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# Proceedings of the 2nd International Conference on Veterinary, Animal, and Environmental Sciences (ICVAES 2020)

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### Proceedings Article

## Effect of the Natural Cat and Dog Liquid Additives on 3% Salt Fed Rats

Elif Rabia Sanli, Musa Yavuz, Zafer Özyıldız, Veli Uygur

Salt poisoning could be important for the pets' life. High salt and low water consumptions can be effective in the formation of salt poisoning. Low levels of salt poisoning increase blood pressure which reduces the quality of life. Plant-origin cat and dog liquid additives are previously used and improve...

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## Sustainable Aquafeed: Alternative Ingredients Produced Locally as Nutrient Complementary in Minimizing the Use of Fishmeal

Noor Khalidah Abdul Hamid

Exploring cost-effective animal-derived protein alternatives is very important to support the growing human population with food resources globally.

Aquaculture has significant contribution to this sector by providing fish and fishery products. This article provides short review on aquafeed, one of important...

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### Proceedings Article

## Evaluation of the Situation of the People Received Service from Equine Facilities Before and During the Covid-19 Pandemic

Ayşe Kocabıyık, Fatih Şahiner, Demet Gökdere, Elif Rabia Şanlı, Musa Yavuz

The covid-19 pandemic influences human social life and their relation to animals. The equine sector is affected by covid-19 pandemic. The aim of this study was to evaluate the general situation of the people receiving service from equine facilities before and during the pandemic. The data were collected...

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### Proceedings Article

## Classical Enterotoxin Genes of *Staphylococcus aureus* Isolated from the Raw Milk of Cows and Goats in Yogyakarta Indonesia

Desy Cahya Widianingrum, Sarasati Windria, Fatkhanuddin Aziz, Siti Isrina Oktavia Salasia

Milk is a highly nutritious food containing several essential nutrients. Consequently, it can be contaminated by pathogenic bacteria, including *Staphylococcus aureus*. This study aimed to analyze the genes encoding nine classical enterotoxins of *S. aureus* isolated from cow and goat raw milk. A total of...

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### Proceedings Article

## Profile of Antigen Excretory-Secretory *Schistosoma Japonicum* in the Development of Elisa Method to Detect Schistosomiasis in Indonesia

Samarang, Made Agus Nurjana, Malonda Maksud, Sri Murtini, Fadjar Satrija

The development of ELISA method to detect patients with schistosomiasis in Indonesia is a detection of the antigens that are derived from the peripheral vessels in the blood serum. This research is conducted in the Napu Plateau of Poso Regency, Central Sulawesi, Indonesia. The goal is to develop ELISA...

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### Proceedings Article

## Rats Development of Contactless Thermal Detector for Animal: Comparison of Three Sensor Types

Ridi Arif, Koekoeh Santoso, Dhani S. Wibawa

One of the important physiological parameters is body temperature, and the increased level is an early indicator of infection. Therefore, it is necessary to develop temperature detection in animals using contactless methods and with precise results. This study aimed to compare 3 types of IR sensors for...

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#### Proceedings Article

### Biochemical Changes of Liver and Kidney After Bone Graft Implant from Black Devil Snail (*Faunus ater*) Shell Material

Erwin Erwin, T. Fadrial Karmil, Teuku Zahrial Helmi, Nuzul Asmilia, Muhammad Isa, Wahyu Eka Sari, Budianto Panjaitan, Sugito, Hathalia Y. Utari, Azzalia N. Diva, Astri Wulandari

Biochemical changes in blood can be used as an indicator in liver and kidney disorder. This study aims to discover the changes of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), creatinine and blood urea nitrogen (BUN) levels after the implant of black devil...

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#### Proceedings Article

### Efficiency of Testosterone Administration on the Performance of Day Old Chick (DOC) Layer: Cockscomb Size, T3/T4 Ratio, and Histopathological Description of Bursa Fabricius

Rizki Fitrawan Yuneldi, Claude Mona Airin, Hendry TSSG Saragih, Pudji Astuti

This study aimed to evaluate the efficiency of single and continuous testosterone injection against cockscomb size, T3/T4 ratio, and histopathological features of the bursa of Fabricius in the male layers. Sixty DOC



layers were divided into 3 groups namely control (C), single testosterone injection with...

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#### Proceedings Article

### Molecular Study of Myostatin Gene in Stallion and Mares of Sandelwood Horses in Sumba Island, East Nusa Tenggara

Cynthia Dewi Gaina, Frist B. H. Francis

Sandelwood horse is one of local horses in Indonesia that has become known for its use in horse racing competition. Many genes are known to have an effect on performances traits, but the role of one gene, Myostatin (MSTN) has not been explored yet. This gene is responsible for embryonic and adult muscle...

