voltage of 100 volts for 40 minutes. The results of electrophoresis were visualized using UV transluminator.

## RESULT AND DISCUSSION

### Isolation and identification dermatophyte fungi

Dermatophyte fungi were isolated from 15 livestock that showed clinical symptoms such as dermatophytosis, namely the presence of circular and crusty lesions on the skin. In the area of the lesion there is sometimes an accumulation of pus which indicates a bacterial infection, this can happen because the animal feels uncomfortable and itchy so that it rubs the area of the lesion against nearby objects and causes injury. The test sample data is presented in table 2.

The skin scrapings were examined natively using 10 - 20% KOH, cultured in agar medium and molecular examination by PCR-RFLP. On the native examination, there is a formation of fungal hyphae in the sample. Cultivation results on agar media showed that there was only 1 positive sample of *T. mentagrophytes* from samples from Kunak, Bogor. Samples from Sukabumi showed *Aspergillus* sp., *Mucor* sp., Hypomicetes mold. Samples from Cisarua (C1-C5) all show *Penicillium* sp. and *Aspergillus fumigatus*, this could happen because when the samples were taken, the animal had been given antifungal therapy, so that the dermatophyte fungi were no longer there, the isolated fungi could be saprophytic fungi that stuck to the skin and hair without causing an infection on the skin. Clinical symptoms resembling dermatophytosis seen in samples from Cisarua, it could be residual skin debris leading to healing.

Based on the results of culturing samples on agar media, dermatophytes grows after 7 days of incubation with white colonies and cream-colored colonies. The upper surface of the colony has a hairy texture like cotton, dense and hard when scratched using ose. In this research, observations of the microscopic structure of dermatophytes could be observed after the culture was incubated for 14 days, this is in accordance with Ainsworth and Austwick (1973) who stated that the formation of macro- and microconidia was easier to observe microscopically after 14 days of dermatophyte culture. Macro- and microscopic views of dermatophyte fungi are shown in Figure 1.





Table 2. Data sample from suspected dermatophytosis livestock

No	Code	Animal	Age (month)	Lesion	Origin of sample
1	S1	Cow	> 24	Back, neck	Ujung genteng, Sukabumi
2	S2	Cow	> 24	Abdomen, tail	Ujung genteng, Sukabumi
3	S3	Cow	> 24	Face, neck	Ujung genteng, Sukabumi
4	S4	Cow	> 24	Back, abdomen	Ujung genteng, Sukabumi
5	S5	Cow	> 24	Head, abdomen	Ujung genteng, Sukabumi
6	S6	Cow	> 24	Head, neck	Ujung genteng, Sukabumi
7	C1	Spotted deer	12	Almost all body	Cisarua, Bogor
8	C2	Spotted deer	18	Abdomen, back	Cisarua, Bogor
9	C3	Spotted deer	18	Neck, abdomen	Cisarua, Bogor
10	C4	Camel	> 24	Abdomen	Cisarua, Bogor
11	C5	Camel	> 24	Abdomen	Cisarua, Bogor
12	K1	Cow	> 24	Abdomen	Kunak, Bogor
13	K2	Cow	> 24	Neck, abdomen	Kunak, Bogor
14	K3	Cow	> 24	Abdomen, back	Kunak, Bogor
15	K4	Cow	> 24	Abdomen, tail	Kunak, Bogor

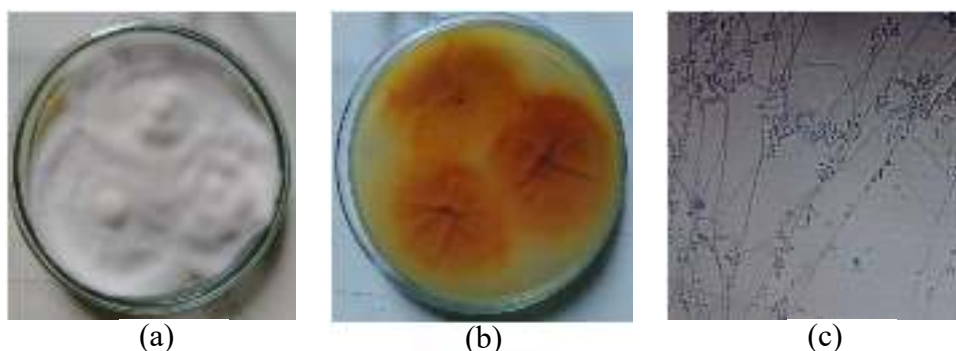


Figure 1. *T. mentagrophytes* colonies were isolated from samples after 14 days of incubation. (a) top view of the colony, (b) basic view of the colony, (c) microscopic view with 400x magnification.

This research used ITS 1 and ITS 4 primers as a general primer for all fungi and the Chylin synthase 1 (CHS 1) gene as a dermatophyte-specific primer capable of detecting dermatophyte fungi at the genus level. The internal



transcribed spacer (ITS) region is part of the nuclear ribosomal gene cluster which is used to mark the DNA of the kingdom of fungi (Schoch et al. 2012).

After 15 samples of skin scrapings were cultured in agar media, 3 isolates were obtained whose colony growth resembled dermatophyte fungi. The three isolates were then extracted by DNA and PCR using ITS and CHS 1 primers as shown in Figure 2.

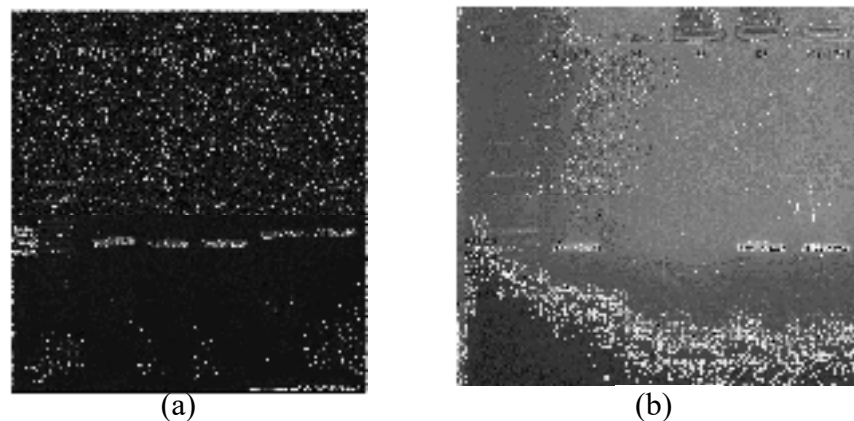


Figure 2. PCR results used primers ITS (a) and CHS1 (b).

Lane 1 is marker, Lanes S1, S3, K3 is isolates from fields, Lane K(+) TI is *T. Interdigitale* from ATCC as positive control, lane K(+)TM is *T. mentagrophytes* from BCC as positive controls

The results in Figure 2 show that on PCR with ITS primers, 3 mold isolates showed a band with amplicon lengths respectively for isolates S1, S3 and K3 were 600 bp, 600 bp and 650bp, with the positive control band showing an amplicon length of 650 bp, whereas in PCR with CHS 1 primers, only K3 isolates showed a band at 350bp amplicon length, with TI and TM positive controls showing 350 bp amplicon lengths, respectively. Based on these results, it can be ascertained that the K3 isolate is a dermatophyte mold because it is amplified by the chitin synthase 1 primer. Nasrin et al (2017) stated that PCR and Chitin synthase 1 (CHS1) gene sequencing are standard tests to determine the presence of dermatophytes in patients. Chitine, an N-acetylglucosamine polymer, is an important part of the cell wall in molds. Chitin synthase is an enzyme that is important for the formation of chitin (Li et al. 2016).

Furthermore, PCR products from K3 isolates were sequenced and sent to 1stBase, Malaysia. The sequencing results were read using the DNA Local Alignment Tool (BLAST) program and the sequence of reference sources in the data in Gene Bank. The results show that the K3 isolate is *T. mentagrophytes*.



## RFLP

The PCR product of K3 isolate was then restricted using DdeI and HinfI enzymes. The resulting cutting pattern is presented in Figure 3.

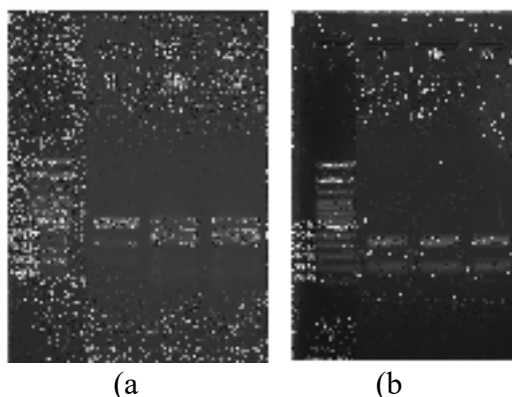


Figure 3. Cutting patterns of *T. mentagrophytes* after digest using DdeI (a) and HinfI (b) enzyme.

Lane 1 is marker, lane TI is *T. interdigitale* from ATCC, lane TM is *T. mentagrophytes* from BCC, lane K3 is isolate from Kunak Bogor

Based on the results in Figure 3, the cutting pattern of *T. mentagrophytes* obtained from field sample isolation after digestion using the DdeI enzyme shows amplicon sizes of 600 bp, 500bp, 400 bp, 250 bp and 100 bp, while digesting using HinfI enzyme it shows amplicon sizes of 450 bp and 200 bp. The cut pattern of DdeI and HinfI enzyme digestion of K3 isolates was identical to the positive control of *T. mentagrophytes* from BCC.

## CONCLUSION

The identification results in this study showed the same results between conventional and molecular identification. The *T. mentagrophytes* isolate from the study had the same ribbon cut pattern as the positive control isolate from BBLitvet Culture Collection.

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**STUDY COMPARATIVE OF SKIN HISTOLOGY OF SUMBA ONGOLE  
(*Bos indicus*) AND BALI CATTLE (*Bos sondaicus*)**

**Vilomena Kusi Toan<sup>1\*</sup>, Filphin Adolphin Amalo<sup>2</sup>, Ingrid Trinidad Maha<sup>2</sup>**

<sup>1</sup>Faculty of Veterinary Medicine, University of Nusa Cendana, Kupang

<sup>2</sup>Laboratory of Anatomy, Physiology, Pharmacology and Biochemistry, Faculty of Veterinary Medicine, Nusa Cendana University, Kupang.

\*Corresponding author: vilomenatoan05@gmail.com

Sumba ongole (*Bos indicus*) and Bali cattle (*Bos sondaicus*) are typical cows in NTT with a semi-arid climate. Cows maintain normal body temperature by maintaining a balance in both heat gain and heat loss through the evaporation mechanism. The skin has a structure that plays an important role in the body's physiological response mechanism, namely the sweat glands. This research was conducted to determine the histological structure of the skin of Sumba ongole and Bali cattle and the distribution of sweat glands. This study used six cows collected from RPH East Sumba and RPH Oeba Kupang. The sample was fixed in 10% formalin, then continued with the HE staining process and microscopic observation. The results showed that there are similarities in the histological structure of the skin of Sumba ongole and Bali cattle with other mammals which have three layers, namely the epidermis, dermis and hypodermis layers. There are differences in the types of sebaceous glands, namely the Sumba Ongole type of unilobular sebaceous glands and in Bali cattle the type of multilobular sebaceous glands. The histological structures of hair follicles, sebaceous glands, and sweat glands are related to one another. The mean number of sweat glands in the *Nuchalis* region of Sumba Ongole cattle ( $13.33 \pm 5.13 / \text{mm}^2$ ) was more than that of Bali cattle, while the *Fascia superficialis dorsalis* region of Bali cattle had a higher average number ( $13.66 \pm 4.16 / \text{mm}^2$ ) than in Sumba ongole cattle.