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#### Proceedings Article

### Conservation of Hawksbill Turtle (*Eretmochelys imbricata*) in Indonesia

#### Lessons Learned and Future Challenges

Adela Hemelikova, Pavel Zoubek, Tomas Ouhel, Awaluddin Awaluddin, Teuku Reza Ferasyi

The Hawksbill turtle (*Eretmochelys imbricata*) is a highly migratory species of subtropical and tropical areas with an important role in the ecosystem. However, the hawksbill turtles are being hunted for their shell to make jewelry and decorative products. The harvest of its tortoiseshell with a combination...

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### Proceedings Article

## Morphological Features and Molecular of *Plasmodium inui* in *Macaca fascicularis* from Bogor, West Java

Upik Kesumawati, Lis Rosmanah, Susi Soviana, Uus Saepuloh, Huda Shalahudin Darusman

Plasmodium is causative agent of malaria in human through the intermediary of female mosquitoes Anopheles spp. Two of the most important human malaria parasites, *P. falciparum* and *P. vivax*, are derived from the complete transmission event of non-human primate malaria species to human. Late data shows...

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### Proceedings Article

## The Use of *Wedelia biflora* Leaf Extract Influences the Platelet Count in Rats with Contusions

Rinidar, Muhammad Isa, Awaluddin

A contusion or bruise occurs when blood vessels are damaged or broken because of a trauma to the skin. The initial phase in responding to a contusion in the body is the entry of platelets into the wound area. This platelet infiltration functions as a hemostasis. This study aimed to determine the effectiveness...

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### Proceedings Article

## Pathological Changes of *Fasciola gigantica* After Treatment with *Nigella sativa* in Vitro

Muhammad Hambal, Alfifatul Hidayah, Ummu Balqis, Farida Athaillah, Muttaqien, Siti Rani Ayuti, Wahyu Eka Sari, Henni Vanda

Black cumin (*N. sativa*) has been traditionally used as medicine for various infectious and non-infectious diseases, such as skin infection, conjunctivitis, hypertension, gastrointestinal problems, diabetes, and diarrhea. This study aimed at finding out the effect of black cumin, *N. sativa* on the survival...

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### Proceedings Article

## Efficacy of *Jatropha* Sap (*Jatropha curcas* L.) Cream on Epithelialization Phase of Cutaneous Wound Healing in Mice Using Masson Trichrome Staining

M. Nur Salim, Ummu Balqis, Rahma Erlis, Dian Masyitha, Erdiansyah Rahmi, Abdul Harris

This study aims to determine the efficacy of 10% *Jatropha curcas* Linn. cream on the epithelialization phase of cutaneous wound healing in mice (*Mus musculus*) using Masson Trichrome staining. This study used nine male mice, aged 2-3 months old with 2 cm incision was made on the back. The mice were divided...

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### Proceedings Article

## Effect of *Veitchia merrillii* Extract on Mortality and Tegument Structure of *Fasciola gigantica*

Henni Vanda, Reza Aulia Anwar, M. Daud AK, Farida Athaillah, Ummu Balq Siti Rani Ayuti, Frengki Frengki

This study was conducted to explore the effect of methanolic extract of *Veithcia merrillii* on mortality time and histopathological changes of *Fasciola gigantica* in vitro. This study used 25 adult flukes of *F. gigantica*, divided into five groups: C0 as negative control (0.9% PBS), C1 as positive control...

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#### Proceedings Article

Descriptive Assessment of Coordination Between Veterinary and Public Health Centers in Responding Report of Potential Rabies Animal Bites at Bener Meriah, Aceh

Teuku Reza Ferasyi, Al Azhar, Erwin Erwin, Awaluddin Awaluddin, Rezky Ramadhan, Agus Nurza

A high case of potential rabid animal bites human is reported in Bener Meriah Regency of Aceh, Indonesia. The application of one health concept in controlling rabies is important at least by providing identical data of potential rabid animal bites in the veterinary (VHC) and public health (PHC) centers....

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#### Proceedings Article

Histopathological Changes in the Gills of *Oreochromis mossambicus* Exposed to Mercury Chloride (HgCl<sub>2</sub>)

Dwinna Aliza, Amalia Sutriana, Nazaruddin Nazaruddin, Teuku Armansyah, Etriwati Etriwati, Muhammad Hanafiah, Hafizuddin Hafizuddin, Denny Irmawati Hasan, Awaluddin Awaluddin, Binti Ulfa

Most of the fish deaths caused by pollutants demonstrate gills damage, since located outside the body that directly exposed to water as a medium of life, this organ is the first to be affected if pollutants contaminated water environment. The objective of this research was to find out histopathological...