### INTRODUCTION

East Nusa Tenggara (NTT) is one of the regions in Indonesia with a semi-arid climate. The distinctive feature of regions with semi-framed climates is the difference in a relatively short rainy season with high rainfall intensity and a longer dry season. During the dry season, exposure to sunlight (UV) is long enough so that the temperature is quite high. The difference in climatic conditions in the dryland islands of East Nusa Tenggara (NTT) has the potential for the development of beef cattle. Cows are used as food products of animal origin, namely Sumba ongole and Bali cows (BPS NTT, 2014; Priyanto, 1998).

Cows maintain normal body temperature by maintaining a balance in both heat gain and heat loss. The exchange of heat in the animal's body has different pathways, among others; radiation, conduction, convection, and evaporation



(Reese, 2004). Sweating is a physiological response due to an increase in body and environmental temperature during physical activity or thermal stress experienced by animals or humans. The integumentary system, especially in the skin where there are sweat glands, plays an important role in the body's physiological response mechanisms. One of the functions of the sweat glands is to regulate body temperature (Mescher, 2012).

### MATERIAL AND METHODS

Skin samples were collected from six Sumba ongole (*Bos indicus*) cows which were slaughtered at the Slaughterhouse of East Sumba Regency and six Balinese cows (*Bos sondaicus*) which were slaughtered at the Oeba Slaughterhouse. Each sample was incised in two regions, namely; the superficial layer of the nuchalis (neck) and dorsalis superficial fascia (back) with a size of  $\pm 5 \text{ cm}^3$ . The sample collection criteria were healthy cows, with a range of 200-300kg body weight and 2-5 years of age.

The sample was fixed in 10% formaldehyde then continued with the process of making histological preparations, Haematoxylin-Eosin (HE) staining and microscopic observation at the Laboratory of the Faculty of Veterinary Medicine, Undana.

### RESULT AND DISCUSSION

Microscopic Overview of the Skins of Sumba Ongole (*Bos indicus*) and Bali Cows (*Bos sondaicus*) consists of epidermis, dermis and hypodermis layers. These three layers are composed of other structural components and each has a different function. A difference in function will help the body's metabolic mechanism of Sumba ongole and Bali cattle to maintain physiological status from heat stress and mechanical stress. Microscopic images of the epidermis layer, dermis layer and hypodermis of Sumba ongole and Bali cattle can be seen in Figure 1.

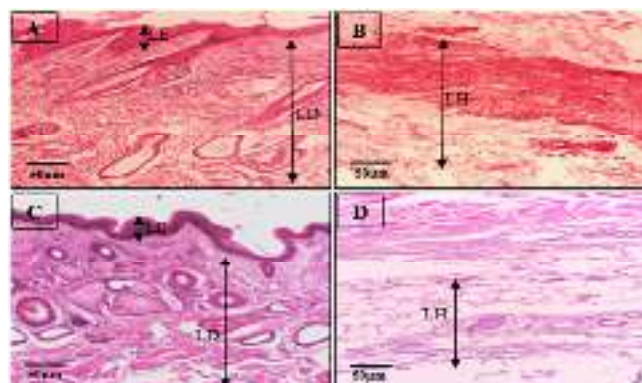


Figure 1. Histological structure of the skin of Sumba ongole and Bali cattle in the nuchalis region. A and B = Sumba ongole cattle, C and D = Bali cattle. Epidermis layer (LE), Dermis layer (LD), Hypodermis layer (LH). HE, 10  $\times$ .



## Epidermis Layer

The epidermal layer in the nuchalis region and the dorsalis superficial fascia in Sumba ongole and Bali cattle have the same histological structure. This layer is composed of 4 (four) stratum, namely; *stratum corneum*, *stratum granulosum*, *stratum spinosum* and *basale stratum* (Figure 2). Stratum lucidum cannot be found because it is only found on the skin of the planum nasale and soles of the feet (Mescher, 2012; Sumena et al., 2010).

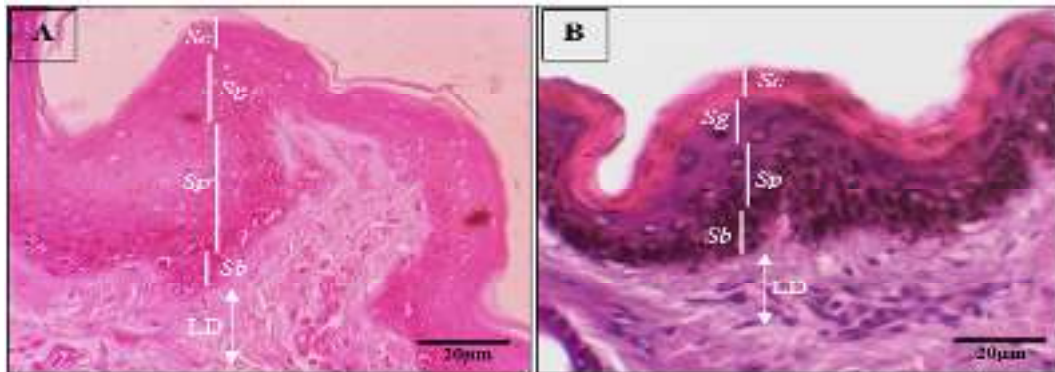


Figure 2. Histological structure of the epidermal layer of the nuchalis region in Sumba Ongole and Bali cattle. A = Sumba ongole cow, B = Bali cow. stratum corneum (Sc), stratum granulosum (Sg), stratum spinosum (Sp), basal stratum (Sb), and dermis layer (LD). HE, 40x.

The stratum corneum in Sumba ongole and Bali cattle consists of many layers of dead, flat and non-nucleated cells. The stratum corneum in Bali cattle appears to be thicker than that of Sumba Ongole. This stratum has the ability to phagocytose harmful pathogenic agents due to penetration through the skin, besides that there are Merkel cells which function as mechanical mechanoreceptors for touch, pain, heat and cold (Mescher, 2012; Humbert and Agache, 2004). According to Mescher (2012), the stratum corneum is a layer that is often peeled off due to the keratinization process.

The stratum granulosum in Sumba Ongole cattle is composed of three rows of polygonal cell layers while in Bali cattle it is composed of two rows of polygonal cell layers. In Bali cattle, the cytoplasm of cells in the stratum granulosum looks more basophilic than in Sumba Ongole cows. According to Rina, (2013) The factors that affect basophilic or acidophilic color differences are caused by acid-base reactions in cell components, small molecular adsorption that will stick to large molecules and different types of solubility depending on the level of solubility in the cell. According to Junqueira and Carneriro (2007), the cytoplasm of the cells in the stratum granulosa contains a basophilic mass called keratohialin granules. This stratum plays a role in the keratinization process and as a barrier against the penetration of most foreign bodies.

The stratum spinosum in Sumba Ongole and Bali cattle is the thickest layer of the epidermis and has epithelial cells that are cuboid to flattened or called polygonal. The cells get squashed the closer they get to the surface. According to Junqueira and Carneriro (2007), between the stratum spinosum there are





intercellular bridges consisting of protoplasm and tonofibrils or keratin. The thickness of the stratum spinosum varies depending on the anatomical location of the animal.

The basal stratum in Sumba Ongole and Bali cattle appears to have a layer of columnar to cuboidal cells. In this stratum there are also melanocyte cells located between the cells of the basal layer. Melanocytes are cells that synthesize melanin and then transfer it to keratinocytes to determine skin pigment. These cells are limited to the skin and are present in the basal layer and in hair follicles (Mescher, 2012). Fiarley (2001) states that the basal stratum functions as a site for cell mitosis or replacement of damaged cells as well as a place of attachment between the epidermis and dermis.

Previous research conducted by Saravanakumar and Thiagarajan (1992), on cattle and buffalo stated that animals with a thin epidermis had better heat tolerance than animals with thick epidermis. This is due to the adsorption ability of the skin which is influenced by the thickness of the skin, hydration, moisture and metabolism of the animals. Absorption can take place through epidermal cells rather than through the intercellular gap (Tortora and Derrickson, 2006).

### Dermis Layer

Based on observations, most of the dermis layer consists of elastic fibers and dense irregular connective tissue. This layer also contains hair follicles, arrector pilli, sebaceous glands, sweat glands and blood vessels which will be discussed based on their respective functions (Figures 3, 4, 5, and 7). The dermis layer on the skin of Sumba ongole and Bali cattle in two regions, namely; The superficial layer of the nuchalis (neck) area and the dorsal superficial fascia of the dorsal region consist of papillary and reticular layers having the same structure (Figure 3). These results have structural similarities in other types of cattle conducted by Hossain et., Al, 2016; Alsodany et., Al, 2019.

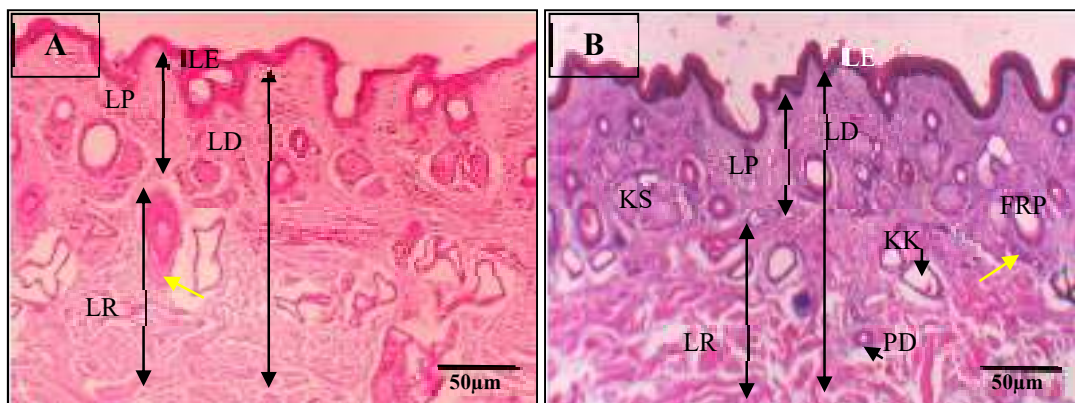


Figure 3. Dermis layer of Sumba ongole and Bali cattle in the nuchalis region. A = Sumba ongole cow, B = Bali cow. Epidermis layer (LE), Dermis layer (LD), Papillary layer (LP) Reticular layer (LR), Hair follicles (FR), Blood vessels (PD), Sebaceous glands (KS), sweat glands (KK), Arrector pilli (yellow arrow). HE A and B 10 ×.



Thickness comparisons were observed in the papillary and reticular layers. The reticular layer appears thicker than the papillary layer and there is a dense, irregular connective tissue that spreads out in the form of a webbing. The characteristics that distinguish these two layers are 1; Papillary layer is located at the top adjacent to the epidermal layer which contains more elastic tissue. The difference in thickness of the papillary layer depends on age and anatomical location. (Sorrel and Caplan, 2004), 2; The reticular layer is under the papillary layer and extends thicker to the hypodermis, there are blood vessels, the number of elastic fibers is less, mostly composed of irregular connective tissue which is denser due to the presence of collagen fibers which have the function of providing strength and flexibility to the skin (Montell et al., 2015; Mobini, 2012; Mir Shabir et al., 2011).

Based on the results of observations of the histological structure in the dermis layer of the skin of Sumba Ongole and Bali cows, there are hair follicles. Types of hair follicles are divided into primary hair follicles and secondary hair follicles. The difference is that primary hair follicles are the main hair follicles that stick out from the dermis layer to the surface of the epidermis layer whereas, secondary hair follicles are follicles that sprout new. The function of hair follicles is to help control body temperature, protect the skin from injury, and circulate blood which will nourish the skin (Alsodany et al., 2019; Mescher, 2012).

Primary and secondary hair follicles in the superficial region of the nuchalis region (neck) and the dorsal superficial fascia in the dorsal region vary in size from smallest to large. The distribution of hair follicles in the two regions also varies in number. According to Adams and Cornje (2003), the number of hair follicles varies in number depending on their anatomical location.

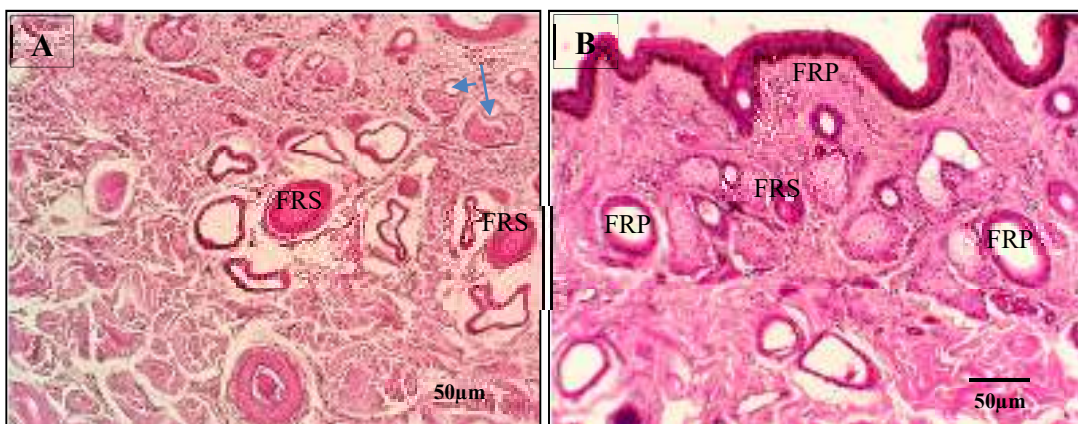


Figure 4. Histological structure of hair follicles in Sumba Ongole and Bali cattle, fascia superficial dorsal region, dorsal region. A = Sumba ongole cow, B = Bali cow. Primary Hair Follicles (FRP), Secondary Hair Follicles (FRS), and Blood Vessels (PD). HE, 10 ×.



The sebaceous glands in sumba ongole cows are a uni-lobular gland type and in Bali cows a multi-lobular gland type (Figure 5). According to Alsodany et al., (2019), there are two types of sebaceous glands, namely uni-lobular and multi-lobular. The unilobular gland type is a type of gland that has a single branching form where one primary or secondary hair follicle is surrounded by 1-2 sebaceous glands. Multi-lobular gland type, where each primary and secondary hair follicle is surrounded by 3-4 sebaceous glands.

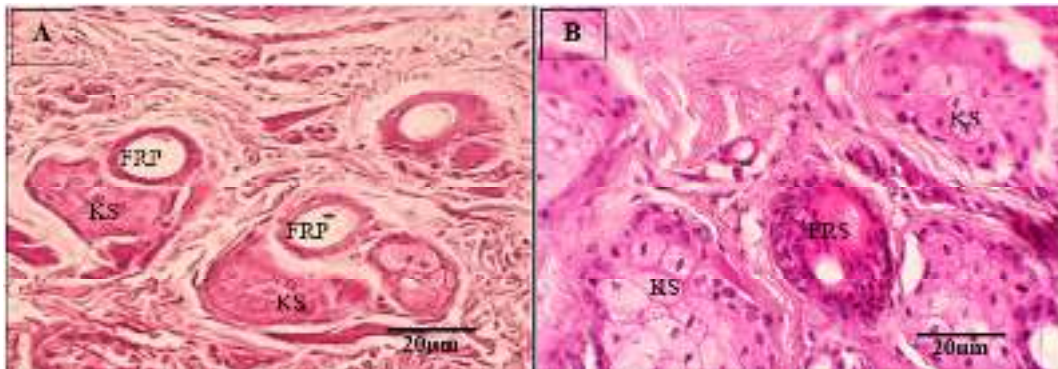


Figure 5. Appearance of sebaceous glands in Sumba Ongole cattle and Bali cattle in the dorsalis superficial region fascia. A = sebaceous glands of uni-lobular type in sumba ongole cattle, B = sebaceous glands of multi-lobular type in bali cattle. Sebaceous glands (KS), Primary hair follicle (FRP), Secondary hair follicle (FRS). HE, 40 ×.

The type of sebaceous glands in Bali cattle has similarities with several animals including buffalo (Umeri and Mamoori, 2016; Ali, 2008), goats (Adib Moradi and Sheibani, 2000), and sheep (Katas and Daglioglus, 2009). Samuelson (2007), states that the sebaceous glands produce sebum (oil) which is secreted by the holocrine. Sebum has a specific function in maintaining the stratum corneum, maintaining skin moisture, antifungal, antibacterial and reducing friction with nearby cells, acting as thermal insulation, contributing to the form of vitamin D and preventing the entry of water into hair and skin.

The more hair follicles, sebaceous glands and sweat glands, the better the body's metabolic mechanism to gain or lose heat due to changes in seasons and changes in temperature (Rohankar et al., 2018). The results of research on camels conducted by Macneill et al., (2005), stated that hair thickness can help protect the animal's body from excess heat and evaporation mechanisms. This shows that the greater the number of follicles in Sumba Ongole and Bali cows, the physiological status to maintain body temperature due to different season conditions will be effective in dry land islands.

### Hypodermis layer

The results obtained, the hypodermis layer in Sumba Ongole and Bali cattle lies under the dermis which consists of loose connective tissue, adipose tissue,



blood vessels and nerves (Figure 6). Adipose cells are round in shape and seem to be pushed aside. The adipose cells in Sumba ongole cattle were large (Figure 6A'), while the adipose cells in Bali cattle were dense and smaller in size (Figure 6B'). According to Karundeng et., Al (2014), adipose tissue density such as shape, size and number varies based on species, age, nutritional status and animal anatomy.

The hypodermis layer is a barrier between the skin and bone (Mescher, 2012; Junqueira and Carneiro, 2007). This layer serves as a physical protection when there is mechanical stress, helps maintain body temperature, a place to store energy, thermal regulation and mobilizes skin obstruction (Karundeng et al., 2014; Mescher, 2012).

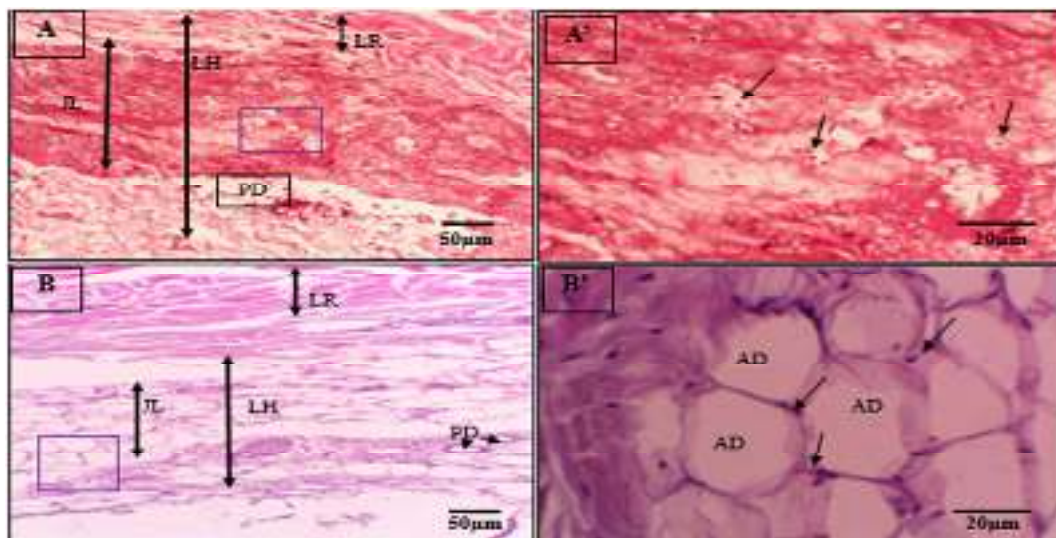


Figure 6. Histological description of the hypodermic layer of the Sumba Ongole cattle and Bali cattle in the Nuchalis region. A, A' = Sumba ongole cattle, B, B' = Bali cattle. Reticular layer (LR), Hypodermis layer (LH), Fat tissue (JL), Adipose (AD), and blood vessels (PD), Adipose cell nucleus (arrows). HE 10 × (A, B) and 40 × (A', B').

### Distribution of sweat glands on the skin of Sumba Ongole (*Bos indicus*) and Bali cattle (*Bos sondaicus*).

The sweat glands in the dermis layer of the skin of Sumba ongole and Bali cattle are distributed between the papillary and reticular layers and are directly related to the hair follicles. The sweat glands are tubular in shape and are embedded in the dermis and are lined with cuboidal cells. The sweat glands observed in the skin of Sumba ongole and Bali cattle are mostly types of merocrine glands. The sweat glands also show myoepithelial cells. According to Monteiro (2007), myoepithelial cells help secrete into the mouth of the hair follicle duct. Microscopic image of sweat glands in Sumba Ongole and Bali cattle is shown in Figure 7.

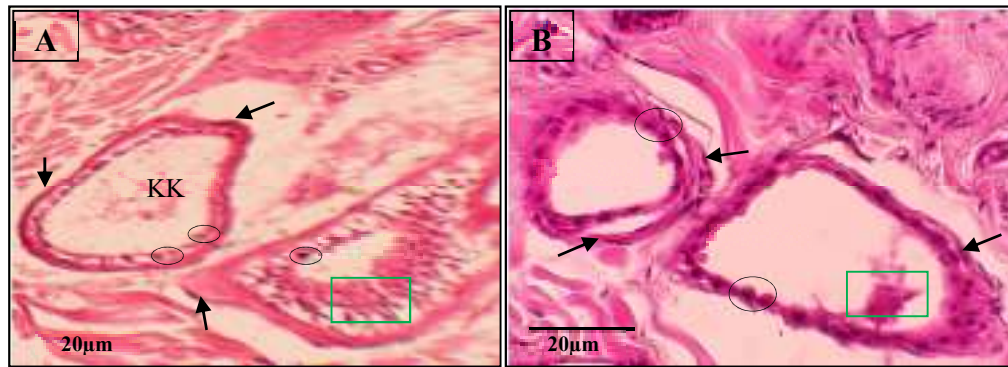


Figure 7. Histological structure of the dorsalis superficial fascia region sweat gland. A = Sumba ongole cow, B = Bali cow. Sweat glands (KK), cuboidal cells (circles), myoepithelial cells (black arrows), and secretory sweat glands (green zone). HE, 40x

Sweat glands are classified into two types namely apocrine and merocrine. The differences between the two glands are based on their distribution, function and structure (Mescher, 2012). Merocrine glands are mostly found in areas where hair is grown and apocrine glands are more sparse and hair-free. The most obvious histological difference between the two types of sweat glands is that the apocrine lumen is larger, and in merocrine, there is a secretory gland consisting of a layer of eosinophil cuboidal cells with a number of secretory granules undergoing exocytosis.