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### Proceedings Article

## Anti-Inflammation Activity of Ethyl Acetate Extract of Malacca Leaves (*Phyllanthus emblica*)

Nuzul Asmilia, Teuku Armansyah, Herrialfian Herrialfian, Rinidar, Abdul Harris, Arman Sayuti, Nazaruddin, Cindy Ary Ristanti, Indriana Oktavani

This study aims to evaluate the anti-inflammation activity of ethyl acetate extract of Malacca leaf (*Phyllanthus emblica*) in mice. The study used 25 mice which were induced by subcutaneous injection of 1 % carrageenan. The mice were divided into 5 treatment groups: K1 was negative control, K2 (positive...

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### Proceedings Article

## Prevalence and Intensity of Trematode Flukes in Different Hair Color of Aceh Cattle

Lian Varis Riandi, Ryan Ferdian, Muhammad Hambal, Muttaqien Bakrie

This study aims to determine the prevalence and number of trematode eggs (*Fasciola gigantica* and *Paramphistomum* spp) in different hair color of Aceh cattle. This research used feces collected from 105 cattle in Mesjid Raya Sub-district, Aceh Besar. The feces samples were divided into 3 groups based on...

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### Proceedings Article

## Histologic and Histomorphometry of Uterine of Gayo Mare

Juli Melia, Welda R. Brilliant, Erdiansyah Rahmi, Rosmaidar Rosmaidar, Hafizuddin Hafizuddin, Amrozi Amrozi

This study was aimed at finding out the histology description and histomorphometry of uterine Gayo mare. The samples were collected from three Gayo mares in Takengon, Central Aceh. The uterine samples were processed by microtechnique and stained with hematoxylin-eosin before examined under a binocular...

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### Proceedings Article

## Ultrasound and Radiograph Mesh Hernia Graft 3D Imaging Correlation; Study in Rabbits

Nanda Yulian Syah, Akhyar Akhyar, Etriwati Etriwati, Erwin Erwin

A Mesh hernia graft is needed to prevent recurrent hernias. This study aimed to observe the biocompatibility of hernia mesh graft as an alternative material for handling hernia cases via surgery for small animals. This study used 6 male rabbits aged 6-8 months and weighted 1.5-2 kg which were divided...

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### Proceedings Article

## Pathomorphology of Papilloma Tumor in Cattle Based on Macroscopic and Microscopic

Etriwati Etriwati, Nazaruddin Nazaruddin, Dwinna Aliza, Denny Irmawati Hasan, Rizki Akbar Siagian

Papilloma was a proliferative neoplasm which benign typically that affecting nearly all animal species. This study aimed to observed changes in the papilloma in cattle based on macroscopic and microscopic to determine the difference between the two samples. The sample used was papilloma which was suspected...

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### Proceedings Article

## Avian Influenza Virus Subtype H5N1 in Pigeons: A Conventional Serological Detection

M. Daud AK, Erina, Mahdi Abrar, Fakhurrrazi, Darniati, Maryulia Dewi, Sugito, Amiruddin, Roslizawaty, T. Reza Ferasyi, Ismail, Abdul Harris

Prior to the novel coronavirus, the subtype H5N1 that emerged in Asia in 2003 and hit Indonesia from 2005 to 2010 has created fears with the public. Attention has not only been detections of domestic fowls but pigeons as well. This study aimed to detect the specific antibody to the avian influenza virus...

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### Proceedings Article

## Histology and Histomorphometry of Kidney on Domestic Chicken (*Gallus gallus domesticus*) During Pre and Post Hatch

Deci Aryani, Dian Masyitha, Zainuddin, Muslim Akmal, Teuku Zahrial Heln  
Yudha Fahrimal, Herrialfian Herrialfian

The purpose of this study was to determine the renal histology and histomorphometry of domestic chickens (*Gallus galus domesticus*) before and after hatching. The study involved in four treatment groups namely incubation period of 7 days (K1), 14 days (K2), and 20 days (K3), 7 days after hatching (K4)...

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The Effect of Neem Leaf (*Azadirachta indica* A. Juss) Extract on Tubules Seminiferous of Rat (*Rattus norvegicus*)

Cut Nila Thasmi, Dian Masyitha, Hafizh Arief, Husnurrisal, Dewi Ardiyanti  
Dalimunthe

This study aimed to determine the effect of neem leaf extract on the seminiferous tubules of white rats. This study used 15 adult white rats which divided into 5 treatment groups. The control group was only given water and each treatment group was given neem leaf extract with the dose of 50 (P1), 100...