The mean distribution of sweat glands in Sumba Ongole and Bali cows in the nuchalis region and the dorsalis superficial fascia region can be seen in Table 1.

No	Cow Species	Regio	
		Neck ( <i>Nuchalis</i> )/mm <sup>2</sup>	Back ( <i>Fascia superficial dorsalis</i> )/mm <sup>2</sup>
1.	Sumba Ongole ( <i>Boss indicus</i> )	13,33 ± 5,13	10,66 ± 2,51
2.	Bali ( <i>Boss soindaicus</i> )	9,66 ± 3,21	13,66 ± 4,16

The difference in the number of sweat glands in Sumba Ongole and Bali cattle is thought to be caused by different races, species, sex, anatomical regions and climatic conditions. According to Rohankar et al., (2018), the number of sweat glands will make it easier for animals to maintain the body's homeostatic status.

Sweating is a physiological response to an increase in body temperature during physical activity or thermal stress in animals and humans and is the most



effective way to regulate body temperature (Mescher, 2012). The sweat glands consist of water and salt, so that in the event of metabolic instability in the body due to the sun's heat, the secretion of the sweat glands will play a role in regulating the degree of the animal's body temperature.

The sweat glands in Sumba Ongole and Bali cattle are numerous and large in size, aiming to regulate body temperature and allow it to withstand heat and cold due to the monsoons on dry land islands. When compared with the sweat glands in buffaloes, (Alsodany et., Al 2019) the sweat glands are limited, single and small. This affects the behavior of buffalo prefer to beetle in water. Water has the ability to absorb heat from the animal's body. The hair on the buffalo body is small in number, which functions to help exchange between water and the animal's body (Umeri and Mamoori, 2016; Samuelson, 2007).

The histological structure of the skin of Sumba ongole and Bali cattle has structural similarities with other mammals, namely consisting of the epidermis layer, the dermis layer and the hypodermis. The sebaceous glands in Sumba Ongole cattle are of the uni-lobular type and in Bali cattle are of the multi-lobular type. Hair follicles, sebaceous glands and oil glands are connected to one another. The sebaceous glands have ducts that lead to the hair follicles, while the sweat glands have myoepithelial cells that are petrified in glandular secretions through the glandular ducts and excreted through the follicles to the surface of the hair. Fat finger density depends on nutritional status, age, sex, and differences in season conditions in the animal's habitat. The mean number of sweat glands of Sumba ongole cattle in the neck region of nuchalis is higher than that of Bali cattle, while in the dorsal region of the superficial dorsalis, the average number of sweat glands in Bali cattle is higher than that of Sumba Ongole. The difference in the number of sweat in the number of sweat glands in Sumba Ongole and Bali cattle is thought to be caused by different races, species, sex, anatomical regions and climatic conditions.

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**STUDY OF MULTIDRUG RESISTANCE (MDR) *E. coli* ON PET DOGS  
IN KUPANG CITY**

**Novalino H.G. Kallau<sup>1\*</sup>, Maxs U.E. Sanam<sup>1</sup>, Tri Utami<sup>2</sup>, Yeremia Y.  
Sitompul<sup>2</sup>**

<sup>1</sup>Department of Animal Diseases Sciences and Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Nusa Cendana, Kupang, East Nusa Tenggara, Indonesia

<sup>2</sup>Department of Veterinary Clinical, Reproduction, Pathology and Nutrition, Veterinary Medicine Faculty, Nusa Cendana University, Kupang city, East Nusa Tenggara

\*Corresponding author: novalino.kallau@staf.undana.ac.id

The health threat from antimicrobial resistance is escalated, not only to animals, but also human. The resistant bacteria can live and spread across the animal population and the community. This situation may be exacerbated when the interaction between human and pet dogs is increasing. This study aimed to detect the incidence of multidrug resistance (MDR) *Escherichia coli* in pet dogs around Kupang City. This research was a cross sectional study by taking samples of rectal swabs from the dogs, then the bacteria were isolated, identified and tested for antibiotic sensitivity. The results of the study showed that from 57 samples of domestic dog rectal swabs, there were 37 positive samples of *E. coli* (64.9%), and 13 samples of those were MDR (35%). The order of antibiotics from the highest to the lowest level of resistance is Ampicillin, Cephalotin, Erythromycin, clavulanic acid Amoxicillin and several other antibiotics in a low percentage. Based on the results, it showed that the MDR *E. coli* is at a point that must be concerned and control measures need to be implemented to control the health threats that may happen in the future.

### INTRODUCTION

The health threat that comes from the emergence of antibiotic-resistant bacteria has increasingly attacked not only animals but also humans. The presence of resistant bacteria can spread among animal populations and communities. This condition becomes more intense when the interaction relationship between domestic dogs and humans increases. Dogs are one of the most domesticated animal species and based on Gompper (2013) the worldwide dog population is around 525 million.

*Escherichia coli* is one of the bacteria that has been known to be found in dogs (as commensal bacteria) and several strains are often pathogenic by giving symptoms of diarrhea in pet dogs (Kjaergaard et.al., 2016). Efforts to control and prevent the spread of antibiotic resistant bacteria will become easier if the source



of spread or the origin of the causative agent of the resistance can be identified (Kristiningtyas *et al.* 2020). Based on this background, the study of MDR *E. coli* in domestic dogs can provide an idea of the extent to which resistance levels can be a threat to public health.

## METHODS

This research is a type of cross sectional study. Sampling of dog rectal swabs was carried out on domestic dogs carried out in 6 districts in Kupang City. Sampling was carried out by simple random sampling (simple random sampling). Data was collected through direct observation of dog fecal swab samples obtained from household dog owners in Kupang City.

The swab samples were then isolated on MacConkey Agar and EMBA media and identified by gram staining and Biochemical tests. Testing the sensitivity of *E. coli* to antibiotics with Kirby Bauer method (11 types of antibiotics produced by OXOID: Amoxicillin + Clavulanic acid, Ampicillin, Cephalotin, Oxytetracycline, Tetracycline, Sulfamethoxasol + trimethoprim, Gentamicin, Erythromycin, Ciprofloxacin, Nalidixic acid, and Cholistin sulfate) (CLSI 2017). The data obtained were then analyzed to see an overview of the prevalence of *E. coli* in the samples found, the level of resistance of *E. coli* to the 11 antibiotics used and to see the prevalence of MDR in the *E. coli* isolates found.

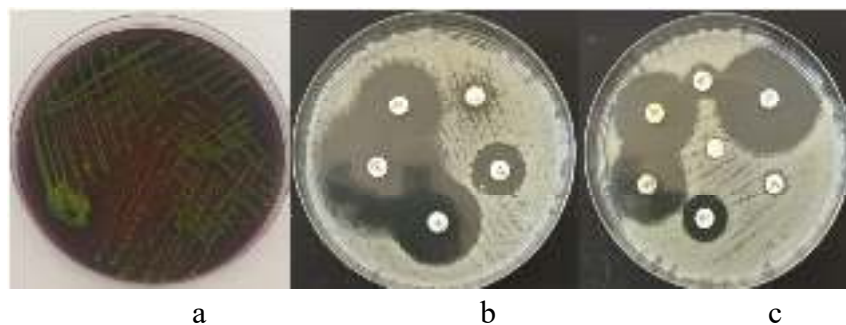


Figure 1. a. *E. coli* in EMBA media, b and c. Result of sensitivity test

## RESULT AND DISCUSSION

The research was conducted in Kupang City by taking samples of the rectal swabs of domestic dogs scattered in 6 districts. The swab samples obtained were processed to isolate and identify the *Escherichia coli* bacteria. The results of identification through staining and biochemical tests showed that 37 (65%) of the 57 samples were identified as *E. coli*. The results of the isolation of *E. coli* on EMBA media are shown in Figure 1a. This number is slightly different from that found by Kristianingtyas *et al.* (2020) with a total of 84 (100%) in several clinics in Surabaya. The results of the observations that the authors have done show that the potential for pet dogs to be infected is due to the method of caring for some dogs, namely being released, causing the dog to roam around and come into



contact with an unclean environment or from other dogs. Another possibility could come from the cleanliness of the pet owner's house. Kallau et al. (2018) show that the presence of *E. coli* is influenced by environmental temperature, hygiene and sanitation where the animal is located.

Pets can potentially spread resistant bacteria to humans and the environment (Carvalho et al. 2016). Antibiotic sensitivity test results of 37 *E. coli* positive samples tested against 11 antibiotics, showed that 10 antibiotics had been resistant with variations ranging from 1 to 7 types of antibiotics. The results of sensitivity tests for 11 antibiotics are shown in Table 1. Figure 1b and 1c showed the result of Kirby Bauer method in Mueller Hinton Agar.

Antibiotics Ampicillin (94.6%), Cephalotin (91.9%), Erythromycin (73%) and Amoxicillin + As. clavulanic (51.4%) was found to be in high levels and has the potential to be a threat to public health that needs attention. Several other antibiotics were also found to have developed resistance and the possibility to increase in the future as seen in Cholistine Sulfate (35.1%), Oxytetracycline and Gentamicin (16.2%). The antibiotics Tetracycline (10.8%), Sulfamethoxazol trimethoprim (8.1%) and Ciprofloxacin were at low levels of resistance. The interesting thing shown in this study is that nalidixic acid antibiotics have not yet experienced resistance in all tested samples. The pattern of antibiotic resistance that appears most often is shown by the Ampicillin-Cephalotin-Erythromycin (AMP-KF-E) pattern 9 times. Several intermediate patterns of *E. coli* can be seen in several antibiotics such as Amoxicillin (13%), Oxytetracycline (11%), Erythromycin (9%). This intermediate pattern can develop into resistance if the use of antibiotics in animals and humans is carried out irresponsibly. The sensitivity test data above shows that several antibiotics such as nalidixic acid (100%), ciprofloxacin (94.6%), Sulfamethoxazol Trimethoprim (91.9%) and Gentamicin still show a high level of sensitivity. This needs to be maintained in the future so that the use of antibiotics can still be optimal.

The data shown above shows that some of the antibiotics that are included in the beta-lactam class have experienced a high level of resistance to Ampicillin, Cephalotin, and Amoxicillin + Clavulanic Acid. Extended Spectrum Beta Lactamase (ESBL) is suspected to be one of the enzymes produced by *E. coli* which hydrolyzes third-generation penicillin and cephalosporin class antibiotics. WHO has also indicated that ESBL is often associated with resistance to other antibiotic classes (WHO 2014).



Table 1. Percentage of antibiotic resistance, number of resistant antibiotics in pet dog in Kupang City, Indonesia.

Types of antibiotics	Antibiotic Class	number and percentage of resistance		number and percentage of intermediates		number and percentage of susceptible	
		Σ	%	Σ	%	Σ	%
AMC	Penicillin	19	51.4	13	35.1	5	13.5
AMP		36	97.3	1	2.7	0	0.0
KF	Cephalosporins	34	91.9	2	5.4	1	2.7
OT	Tetracycline	6	16.2	11	29.7	20	54.1
TE		4	10.8	1	2.7	32	86.5
SXT	Diaminopyrimidine-Sulfonamide	3	8.1	0	0.0	34	91.9
CIP	Fluorokuinolon	1	2.7	1	2.7	35	94.6
NA		0	0,0	0	0,0	37	100.0
CN	Aminoglycosides	6	16.2	0	0.0	31	83.8
E	Makrolide	27	73.0	9	24.3	1	2.7
CT	Polymixin	13	35.1	0	0.0	24	64.9

Note: Amoxicillin+Clavulanic acid (AMC), cephalothin (KF), Oxytetracycline (OT), tetracycline (TE), trimethoprim-sulfamethoxazole (SXT), Ciprofloxacin (CIP), Nalidixic acid (NA), Streptomycin (S), Gentamicin (CN), Ciprofloxacin (CIP), Erythromycin (E), Colistine sulphate (CT).

The increased incidence of resistance to *E. coli* can be due to excessive use of this antibiotic (Byarugaba et al. 2011). The presence of *E. coli* bacteria that are resistant to erythromycin in dogs can also come from humans and the environment. The *erm* gene contained in *E. coli* bacteria resistant to the antibiotic erythromycin is responsible for this occurrence in the environment (Koike et al. 2010).

The prevalence of MDR in *E. coli* found in domestic dogs in Kupang City shows the data is 37.8% (14/37 samples). Figure 2 shows the MDR pattern in *E. coli* bacterial isolates. The incidence of MDR in pet dogs is often associated with the emergence of the ESBL enzyme (Kristianingtyas 2020) and is also associated



with the emergence of resistance to the type of Fluoroquinolone antibiotics (Liu et al. 2012). The increasing prevalence of MDR can further worsen the health conditions of humans and animals. Continued efforts should be made to prevent and control it by regularly carrying out antibiotic sensitivity tests (Quinteros et al. 2003).

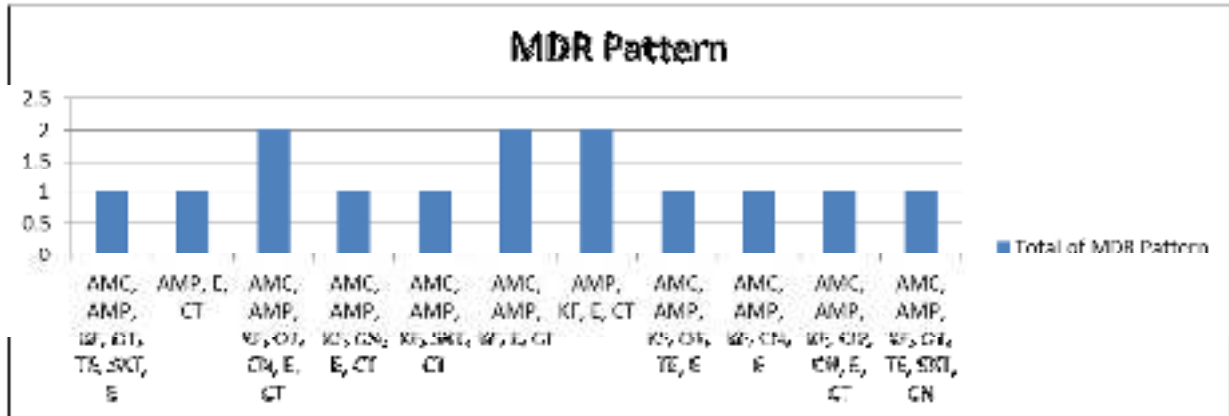


Figure 2. Amoxicillin+Clavulanic acid (AMC), cephalothin (KF), Oxytetracycline (OT), tetracycline (TE), trimethoprim-sulfamethoxazole (SXT), Ciprofloxacin (CIP), Nalidixic acid (NA), Streptomycin (S), Gentamicin (CN), Ciprofloxacin (CIP), Erythromycin (E), Colistine sulphate (CT).

### CONCLUSIONS

In conclusion, this study showed an *E. coli* prevalence of 65% of the total sample rectal swabs obtained. The incidence of resistance in a high level occurred in several antibiotics such as the Antibiotic Ampicillin (94.6%), Cephalotin (91.9%), Erythromycin (73%) and Amoxicillin + As. clavulanic (51.4%) among other antibiotics in a low percentage of resistance. The prevalence of MDR shows a value of 37.8%. These data indicate that the danger of resistance is a potential threat to animal and human health in the future if the use of antibiotics is not used wisely and efforts to improve hygiene and good sanitation are necessary to prevent the spread of resistant bacteria. This survey needs to be carried out continuously to monitor the development of resistance that occurs in animals.

### ACKNOWLEDGEMENTS

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**THE EFFECT OF BITTERMELON (*Momordica charantia* L) FRUITS  
INFUSION INTAKE ON HEPATIC SERUM LEVEL**

**Cynthia Dewi Gaina<sup>1\*</sup>, Nemay Anggadewi Ndaong<sup>2</sup>, Agustina Dwi Jayanti<sup>3</sup>,  
Agung Budiyanto<sup>4</sup>**

<sup>1</sup>Laboratory of Clinic, Reproduction, Pathology and Nutrition, FKH UNDANA

<sup>2</sup>Laboratory of Anatomy, Pharmacology and Biochemistry, FKH UNDANA

<sup>3</sup>Laboratory of Anatomy, Pharmacology and Biochemistry, FKH UGM

<sup>4</sup>Laboratory of Clinic, Reproduction, Pathology, FKH UGM

\*Corresponding author: cynthia.gaina@staf.undana.ac.id

Research on the effect of bitter melon (*Momordica charantia* L) infusion on male Wistar rats on SGPT levels has been conducted. This research aims to identify the effect of bitter melon infusion intake for 30 days on liver function damage. The study used 24 male rats divided into 4 groups, the normal group (sterile distilled water), the first dose test group (1250 mg / kg bw), the second dose test group (2500g / kg bw) and the third test group (5000mg / bw). The blood sample was obtained from the orbital sinus. The infusion was orally administered once a day for 30 days. The results showed that the level of SGPT was not statistically significant different ( $p>0.05$ ) compared to the other treatment groups, which had been given doses of 1250mg / kg , 2500mg / kg bw and 5000 mg / kg bw which start on day-1. Day-7, day-14 and day-30. This shows that in each dose group, the infusion did not cause liver damage which correlated with an increase in SGPT levels. This shows that the effect of bitter melon infusion up to 5000 mg/kg BW has no effect on the test animals.

### INTRODUCTION

Bittermelon fruit plant is an alternative herbal ingredient that has been shown to be beneficial in reducing testicular function in Sprague-Dawley rats (Yama et al, 2011). Bittermellon fruits is one of the plants from the cucurbitaceae group which is proven to be able to inhibit the spermatogenesis process as a result of the active ingredient contained in it, namely kukurbitasin B which is a triterpenoid group and has a bitter taste (Odosuga et al 2014; Ilyas, 2004). The bitter taste of bitter melon fruit is caused by the content of Cucurbitacin (momordicosides K and L), which can inhibit cell growth and development (West, et al. 2000). Cucurbitacin, which is classified as triterpene glycosides has the basic structure of cyclopentanoperhydrophenanthrene which is also owned by steroids which acts as an inhibitor of spermatogenesis and is reversible (Robinson, 1995). It is reported that the use of bitter melon as an alternative to the castration method cause liver function damage. Therefore, a study was conducted to determine the safety level of bitter melon infusion through sub chronic toxicity tests on male



Wistar rats against SGPT levels. The research objective was to determine the effect of giving bitter melon infusion for 30 days on SGPT levels in Wistar rats.

### METHODOLOGY

The materials used in the study were bitter melon fruit, sterile distilled water, filter paper, physiological NaCl, 96% ethanol and rat diet. The tools used in this study were mouse cages, food and drink containers, gavage, analytical scales, beaker glass, object glass, cover glass, gloves and infusion pans.

The bitter melon fruit in the study was obtained from a local market in the city of Kupang. The bitter melon fruit was cut into small pieces and blended, put in an infusion jar, 100 ml of aquades are added, then heated over the hands of water for 15 minutes calculated from the temperature starting to reach 90<sup>0</sup>C, then it is occasionally stirred and sprinkled while pans through flannel cloth (DepKes RI, 1995).

Before the experiment, Wistar rats were adapted for 1 week to observe their health by weighing their body and observing their normal behavior. The 24 healthy mice used in this study weighed 100-200 grams. These mice were obtained from the Pharmacology Laboratory of Brawijaya University, Malang. Determination of the number of samples used using the Ferderer formula  $(n-1) (t-1) > 15$ ; with t: number of groups and n: minimum number of samples per treatment.

Blood was drawn from the orbital sinus of the eye with 1cc hematocrit and collected in an Eppendorf tube, then serum was taken for further examination. After that, read the SGPT activity with the reagent Kit. From the research data, statistical analysis was carried out using ANOVA.

### RESULTS & DISCUSSIONS

In this study, rats and bean goats were divided into 4 treatment groups with details of the control group, test group I 1250 mg / kg bw, test group II 2500 mg / kg bw and test group III 5000 mg / kg bw. The infusion dose of bitter melon used for mice was then converted to the dose of goat. In this study, there were 24 experimental rats and 12 goats. In this study, the observation of SGPT images from 24 mice was carried out. The results can be seen in table 1.

Group	SGPT (Mean ± SD (U/L) day-		
	7	14	28
Control	79,40±13,29	47,76±10,72	71,1±10,31
Dose 1250mg/kg bw	81,50±2,2,5	75,4±8,01	67,06±6,24
Dose 2500mg/kg bw	86,90±1,00	63,94±6,08	62,28±6,14
Dose 5000mg/kg bw	77,16±6,18	54,57±2,77	56,73±9,97





Serum Glutamic Pyruvic Transminase (SGPT) is an intracellular enzyme that normally must be in cells. SGPT is a type of transaminase enzyme that catalyzes chemical reactions that occur in cells. Transaminase enzymes or also called aminotransferase enzymes are enzymes that catalyze the transamination reaction. If there are toxic compounds in liver cells, there will be a change in the permeability of the cell membrane, which will cause the enzymes in the cells to come out of the cells and be in the blood. SGPT examination is an indicator that is more sensitive to liver damage than AST. This is because the main source of the GPT enzyme is in the liver, while the GOT enzyme is found in many tissues, especially the heart, skeletal muscle, kidneys and brain (Cahyono 2009). The description of SGPT activity in test animals that was administered bitter melon fruit infusion was not statistically significant different ( $p>0.05$ ) from the other treatment groups, which had been given doses of 1250mg / kg bw, 2500mg / kg bw and 5000 mg / kg bw on day 7, day 14 and 30t day. This result showed that that each dose group did not cause liver damage because there was not an increasing in SGPT levels. This shows that the effect of bitter melon infusion up to 5000 mg / kg bw has no effect on the test animals.

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## STUDY ON THE DESIGN AND STRUCTURE OF TRADITIONAL CHICKEN HOUSING IN KUPANG REGENCY

Maxs Urias Ebenheizer Sanam, Aji Winarso\*

Department of Animal Diseases Sciences and Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Nusa Cendana, Kupang, East Nusa Tenggara, Indonesia

\*Corresponding author: [ajiwinarso@staf.undana.ac.id](mailto:ajiwinarso@staf.undana.ac.id)

One factor that also influences the success of chicken farming is housing. This study aims to describe the design and structure of traditional housing based on local materials in native chicken farms in Kupang Regency. The research was carried out through interviews with breeders and observations of native chicken cages made by a group of native chicken breeders in Camplong II Village, Fatuleu Subdistrict, Kupang Regency. The results showed that the semi-intensive cage was made as a slatted cage (30-40 cm from the ground), measuring about 2.5m x 1m x 1.5 m (length x width x height), with the feet not planted in the ground for easy movement. The cage can be divided into two or three spaces. Cages are made of materials in the form of logs for poles, gewang (*Corypha utan* Lamarck) leaf stalks for walls, ceilings, and floors. Cage roofs are made of lontar/palm leaves (*Borassus flabellifer* Linn). A variety of materials such as bamboo and wooden planks can also be found. The arrangement of gewang leaf stalks on the wall is made not too tight (2-3 cm apart) so that it provides good ventilation and adequate lighting. Likewise, making floors, by providing a gap so that droplets can fall to the ground. Palm leaves roofs provide protection from extreme heat during dry season and rain during wet season.