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#### Proceedings Article

Case Study of Bovine Papilloma Virus in Aceh Cattle in Lhoknga  
Aceh Besar

Budianto Panjaitan, Syafruddin, Roslizawaty, Muhammad Hasan, Herrialfian  
Herrialfian, Dwinna Aliza, Hafizuddin, Rahman Alfarisyi

Bovine papillomavirus (BPV) induces benign tumours of cutaneous or mucosal epithelial in cattle and generally does not cause serious clinical problems in the



host. A case of BPV reported in 2-year-old female Aceh cattle in Lhoknga, A Besar. The clinical examination found various sizes of lumps on...

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#### Proceedings Article

### Protein Concentration of Excretory-Secretory and Somatic Antigen of *Paramphistomum* spp.

Siti Rani Ayuti, Muhammad Hambal, Ummu Balqis, Henni Vanda

This study was aimed at finding out protein profile of excretory-secretory and somatic antigen of *Paramphistomum* spp. As many as 60 *Paramphistomum* spp. taken from cattle in Banda Aceh Municipality Slaughterhouse were placed into 80 ml Roswell Park Memorial Institute (RPMI) 1640 and incubated for 12,...

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#### Proceedings Article

### The Relationship Between the Adequacy of Iron Tablet Supplementation and Hemoglobin Level in Pregnant Women

Afwandi Afwandi, Sugito Sugito, Nurliana Nurliana, Rinidar Rinidar, Teuku Reza Ferasyi

Anemia is problem commonly occurs in pregnant women. Pregnant women are said to be anemic if their hemoglobin levels are less than 11 g/dl. This condition occurs because of the insufficient intake of iron (Fe) to meet the increased need of the body during pregnancy and childbirth. The incidence of Fe...

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## The Prominent Role of Pituitary Adenylate Cyclase-Activating Polypeptide in Spermatogenesis and Function of Spermatozoa: A Mini Review

Muslim Akmal, Zulkarnain, Gholib Gholib, Teuku Zahrial Helmi, Sugito

Spermatogenesis is a great, complex, and long process. This process take place in the tubulus seminiferus of the testis and consists of three phases i.e. proliferation, meiosis, and spermiogenesis. In proliferation phase, the number of cells were multiplied, while in the meiosis occurs the completion...

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### Proceedings Article

## Morphoanatomy and Gonadosomatic Index (GSI) of Testis of Turkey (*Melagris gallopavo*) at Different Ages

Dian Masyitha, Muslim Akmal, Gholib Gholib, Sri Wahyuni

Testis is a male reproductive organ that responsible for producing the spermatozoa and regulating the reproductive hormones. Study on morphoanatomy and gonadosomatic index (GSI) can provide overview on the development of male reproduction. The study was conducted to determine the testicular morphoanatomy...

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## Identification and Characterization of Protamine 3 (*prm3*) Gene in Aceh Bull Testis

Rumi Sahara Zamzami, Muslim Akmal, Teuku Zahrial Helmi, Rusli Rusli, Sri Sugito, Tongku Nizwan Siregar, Sri Wahyuni

The incidence of infertility in breeding male cows highly contributes to economic loss in breeders. To date, there is still a lack of a precise and accurate molecular biomarker to predict the fertility rate on each ejaculation. Therefore, it is important to accurately identify genes related to the fertility...

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### Study of the Effect of Electroacupuncture Combination in Total Erythrocyte, Hemoglobin, and Hematocrite Values in Domestic Cat

Mudhita Zikkrullah Ritonga, Muhammad Jalaluddin, Wahyu Eka Sari, Triva Murtina Lubis, Indah Novicia Putri, Nurliana, Arman Sayuti, Teuku Zahrial Helmi

Acupuncture is an alternative treatment originating from China. According to Traditional Chinese Medicine (TCM) the condition of blood insufficiency including blood deficiency and blood stagnation. The study aimed to determine the effect of electroacupuncture on erythrocyte number, haemoglobin level...

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### Identification of Pathogenic Bacteria in Snapper Fish (*Lutjanus* sp.) from Banda Aceh Waters

Wahyu Eka Sari, M. Nur Salim, Teuku Reza Ferasyi, Muhammad Hambal, Henni Vanda, T. Zahrial Helmi, Mudhita Zikkrullah Ritonga, Sukmawan Fajar Santosa,

Windy Budi Setiawandi

Snapper (*Lutjanus* sp.), which is an important marine fish resource, is known to have relatively low movement activity and form relatively small groups. These characteristics make these fish susceptible to various diseases caused by pathogenic bacteria. This study aims to identify the pathogenic bacterial...