### INTRODUCTION

The prospect of developing native chicken farms today is quite good. There is a trend of increasing demand for free-range chicken products, for example people's preference for the taste of native chicken meat and eggs. More and more restaurants also need a supply of native chicken meat (Suprijatna 2010). Therefore, the development of native chicken farms in the community can be a tool to alleviate poverty and malnutrition.

In general, native chickens are raised by people in rural areas with an extensive traditional system, with a minimal role of breeders. However, to increase production, chickens are now being raised semi-intensively. Chickens are housed at night or when the weather is bad and released during the day or when the weather is good so that the chickens roam around, also the farmers give regular feeding (Suprijatna 2010).



Along with semi-intensive chicken farming system, the existence of an adequate housing has become increasingly important. A good housing will determine the success of a native chicken farm. This study aims to describe the design and structure of traditional housing based on local materials in semi-intensive native chicken farms in Kupang Regency.

## METHODS

The research was carried out through interviews with breeders and observations of native chicken cages made by a group of native chicken breeders in Camplong II Village, Fatuleu Subdistrict, Kupang Regency.

## RESULTS AND DISCUSSION

Farmers need to pay attention to the housing design, its size and the materials (Astiningsih, 2004). A good cage can at least provide adequate space, lighting, ventilation, and protection (from extreme weather, predators, and thieves) (Sonaiya & Swan, 2004). Housing design should also facilitate worker activities (Nadzir et al., 2015)

The results showed that the semi-intensive chicken housing was made as a slatted cage (30-40 cm from the ground), measuring about 2.5m x 1m x 1.5 m (length x width x height), with the feet not planted in the ground for easy movement. The cage can be divided into two or three spaces. The division of the cage into a small space makes it easier for breeders to reach all corners of the room by hand.



Figure 1. Semi-intensive native chicken housing in Camplong II Village based on local materials

Cages are made of materials in the form of logs for poles, gewang (*Coryphatan* Lamarck) leaf stalks for walls, ceilings, and floors. A variety of materials such as bamboo and wooden planks can also be found. These materials are strong enough to protect chicken from predators.

Cage roofs are made of lontar/palm leaves (*Borassus flabellifer* Linn). Palm leaves roofs provide protection from extreme heat during dry season and rain during wet season. Roof material affects the temperature level in the cage



(Astiningsih, 2004). Roof materials made of natural materials such as coconut leaves provide a cooler temperature (Nadzir et al. 2015) and are comfortable than tin roof (Astiningsih, 2004).

The arrangement of gewang leaf stalks on the wall is made not too tight (2-3 cm apart) so that it provides good ventilation and adequate lighting. Likewise, making slatted floors, by providing a gap so that droplets can fall to the ground. The advantage of this loose floor is that the floor is always clean, and the air exchange will be even better because the floor also functions as a ventilation hole (Putri et al. 2017). Since the cleanliness of the floor is maintained, it can reduce the risk of parasite infection. However, it should be noted that the distance between the floors so that the chicken feet are not caught on the floor, especially young chickens (Nadzir et al. 2015).

## CONCLUSIONS

Semi-intensive chicken housing in Camplong II Village is good enough to provide basic requirements of native chicken housing.

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**COMMUNITY EMPOWERMENT IN SUPPORTING LIVESTOCK-BASED AGRO-TOURISM, NGGORANG VILLAGE, WEST MANGGARAI**

**Annytha Detha<sup>1\*</sup>, Nemay Ndaong<sup>2</sup>, Nancy Foeh<sup>3</sup>, Grace Maranatha<sup>4</sup>, Frans Umbu Datta<sup>2</sup>**

<sup>1</sup>Department of Animal Diseases and Veterinary Public Health, Faculty of Veterinary Medicine, Nusa Cendana University, Kupang, Indonesia

<sup>2</sup>Division of pharmacology and biochemistry, Veterinary Faculty, Nusa Cendana University

<sup>3</sup>Division of Animal Reproduction, Veterinary Faculty, Nusa Cendana University

<sup>4</sup>Faculty of animal husbandry, Nusa Cendana University, Kupang, Indonesia

\*Corresponding author: detha.air@staf.undana.ac.id

The purpose of this community service activity is to support Agro-tourism of Nggorang Village, Komodo District, West Manggarai to maximize community skills in increasing the productivity of cattle farming. The type of empowerment is training in animal feed production, and the provision of supporting facilities for areas with tourism-based grazing. The approach methods offered to support the Partner Village Service Program are education and training, simulation model pilots and mentoring. Pilot model activities to increase the adoption rate of farmer groups in creating integrated grazing areas. In addition, partners and teams independently will build infrastructure facilities to support grazing land and animal health service centers. Based on the results obtained in this activity, there is an increase in community skills in implementing grass-based feed making, and an increase in understanding of the benefits and improvement of grazing land functions that adopt the concept of livestock-based tourism by building livestock supporting facilities.

### INTRODUCTION

West Manggarai is a district located in the western region of East Nusa Tenggara Province which has very promising livestock and agriculture potential. Based on West Manggarai Food Production Data, the main agricultural crops are Rice and Corn Production. Based on the previous study, it was stated that Nggorang has the potential for animal feed and has the potential to develop livestock-based tourism areas (Detha et al., 2020; Detha et al., 2019; Ndaong et al., 2019). Nggorang Village is an ideal area for livestock development and utilization of agricultural waste because it is supported by several factors, including existing water sources that can meet community water needs throughout the year and agroecosystems which are still dominated by grasslands.



Nggorang Village also has high potential for livestock resources, especially cattle and buffalo, and the potential of these resources is inseparable from the farming system that is inseparable from the life of farmers, as well as the carrying capacity of food availability, the availability of forage from pasture, rice fields and grass around the agricultural area and agricultural waste (straw) is quite high. Natural resources in Nggorang Village can be a source of income that can improve the economy of the village community. Because knowledge about the use of local food and the availability of livestock and land owned based on the downstream results of the research that has been produced can significantly increase the capacity of Nggorang Village through partner farmer groups (Detha et al., 2019; Detha et al., 2018; Foeh et al., 2019). This activity is expected to optimize livestock productivity through the independence and adequacy of animal feed, increasing the number of births, increasing the beauty of the area through the use of livestock waste which has a significant impact on improving the quality of life of the people in Nggorang Village. Village, Komodo District, West Manggarai, NTT.

## METHODS

The approach method offered to support the intended Partner Village Service Program is in the form of education and training accompanied by making a pilot model and mentoring and monitoring evaluation. Education and training focuses on the technique of making feed from local resources and techniques for making ammonia by utilizing abundant forage during the rainy season, so it is hoped that breeders can apply this properly to be able to meet feed needs and no longer raise livestock. Pilot model activities are aimed at increasing the adoption rate of farmer groups in making ammonia feed that comes from local resources owned by breeders or easily obtained by breeders. This pilot model is carried out in a pilot cage made in the farmer's land.

In this training and education, natural fermentation material will be introduced as a downstream from the results of the Research Dedicated Team that has been produced so that it can be useful for farmers or breeders (Datta et al., 2019; Foeh et al., 2020). Consistent assistance activities to ensure the sustainability of the program. This activity will be carried out starting from the preparation and implementation of the Ammonia demonstration plot activities so that the amount of production costs and profits obtained by partner traders can be found. Pilot model activities to increase the adoption rate of farmer groups in creating integrated grazing areas, independently partners and teams will build grazing sites that can provide multiple benefits or functions, namely as grazing land and animal health service centers (Detha et al., 2018, 2020).



## RESULTS AND DISCUSSION

This training focuses on techniques for making feed from local resources and techniques for making ammonia by utilizing abundant forage during the rainy season, so it is hoped that breeders can apply this properly to meet feed needs and no longer raise livestock. Pilot model activities are aimed at increasing the adoption rate of farmer groups in making ammonia feed that comes from local resources owned by breeders or easily obtained by breeders. This pilot model is carried out in a pilot cage made in the farmer's land. Consistent assistance activities to ensure the sustainability of the program. This activity will be carried out starting from the preparation and implementation of the Ammonia demonstration plot activities. Pilot model activities to increase the adoption rate of farmer groups in creating integrated grazing areas, independently partners and teams will build grazing sites that can provide multiple benefits or functions, namely as grazing land and animal health service centers. This pilot grazing area consists of a clamp cage, a water storage tank, and a feed manufacturing tank.



Figure 1. Ammonia and Silage storage tanks



Figure 2. Water storage tank

SUPPORTED BY





Figure 3. Location of construction of the clamp cage

### CONCLUSION

Based on the results obtained in this activity there is an increase in community skills in implementing grass-based feed making, and an increase in understanding of the benefits and improvement of grazing land functions that adopt the concept of livestock-based tourism with the construction of livestock supporting facilities.

### ACKNOWLEDGMENTS

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**THE RED BLOOD CELL DISTRIBUTION WIDTH IN GOATS (*Capra hircus*) WITH MORINGA OLEIFERA FEED SUPPLEMENTATION**

**Nemay Ndaong<sup>1\*</sup>, Desmon Hurek<sup>1</sup>, Annytha Detha<sup>2</sup>, Frans Umbu Datta<sup>1</sup>,  
Nancy Foeh<sup>3</sup>**

<sup>1</sup>Anatomy, Physiology, Pharmacology and Biochemistry Laboratory, Faculty  
Veterinary Medicine, Nusa Cendana University

<sup>2</sup>Laboratory of Veterinary Disease and Veterinary Public Health, Faculty of  
Veterinary Medicine, Nusa Cendana University, Kupang

<sup>3</sup>Clinical, Reproductive, Pathology and Nutrition Laboratory, Faculty of  
Veterinary Medicine, Nusa Cendana University, Kupang

\*Corresponding author: [nemayndanong@gmail.com](mailto:nemayndanong@gmail.com)

The red blood cell distribution width (RDW), as part of an automated complete blood count (CBC), is a routinely available parameter on hematology analyzers. This parameter is the most commonly reported index of the variation in red cell volume and can be used to detect subtle degrees of anisocytosis and anemia. This study was to determine the effect of moringa oleifera feed supplementation in goats (*Capra Hircus*). The study used 16 female goats (*Capra hircus*), aged 6 months with a bodyweight of  $\pm$  10-12 kg. The female goats were divided into 2 treatment groups with 3 replications, namely the first group was given ammoniated, concentrated; the second group was given ammoniated, concentrated, supplementation moringa flour. The treatment for each goat is given for 50 days. Before the treatment of goats, preparations for tools and materials are needed in the study as well as processes such as weighing, production of Moringa oleifera flour, making concentrates, making ammoniation, making moringa flour, adapting to goats. Blood sampling is done on day 0 (H0), day 25 (H25), and day 50 (H50). For each withdrawal,  $\pm$  3 mL of blood is drawn and then put into Ethylenediamine Tetraacetic Acid (EDTA) tubes. Blood smear examination of both treatment groups shows poikilocytosis and anisocytosis. Could be concluded that the values of the red blood cell profile of female goats on the two treatment groups did not differ statistically, this means the use of Moringa flour had no significant effect on the red blood cells.