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**Proceedings Article**

## Diagnose Enforcement of Case Canine Transmissible Venereal Tumor (CTVT) on Domestic Dog

Awaluddin Awaluddin, Etriwati Etriwati, S.N Habiburrahman, Rusyidi Rusyidi, Ummu Balqis, Cut Dahlia Iskandar, Denny Irmawati Hasan

Canine Transmissible Venereal Tumor (CTVT) also known as venereal sarcoma is an inflammation mediated by reticuloendothelial tumors and infects the canine genitalia. This study aimed to diagnose CTVT cases in domestic dogs by looking at clinical symptoms, macroscopic, hematologic, blood chemistry and...

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**Proceedings Article**

## The Activity of *Jatropha curcas* Cream on Day 5 of Skin Wound Healing in Mice Infected with *Staphylococcus aureus*

M. Nur Salim, Dian Masyitha

This research aimed to determine *Jatropha* sap cream's activity on day 5 of the healing process of skin wounds of mice infected with *Staphylococcus aureus*. Twenty-seven male mice (*Mus musculus*) aged 2 months and weighed 30-40 g were used. All mice underwent a 20 mm incision in the dorsal area (back) and...

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### Proceedings Article

## Molecular and Phylogenetic Analysis of Hemagglutinin Gene of Avian Influenza a Viruses Subtype H5N1 Isolate from Quail

Teuku Zahrial Helmi, Wayan Tunas Artama, Aris Haryanto, Rumi Sahara Zamzami

Based on the level of infection, the Avian Influenza (AI) virus can be grouped into 2 levels of infection, namely highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI). The pathogenicity of AI viruses was determined by the amino acid sequence at the site of the hemagglutinin...

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### Proceedings Article

## Resistance Test for *Anopheles* spp. to Cypermethrin Insecticide Using Bottle Bioassay Test Method

Sabri, Muhammad Hanafiah, Rinidar, Yudha Fahrimal, Teuku Zahrial Helmi

The resistance of *Anopheles* spp to insecticides is the most important and dangerous problem that affects malaria control programs. One of the methods used to detect insecticide resistance is the bottle bioassay test method from Centers for Disease Control (CDC) which can be applied as part of a broader...

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### Proceedings Article

## Decreasing Blood Glucose Levels Using *Muntingia calabura* L Leaf Extract in Rats with Diabetes Mellitus

Nurlena Andalia, M. Nur Salim, Nurdin, Ummu Balqis

The aim of this study was to determine the decrease in blood glucose levels using *Muntingia calabura* L. leaf extract in hyperglycemic rats. This study used a completely randomized design with six treatments and four replications. The experimental animal used 24 male rats (*Rattus novergicus*) aged 3 months,...

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### Proceedings Article

## Isolation and Identification of *Escherichia coli* Serotype O157 from Swabs of Rectal Faeces in Aceh Cattle

Mahdi Abrar, Teuku Reza Ferasyi, Amiruddin, Fakhurrazi, Erina, Rusli Sulaiman, Rina Aulia Barus, Teuku Shaddiq Rosa, Rezky Ramadhan

*Escherichia coli* Serotype O157 (hereafter, *E. coli* O157) is known as a very important food-borne pathogen and the case spread world-wide in human. This study was aimed to isolate the bacteria of *E. coli* O157 from faecal sample of rectal swabs in aceh cattle. A number of 85 rectal swab samples were collected...

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## Identification of Gastrointestinal Protozoa in Long-Tailed Macaque (*Macaca fascicularis*) in Sabang

Farida Athallah, Alfi Wiratama Elpasha Ginting, Erdiansyah Rahmi, Muhammad Hambal, Muhammad Hasan, Erwin Erwin, Henni Vanda

This study aimed to determine the types of gastrointestinal protozoa that infested long-tailed macaque (*Macaca fascicularis*) in Sabang Municipality study could be used as a basis of consideration for controlling zoonotic diseases caused by gastrointestinal protozoa. This research was conducted...

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#### Proceedings Article

### Effect of Visitor Number on the Behavior and Cortisol Metabolites Concentrations of Sambar Deer (*Cervus unicolor*) in Captivity

Gholib Gholib, Sri Panggabean, Sri Wahyuni, Fadli A Ghani, Abdullah Hamzah, Erdiansyah Rahmi

The effect of the visitor on animal behavior in captivity has been widely reported. However, whether this visitor-effect also influences stress for captive animals is still limited and absent for captive Sambar deer (*Cervus unicolor*). Therefore, the objective of this study was to examine the effect of...

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#### Proceedings Article

### Spleen Histopathological Study of Mice (*Mus musculus*) Infected with *Plasmodium berghei* After Treatment with Malacca Leaves extract (*Phyllanthus emblica*)

Amalia Sutriana, Rasmaidar, Nuzul Asmilia, Cut Nila Thasmi, Dwinna Aliza, Wahid Muharram

This study aimed to evaluate the effect of ethanolic extract of malacca leaves (*Phyllanthus emblica*) on histopathology of mice (*Mus musculus*) spleen infected

with *Plasmodium berghei* (*P. berghei*). This study used 15 male Balb/c strain mice aged 2 months, allotted into 5 treatment groups. The mice in group...

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## Glycoconjugate Residues Type in Endometrium Aceh Cattle (*Bos indicus*) During Estrus Cycle

Muhammad Jalaluddin, Teguh Budipitojo, Dwi Liliek Kusindarta, Fajar Shodiq Permata, Priyo Sambodo, Hamny Hamny, Sri Wahyuni, Mustafa Sabri, Teuku Reza Ferasyi, Erdiansyah Rahmi, Awaluddin Awaluddin

The ability of aceh cattle to reproduce in extreme environments is thought to be supported by their reproductive system. This research was conducted to study the reproductive system of Aceh cattle by identifying the types and distribution of glycoconjugates in the endometrium of Aceh cattle at various...

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## Study of Cerebellum Structure and Histomorphometry of Local Chicken (*Gallus gallus domesticus*) Before and After Hatching

Arief Maulana, Dian Masyitha, Muslim Akmal, Sri Wahyuni, Zainuddin, Rosmaidar

Cerebellum is an organ that plays an important role in controlling and coordinating motoric nerves and maintaining body balance. This study aimed to observe the histological and histomorphometric structures of the cerebellum in native chickens before and after hatching. The samples of this study were...



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## Histology and Histomorphometry of Testes in Turkeys (*Meleagris gallopavo*) Based on Age Level

Nasya Nurma Zushita Firwan, Muslim Akmal, Dian Masyitha, M. Nur Salim, M. Jalaluddin, Tongku Nizwan Siregar

This study aims to determine the histological and histomorphometric features of the turkey testes based on age levels. Turkey testes were taken from 21 male turkeys which were grouped into 3 different age groups; 12, 20 and 33 weeks. The testes were then processed into histological preparations with...

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## The Prevalence of Gastro-Intestinal Nematode Parasite and Egg-Count in Cattle with Different Skin Colors

Riyan Ferdian, Lian Varis Riandi, Muhammad Hambal, Muttaqien, Henni Vanda, Wahyu Eka Sari, Rusli, Amiruddin, Irfan Mahyidin

This study was aimed to find out burden of nematode infection in aceh cattle with different skin colors. The samples were 105 cattle consisting of adult male and female. Each of skin color consisted of 15 males and 20 females obtained from Aceh Besar District. The samples were examined in the laboratory...

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## **Detection of 37 kDa *Outer Membrane Protein H (ompH)* gene of *Pasteurella multocida* Type B Local Isolate From Nusa Tenggara Timur (NTT)**

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### **Abstract**

Haemorrhagic Septicaemia (HS), in Indonesia known as Ngorok disease is a fatal acute septicemia disease in cattle and buffalo caused by *Pasteurella multocida*. Omp has a number of important roles for bacterial cells, such as uptake of nutrients, transporter for molecules in and out of cells and interactions with the host and environment. Outer membrane Protein H (OmpH) in *P. multocida* is one of the major proteins in the envelope which has been purified and characterized as porin immunodominant. The purpose in of this study was to detect the 37d kDa outer membrane protein H (ompH) gene from *P. multocida* local isolate from Nusa Tenggara Timur (NTT) and compare it with vaccine strain. Detection of 37 kDa ompH of *P. multocida* was done using PCR. Sample used in this study were *P. multocida* local isolate from NTT and vaccine strain (Katha). Isolation and identification were performed using bacteriological culture and biochemically characterization. Results from PCR showed a 37 kDa ompH gene on *P. multocida* local isolate and vaccine strain is 946 bp.

*Keywords: haemorrhagic septicaemia; P. multocida type B; outer membrane protein H (ompH) gene; PCR*

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### **1. Introduction**

Haemorrhagic Septicaemia (HS), in Indonesia known as Ngorok disease is a fatal acute septicemia disease in cattle and buffalo caused by

*Pasteurella multocida*. Hemorrhagic Septicemia in an economic perspective become the most important bacterial disease in Middle East, South Africa, Southeast Asia including, Thailand, Philippines, Malaysia and Indonesia (Jabeen *et. al.*, 2013).

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Haemorrhagic septicemia caused by infection of *P. multocida* strains belonging to serogroup B and E. *P. multocida* is a coccobacillus, Gram negative, non motile bacteria, producing toxin, and found as part of the commensal microflora in the upper respiratory tract of domestic and wild animals (Hotchkiss *et al.*, 2010).