### INTRODUCTION

The red blood cell distribution width (RDW) is a simple, and inexpensive parameter, which reflects the degree of heterogeneity of erythrocyte volume (conventionally known as anisocytosis), and is traditionally used in laboratory hematology for differential diagnosis of anemias based on MCV and RDW (Evans & Jehle, 1991). Anemic conditions are caused by the physiological status of livestock (pregnant, lactation), disease, or nutritional deficiencies. Nutritional



deficiencies result in reduced production of red blood cells so that the nutrients delivered by the blood to tissues and cells low, disturbance in the physiology of livestock and also affects the image of red blood cells. The increased RDW reflects the deregulation of erythrocyte homeostasis involving impaired erythropoiesis and abnormal red blood cell survival, which can be associated with various underlying metabolic disorders such as shortening of telomere length, oxidative stress, inflammation, nutritional status, hypertension, erythrocyte fragmentation, and changes in erythropoietin function (Salvagno et al., 2015).

Moringa oleifera plant is a multipurpose plant, with nutrient-dense content and pharmacological effects. Moringa is used as animal feed and provides positive results for livestock health (Dosom et al., 2018). Moringa is known to contain 46 powerful anti-oxidants that protect the body from free radicals, contains 18 amino acids (8 of which are essential) that the body needs to regenerate new cells, 36 anti-inflammatory compounds, and 90 natural nutrients such as vitamins and minerals (Krisnadi, 2015). The benefits and properties of the Moringa oleifera plant are found in all plant strains, including leaves, stems, roots, and seeds (Am et al., 2015). The nutrient content is high enough to make moringa have functional properties for health and overcome nutritional deficiencies. Therefore this study aims to determine the effect of Moringa flour supplementation in goats (*Capra hircus*) based on RDW.

### MATERIALS AND METHODS

The study included female goats (*Capra hircus*), ranging between 5 and 6 monthss of age. They were non-pregnant dry goats. All animals were well fed, clinically healthy, and free of internal and external parasites. They were treated for endoparasite control and their health status was regularly monitored by veterinarians. The goats were divided into 2 groups: 16 goats (8 goats each groups) with a body weight of 10-12 kg (group P1 and P2). Table 1. Treatments to each Goat (*Capra hircus*).

Treatment	Amount of feed (gr/head/day)					TOTAL
	Moringa Flour	Corn Flour	Bran Flour	Fish Flour	Minerals	
(P1)	-	54	54	72	1	180
(P2)	36	54	54	36	1	180

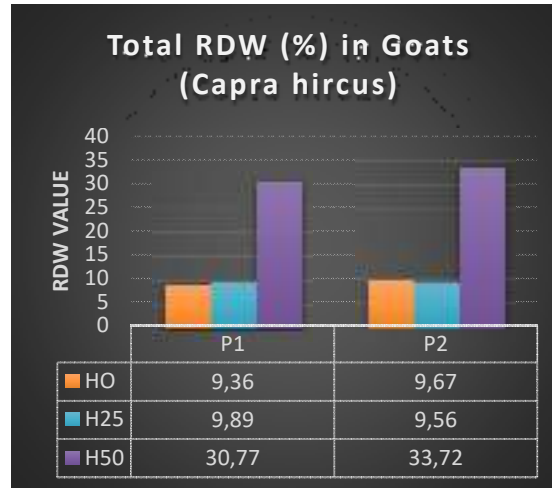
All goats were sampled three times (D0, D25, D50) blood samples were taken from each animal every morning (starting at 08.00 and ending at 11.00) by stabbing the jugular vein with a 22 G needle into a vacutainer tube: blood collected as much as 3 mL. Determination of the RDW value using a Hematology analyzer (MEK-8222J / K CELLTA-F). Erythrocyte appearance with blood smear



preparations. The results in the form of erythrocytes morphological images were analyzed descriptively and quantitative data were analyzed using the T-test.

## RESULTS

The results of the examination of the RDW value of goats (*Capra hircus*) obtained the P1 range, namely 9.36-30.77% and P2, namely 9.67-33.72%. The results of checking the RDW values are presented in diagram 1.



The results of the analysis with the independent T-test, day 0 seen from the sig value (2-tailed) is  $0.8 > 0.05$ , day 25 is  $0.6 > 0.05$ , day 50 is  $0.5 > 0.05$ . These results indicate that statistically giving Moringa flour supplementation in the feed has no significant effect on the RDW value. The results of the examination of the RDW value in goats (*Capra hircus*) in Indonesia, have a lower value than the Damascus goat (31.35%), Barbara goat (34.05%), BlackArabi goat (34.45%), WhiteArabi goat (35, 25%) (Samira et al., 2016).

A blood film or peripheral blood smear is a thin layer of blood smeared on a microscope slide. The peripheral blood smear is usually examined to investigate hematological problems. the proportion of blood- count samples that require a blood smear. Nevertheless, blood smear remains a crucial diagnostic aid. From the peripheral blood smear, we can examine the number, anemia, erythrocyte morphology, different leucocyte, and other abnormalities (Shagana, 2014). Goat have an erythrocyte size of 3.2-4.2 $\mu$ m with a life span of 125 days (Adili et al., 2016). Figure 1. Peripheral Blood smear of goat (*Capra hircus*)

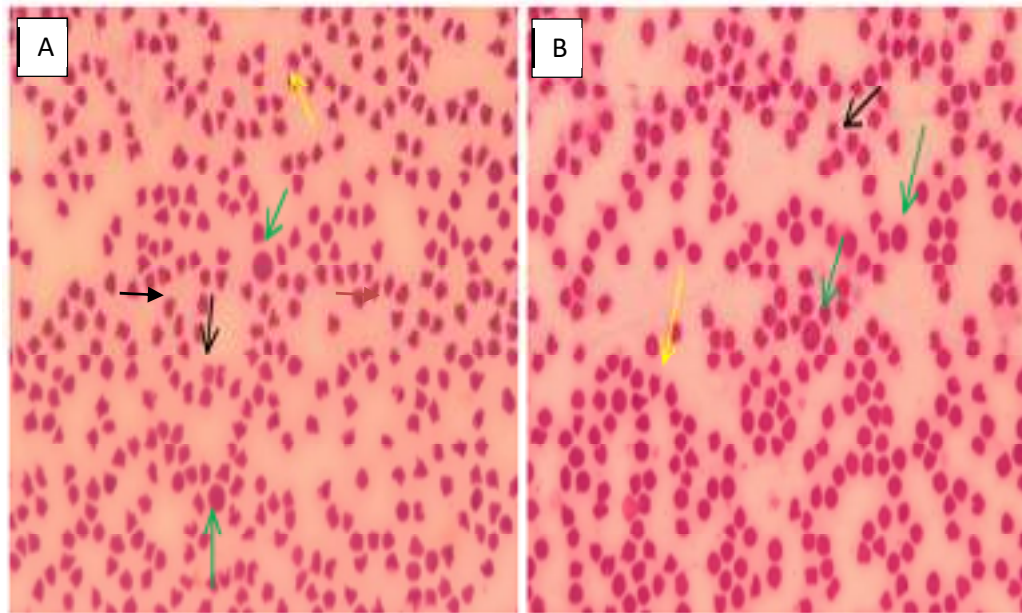


Figure 1: ( ) Acantocit, : ( ) ecinosit, : (→) Makrositik

The observation result of goat blood smear in Figure 1. shows that there are variations in normochromic and anisocytosis variations of poikilocytosis (acanthocytes and ecinocytes) and macrocytic. Anisocytosis refers to increased heterogeneity above what is considered "normal" (Evans & Jehle, 1991). Acanthocytes are cells that are shaped like spines with blunt ends. Acanthocytes are caused by changes in cholesterol and phospholipid concentrations in red blood cell membranes and often occur in young goats and heifers, whereas ecinocytes are erythrocytes that have small sizes or fragmentocytes that have one or more spines on the surface of erythrocytes which are formed when erythrocytes are dehydrated pH increases, and intracellular calcium increases (Ford, 2013).

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**SAMBILOTO AND CURCUMIN ADMINISTRATION AS  
COMBINATION THERAPY FOR THE TREATMENT OF MALARIA IN  
BERGHEI PLASMODIUM INFECTED MICE**

**Anita Lidesna Shinta Amat<sup>1\*</sup>, Aty Widyawaruyanti<sup>2</sup>, Ahmad Fuad Hafid<sup>2</sup>**

<sup>1</sup>Medical Faculty of Universitas Nusa Cendana

<sup>2</sup>Pharmacy Faculty of Universitas Airlangga

\*Corresponding author: anita\_amat@staf.undana.ac.id

Combination therapy is the method of overcoming the global challenge of drug-resistant malaria. Three models of combination therapy of ethanolic extract 80% sambiloto (*Andrographis paniculata* Nees.) and curcumin were evaluated *in vivo* for potentiation against mice infected *Plasmodium berghei* by oral route. A five-day dosage (D0-D4) of combination ethanolic extract 80% sambiloto 100 mg/kg body weight and curcumin 100 mg/kg (I model) produce inhibition of parasite's growth for 25,99% and survival monitored for 11-13 days. A five-day dosage (D0-D4) of combination ethanolic extract 80% sambiloto 100 mg/kg body weight and curcumin 10 mg/kg (II model) produce inhibition of parasite's growth for 23,83% and survival monitored for 9-12 days. The third combination is ethanolic extract 80% sambiloto 100 mg/kg body weight and curcumin 1 mg/kg (III model) produce inhibition of parasite's growth for 9,03% and survival monitored for 9-11 days.

### INTRODUCTION

Malaria is still a health problem in many countries. Indonesia still has 5 malaria endemic areas, one of which is East Nusa Tenggara. The resistance to standard drugs encourages the search for new antimalarial alternatives. One of them is therapy derived from natural ingredients in the form of plants with anti-malarial medicinal properties. The goals of this therapy are to increase therapeutic effectiveness, reduce toxicity and reduce resistance.

Previous research stated that andrographolide as the main ingredient in the bitter plant can provide antimalarial activity *in vitro* and *in vivo*. In addition, it was also known that ethyl acetate sambiloto fraction had better antimalarial activity compared to andrographolide isolates.

Another plant that is widely used as medicine is the Zingiberaceae family, especially the *Curcuma* genus. From previous research, it was stated that curcumin can inhibit the growth of parasites that are resistant to chloroquine with  $IC_{50} \approx 5 \mu M$  and oral administration in the minutes of being infected with *P. berghei* at a dose of 100 mg / kg BW can reduce the level of parasitemia 80-90% and improve survival (Reddy dkk. 2005).



Sambiloto extract and curcumin both have anti-malarial properties. This allows a combination administration to be more effective than a single administration. Both have the ability to inhibit the growth of *Plasmodium berghei* parasites in vivo in mice infected with *Plasmodium berghei*. In terms of the synergistic effect of drugs, for infectious diseases there may be a decrease in resistance to drug combinations and a decrease in toxicity effects.

Combination therapy is one of the methods used to address the global challenge of malaria drug resistance. The three models of combination therapy of 80% ethanol extract of sambiloto (*Andrographis paniculata* Nees.) And curcumin given orally have been obtained in vivo to kill *Plasmodium berghei* which infects mice. This study demonstrated the antimalarial activity of a combination of 80% ethanol extract of sambiloto and curcumin.