The complex interactions between host factors and bacterial virulence factors, such as lipopolysaccharide (LPS), capsule, putative hemolysin, and outer membrane protein (omp) has an effect on the pathogenesis of the disease (Joshi *et al.*, 2013). Omp has a number of important roles for bacterial, such as nutrient uptake, transport of molecules in and out of cells and interactions with the environment and host (Hatfaludi *et al.*, 2010).

Outer membrane Protein H (OmpH) in *P. multocida* is one of the major proteins in the envelope which has been purified and characterized as immunodominant porin. Research has been conducted on the prospect of using OmpH as a subunit vaccine in its original and recombinant form. Immunoblotting studies by Tomer *et al.* (2002) was identified that 37 kDa polypeptide was the most antigenic in the omp profile of all serotype B:2 isolates, which was applied a P-52 whole-cell hyperimmune anti-*P. multocida* strain made on rabbits and buffalo. Further research conducted by Tan *et al.* (2010) showed that 37 kDa ompH was more reactive to polyclonal antiserum of mice and recombinant ability of 37 kDa ompH in protecting mice against *P. multocida* approximately 100% which was immunized intraperitoneally and 80% which was immunized subcutaneously and intraperitoneally. Therefore, OmpH is one of the most important bacterial antigens and promising candidates for the development of the vaccine and the diagnostic kit of HS.

The problem of HS disease in Indonesia is still commonly endemic, although vaccination has been done once a year using the HS vaccine made from the Katha strain. DNA Genomic analysis of *P. multocida* by Supar and Arianti (2007) showed that isolate of *P. multocida* from cattle and buffalo that suffered HS in Indonesia, DNA profile was different from Katha vaccine strain from Burma and different to isolate / strain referens from other region

The purpose of this study was to detect the 37 kDa outer membrane protein H (ompH) of *P. multocida* local isolate from NTT and compare it with vaccine strain.

## 2. Material and methods

### 2.1 Sample

Sample of *P. multocida* isolate originated from NTT (Nusa Tenggara Timur) were obtained from Balai Besar Veteriner Denpasar, Bali. Sample was cultured on Blood Agar medium by streak and then incubated for 24-48 hours at 37° C in incubator. Previously, this isolate had been tested for its capsular type. Haemorrhagic Septicaemia vaccine (*P. multocida* Katha strain) from Pusat Veterinaria Farma (Pusvetma) Surabaya was used as a vaccine strain sample.

### 2.2 Re-Identification of *Pasteurella multocida*

Inoculation on MacConkey Agar medium was performed to determine the growth properties of *P. multocida*. Which is *P. multocida* do not grow on Mac Conkey media. Furthermore, the examination using biochemical test, namely: TSIA test, SIM test, SCA test, Urease test, and Sugar test (Fajrin, 2016).

### 2.3 DNA Extraction

Total genomic DNA of *P. multocida* was extracted with QIAamp® DNA Mini kit according to standard protocol (Qiagen, 2016). Briefly, bacteria that have been cultured on Nutrient Agar (NA) at 37°C overnight, were inserted into tube. After addition of bacteria / vaccine incubated at 60 °C for 30 minutes. The 200µL AL buffer is added to the tube, then vortex. The mixture of the ingredients in the tube is transferred to the spin column, then adds 200 µL of 96% ethanol. After that, centrifuged at 8,000 rpm for 1 minute and washed using AW1 500µL. Then centrifuged back at 8.000rpm for 1 minute and washed again using AW2 as much as 500µL. After that centrifuged at 13,000 rpm for 3 minutes and the bottom of the spin column removed. After that, it was centrifuged again for 1 minute at 13,000 rpm and transferred to an empty sterile tube. After that added 50µL AE Buffer and incubated at room temperature for 1 minute. Then centrifuged at 8,000 rpm for 1 minute

### 2.4 Detection of ompH gene by PCR assay

Amplification of the ompH gene was performed using both ompH-F and ompH-R primer in 20µl PCR mixture. The primers used was developed from the primers used by Tan *et al.* (2010). Primer ompH-F forward 5'-CAGCAACAGTTTACAATCAAGACGGTAC -3' and ompH-R reverse 3'-GAAGTGTACGCGTAAACC -5' with gene target 946 bp. The thermal cycling conditions are: 1 cycle at 95°C for 5 minutes; 40 cycles at 94°C for 30 sc, 50°C for 1 minute, and 72°C for 1.5 minutes; 1

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cycle at 72°C for 10 minutes. This PCR profile is repeated three more times to ensure specific PCR products. The electrophoresis of PCR product was analyzed in 1% agarose gel.

**3. RESULT**

*3.1 Reculture and Re-identification of Pasteurella multocida*

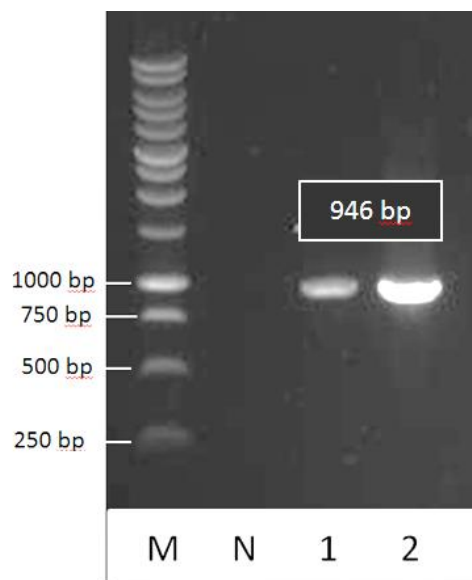
The result from reculture of *P. multocida* on Blood Agar incubated 37 ° C for 24-48 hours as shown in (Fig. 1.) Colonies of clear shiny bacterial bacteria, does not hemolyzed red blood cell and does not growing on Mac Conkey Agar medium can be said suspect *P. multocida*. The suspect colonies of *P. multocida* were then confirmed by biochemical test, the TSIA was obtained in alkaline slat and butt, yielding no gas and H<sub>2</sub>S. In the Indol test the pink ring appeared on the surface after Kovach's reagent was added. In Urease Test this bacteria does not produce urease enzymes so it can not change the color of Urease media. In the Simon Citrate Agar Test this bacteria can not use citrates as carbohydrates so as not to change the color of the media Simon Citrate Agar.



**Figure 1.** Morphologi colonies of *Pasteurella multocida* on Blood Agar media

*3.2 Detection of ompH by PCR assay*

The *ompH* gene was successfully amplified from local isolate *P. multocida* and vaccine. The PCR product was obtained with the same band size of 946 bp of both samples (Fig. 2).



**Figure 1.** PCR detection of *ompH* gene of *P. multocida*. Lane M: 1kb molecular ladder (Vivantis); N negative control; 1 *P. multocida* from vaccine; 2 local isolate of *P. multocida*

**4. DISCUSSION**

In Indonesia, Haemorrhagic septicemia disease is still commonly endemic, although vaccination has been done once a year using vaccines made from Katha strains. (Ghani *et al.*, 2016). Omps are exposed to the outside of the bacterial cell and are the first line of contact between the bacterium and environment. Omps has numbers of important role, such as acting as adhesion factors in virulence, channels for nutrients uptake, siderophore receptors and enzymes (Rollauer *et al.*, 2015). Identification of *ompH* as one of the virulence factors is helpful for the determination of pathogenesis mechanisms and development of prevention and control measures of the disease such as the establishment of an efficient vaccine (Furian *et al.*, 2013).

*OmpH* or porin H of *P. multocida*, is the major outer membrane protein in the envelope. Research has been conducted in purification and characterization this protein and because its structure and functional related to the superfamily of porins of Gram negative bacteria, this protein classified as Porin (Ganguly *et al.*, 2015). In native conformation, porin H is a homotrimer, stable in sodium dodecyl sulfate (SDS) at room temperature, and is dissociated into monomers upon boiling. The molecular masses of *ompH* range between 34 and 42 kDa depending on the serotype of bacteria and

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the electrophoretic system used for analysis (Lou *et al.*, 1997)

In an earlier study, 37 kDa ompH were identified as major immunodominant proteins and has been shown to provide immunity, hence primers used in this study are specific for 37 kDa ompH genes. In this study the ompH gene of *P. multocida* local isolate and vaccine strain was detected using PCR. PCR product obtained result of 946 bp for both samples. Research by Singh *et al.* (2011) in isolate *P. multocida* P-52 (vaccine strain for HS), the ompH gene was obtained approximately 1.2 kb. While on research by Tan *et al.* (2010) which were the primer developed in this study 37 kDa ompH gene was obtained for 980 bp. The difference from the primer use in each study had an effect on the result of PCR of the ompH gene itself.

In ompH gene detection in this study, *P. multocida* local isolate and vaccine strains showed the same band. But it is not known whether they have the same DNA sequence.

From the results obtained in this study, it is necessary to further investigate the prediction of epitope genes encoded by 37 kDa ompH for development in a vaccine consistent with the incidence of Haemorrhagic septicemia in Indonesia

### Conclusion

In this study, 37 kDa ompH gene from local *P. multocida* isolate and vaccine strain (katha) was obtained with PCR yield of 946 bp

### Acknowledgments

We would like to thank Balai Besar Veteriner Denpasar-Bali, Department of Microbiology and Mycology and Bacteriology and Mycology Laboratory of Veterinary Medicine Faculty of Airlangga University for the permission to use the facility.

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