### MATERIALS AND METHODS

Herba sambiloto (*Andrographis paniculata* Nees) was obtained from Pacet, East Java. Sambiloto herbs were extracted using the 80% ethanol extraction method as a solvent. Curcumin from Turmeric (*Curcuma longa*) uses ingredients from Sigma Chemical, St. Louis, MO. The reference material was chloroquine diphosphate at 10 mg / kg BW (Sigma C-6628, 25 grams, Lot 7740650).



Picture 1. Sambiloto Herb

The examination of antimalarial activity used the Peter Test (The 4-day suppressive test of blood schizontocidal action) in which the experimental animal was infected with *Plasmodium berghei* and then given therapy for 4 days.

The research design was divided into 3 test groups, where each test group was divided into 7 male mice *Mus musculus* BalB / c strain. The mice used were 2-3 months old and weighing 20-30 g. Age and body weight of the mice influenced the immune system in the body of the mice against *Plasmodium berghei* infection. Observation of the test results by making a thin smear of blood from the tails of mice with staining on a slide and counting the number of parasite-infected erythrocytes in 5000 erythrocytes with a 1000 times magnification microscope. Then calculated the percentage growth and percentage inhibition of each test group.

The therapy was given for 5 days (D0-D4) from a combination of 80% sambiloto ethanol extract at a dose of 100 mg / kg BW and curcumin at a dose of





100 mg / kg (model I). Furthermore, 5 days of therapy (D0-D4) a combination of 80% sambiloto ethanol extract at a dose of 100 mg / kg BW curcumin at a dose of 10 mg / kg (model II). The third combination of ethanol extract 80% sambiloto at a dose of 100 mg / kg BW and curcumin 1 mg / kg BW (model III).

## RESULT AND DISCUSSION

Antimalarial activity testing has been carried out on mice infected with *Plasmodium berghei* by using a combination of 80% ethanol extract of sambiloto and curcumin. A suspension was prepared with a dose ratio of 80% ethanol extract of 100 mg / kg BW of bitter extract and 100, 10 and 1 mg / kg of curcumin. The choice of dosage is based on the results of research by Retnowati (2007) which states that 80% ethanol extract of sambiloto at a dose of 100 mg / kg BW can inhibit parasitemia by 65.14% and in Reddy's (2005) study, curcumin at a dose of 100 mg / kg BW can inhibited 80-90% parasitemia and significantly increased the viability of mice.

The 80% ethanol extract of sambiloto and curcumin were diluted respectively first in DMSO with a concentration of 0.5% then dissolved in 0.5% CMC Na to obtain a suspension form. While chloroquine diphosphate at a dose of 10 mg / kb BW was chosen as a positive control because chloroquine diphosphate is a standard drug for the treatment of malaria in Indonesia and the use of a dose of 10 mg / kg BW is based on research conducted by Phillipson (1991) which has been able to inhibit the growth of *P. berghei*. in mice with up to 100% inhibition. In addition, negative control was also used, namely DMSO suspension 0.5% in CMC Na 0.5% to avoid false positives by the solvent and as a comparison in calculating parasite growth.

Based on the data on the growth of parasites on the Do surface, it was found that the number of erythrocytes infected with parasites was varied, possibly because one of them was the different weight of the mice causing the immune system of each mice to be different. The treatment is given after the number of infected erythrocytes reaches 1-5%.

The results showed that during treatment, from Do to D4 in each test group given a combination of 80% sambiloto ethanol extract at a dose of 100 mg / kg BW of mice with curcumin at doses of 100, 10, and 1 mg / kg BW, it was seen that the number of parasites did not decrease. however, the growth of parasites also did not increase drastically when compared to the negative control group. At D6 - D15 after the treatment was stopped for the test solution group combination of 80% sambiloto + curcumin ethanol extract in 3 kinds of doses, there was a sharp increase in parasitemia. In the group given the combination test solution of 80% sambiloto ethanol extract at a dose of 100 mg / kg BW with curcumin of 100, 10, and 1 mg / kg BW, there was an increase in parasitemia. But each test solution group has a different inhibitory value. The percentage of inhibition of D0 - D5 in the group given the test solution I (80% ethanol extract of 100 mg / kg



BW and curcumin 100 mg / kg BW) was 25.99% with an average of mice able to survive up to 11-13 days. While the group that was given the test solution II (ethanol extract 80% sambiloto 100 mg / kg BW and curcumin 10 mg / kg BW) had an inhibitory percentage of 23.83% with an average of mice able to survive up to 9-13 days. The last group of test solution III (ethanol extract 80% sambiloto 100 mg / kg BW and curcumin 1 mg / kg BW) had an inhibition percentage of 9.03% with an average of mice able to survive up to 9-12 days. When compared with the vitality of mice in the negative control, none were able to survive until the 9th day. This shows the role of curcumin in the immune system as an immunomodulator (Sharma, et al., 2005), although parasites continue to increase, mice are still able to survive. On day 10, there was an increase in the viability of test solution II compared to test solution I with a decrease in the amount of curcumin, this proves that sambiloto provides immunomodulatory activity (Hariyati, Y. 1990).

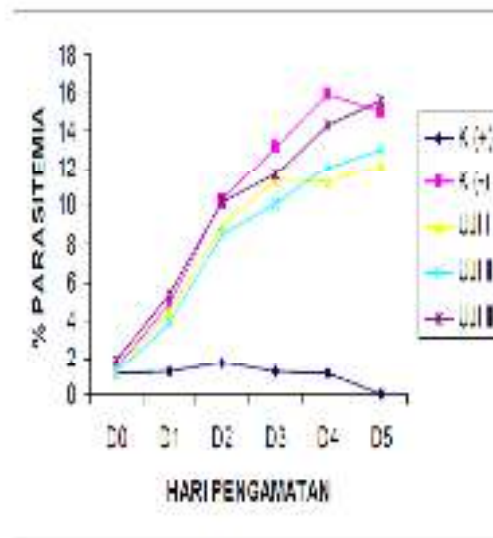
In positive control, starting from D4, there was a decrease in parasitemia to D5 and after that it increased again but not up to the percentage of parasitaemia. The average of mice that died on day 10 to 11 and the percentage of inhibition calculated from D0 - D5 was 100%. This is because chloroquine diphosphate has a very long  $t_{1/2}$  of 127 hours (Ritchel, 1986) so that when its administration is stopped there are still a number of drugs in the body for quite a long time and can provide inhibition of parasite growth.

Research by Reddy (2005), mice given curcumin 100 mg / kg BW were able to survive until the 20th day but in a combination of ethanol extract 80% sambiloto 100 mg / kg BW and curcumin 100 mg / kg BW were only able to survive between the 11th day. until the 13th day. This may be because the  $t_{1/2}$  of the 80% sambiloto ethanol extract is shorter so it cannot maintain the extract levels in the blood to prevent parasite growth. Curcumin in the mixture that is given orally undergoes metabolism in the liver and intestinal wall is converted into hexahydrocurcumin so that its bioavailability in the blood decreases and its activity is not as good as curcumin (Sharma, et al., 2005). In addition, curcumin is combined with sambiloto in the form of an extract, in which there are several compounds with certain levels that may interact with curcumin so that it can inhibit the activity of this combination as an antimalarial.

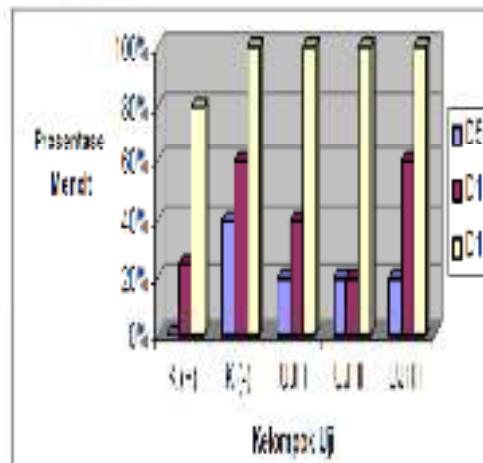
It is known that there is inhibition of parasite growth in the extract combination ethanol 80% sambiloto 100 mg / kg BW and curcumin 100, 10 and 1 mg / kg BW but not potential as an antimalarial combination therapy because the percentage of inhibition is less than 30%. Although the parasitemia in test groups I, II and III continued to increase, the mice were able to survive between day 9 and day 13. This combination therapy can be used as a supplement because it can show activity of sambiloto and curcumin as immunomodulators. In addition, it is also necessary to develop a combination therapy model of 80% sambiloto ethanol extract with other antimalarial drugs, in order to increase the antimalarial activity



produced so that it can potentially be an alternative malaria therapy and can reduce the risk of resistance to standard drugs.



Picture 2. Graphic of Parasite Mean Growth Procent from Combination Therapy of Sambiloto 80% Ethanol Extract + Control Curcumin



Picture 3. Percentage diagram of mice that died from combination therapy of 80% ethanol extract of Sambiloto + curcumin and control at D5, D10 and D15.

### CONCLUSION

This study shows that model I produces the highest parasitemia barrier among other models, namely 25.99%.

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## EMPOWERING FARMER COMMUNITY IN CAMPLONG II VILLAGE THROUGH SMALL SCALE FARMING

Cynthia Dewi Gaina\*, Frans Umbu Datta, Maxs UE Sanam, Filphin Adolfin Amalo

Faculty of Veterinary Medicine, Universitas Nusa Cendana, Kupang, Indonesia

\*Corresponding author: cynthia.gaina@staf.undana.ac.id

Farmer community empowerment in agriculture and husbandry development plays a significant role. Community empowerment in Camplong II village is expected to reduce poverty, especially among farmers. The purpose of this article is to show the village activity that is empowering local farmer community in Camplong II village through community empowerment, such as training, re-designing and monitoring. Based on the findings, the paper shows that the farmers' group empowerment strategies such as farmer's group learning and innovation have a positive influence on improving their communities.

### INTRODUCTION

Generally, agriculture sector faces is highly dependent on socio condition, economy, environment, quality and performance of their community (Cullen et al, 2007). Village farmers in Camplong II as the main actors of income sources in small family has been dealing with uncertainty and risks (Gaina *et al*, 2020).

This study aims to study the empowerment of Camplong II village community through small farming to improve better farming, it enhances farmer's life and community life that is in connection with government programs.

### METHODOLOGY

The study was carried out through interviews with farmers, observations and simple farming methods in Camplong II Village, Fatuleu Subdistrict, Kupang Regency.

### RESULTS AND DISCUSSIONS

Generally, sustainable local agricultural practices, traditional plants were used to feed livestock. However, the introduction of small farming methods assist farmer could gain more benefit to feed both family and livestock (Dlamini, 2007). A mixture of recent knowledge (Fariyanti dkk, 2007) and traditional methods was used in this study. Agricultural technology, like natural fertilizer, Bokashi from cow faeces is cheaper hence attractive to farmers and make it is useful for their community (Gaina, 2019).



This study shows that the recommendation to improve the knowledge through socialization and woman empowerment clearly proves that they still need both these methods and the challenges is knowledge gap and guidance on what to use in Camplong II Village for sustainability outcome. The most critical suggestion is to identify those methods which are better in preparing the local farmers. Thus, the capacity of the farmers should be practical and applicable.

There is a significant correlation between small farming condition and income of villages farmers (Darwis & Rusastra, 2011). Farmers need strengthening of capacity in managing local resources through social and recent appropriate technology that has prospects to develop this local community.

### CONCLUSIONS

The inclusion and implementation of small farming techniques in Camplong II village led to beneficial results. While some the people and its changes may not appear significant, these small steps were a giant leap towards villagers' empowerments in Camplong II village.

### ACKNOWLEDGMENT

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