INTERNATIONAL SEMINAR

ADVANCED TECHNOLOGY ON VETERINARY AND LIFE SCIENCE

PROCEEDINGS

Organized By
Faculty of Veterinary Medicine, Universitas Gadjah Mada

Yogyakarta, March 12\textsuperscript{nd}, 2011
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PREFACE

The focus of the veterinary and life science researcher has shifted substantially since the realizing that the research in animal and life science has a big part of human prosperity life. Following general social trends and specific structural changes in agricultural production, zoonosis disease, several technology application on animal have risen. More and more the question has arises as to the whether curative medical treatment of individual animals makes economic sense. Increasingly veterinarians and other reasearcher in animal and life science are expected to provide preventive management, not only in regard to animal health, but also to ensure the production of high quality food and the appropriateness of the management system of animal and life things.

This publication reports papers presented at the International Seminar of The advanced Technology of Veterinary and Life Science, organized by and held at the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia on March 12, 2011. Three sessions of 66 papers were held at the conference: plenary seassion featuring keynote speakers and invited papers, oral presentation pappers and poster presentation seassion. This proceeding features 60 theme of three filed researchs, basic, preclincial and clinical sciences.

We particullary I acknowledge with sincere gratitude the invaluable contribution the authors who submitted the manuscripts and the member of scientific comittee, who suggested the topics and authors to be included and edited the manuscripts. I wish to thank my fellow Organizing Committee to their
from Canine Amniotic Fluid that have Characteristics of Pluripotent Stem Cells, Approach to Transgenic Research in Cow and Pig, and a lot of topic in parallel session. I hope this seminar is a forum to share information and build scientific collaboration in the field of veterinary Medicine and Life Science. We hope also seminar could give us new information in advanced biotechnology. This topic will be interesting and can draw students as well as faculty member eagerness to realize that this topic is very important in the future animal and human civilization. Beside that, I hope through this seminar, we could strengthen collaboration between Universitas Gadjah Mada and Seoul National University.

Finally, I would like to thank and deepest gratitude to all of you, our guests from Korea, and organizing committee for the responsibility this seminar. I look forward to collaborating between Universitas Gadjah Mada and Seoul National University in education and research, including the possible project for animal cloning. So, I wish all ladies and gentlemen’s here to be enjoyed and great pleasure.

Wassalamualaikum wr.wb.

Faculty of Veterinary Medicine

Gadjah Mada University

Dean,

Prof. Dr. drh. Bambang Sumiarto, SU.,M.Sc
efforts towards the success of the conference. Last but not least, I hope that the seminar leaves us and all participants with memorable and fruitful experience.

drh. Agung Budiyanto, M.P., Ph.D.
Chairperson of the Organizing Committee
WELCOMING SPEECH FROM CHAIR PERSON OF
THE ORGANIZING COMMITTEE

Distinguished guests

Direktur Kesehatan Hewan on behalf Direktur Jendral Peternakan and Kesehatan Hewan, Republik Indonesia, Dr. drh. Pudjiatmoko

Dean of Faculty of Veterinary Medicine, Universitas Gadjah Mada Yogyakarta, Prof. Dr. drh. Bambang Sumiarto, S.U., MSc.

Good morning and may God be with us

It is my great privilege to greet you all to the International seminar of International Seminar of The Advanced Technology of Veterinary and Life Science, held at Faculty of Veterinary Medicine Gadjah Mada University.

This seminar is principally designed to enhance the contribution of veterinary and life science to the development of basic knowledge and applied sciences related to the efforts raising the quality of human safety and prosperity life. Technology in this fields has great important part to supporting the development of human life following very quick changing of the world. I hope this event will be a great opportunity and a wonderful venue for us to lay down a cooperative framework and to establish scientific collaboration between scientists. An impressive roster of distinguish speakers and attendants from Korea, Malaysia, Libya and all of Indonesian area has been gathered in this conference.
Hereby, on behalf of the organizing Committee, I acknowledge to these following speakers Dr. drh. Pudjiatmoko on behalf Dr. drh. Prabowo Respatyo (Dirjen Pternakan dan Kesehatan Hewan Republik Indonesia), Prof Byeong Chun Lee and Goo Jang Ph.D. for delivering their valuable scientific information.

I also gratefully thank to the Dean and Vices Dean of Faculty of veterinary medicine, Universitas Gadjah Mada for giving us opportunity and support to organize this conference. My great thanks to the sponsor of the conference from Bank Mandiri UGM. Heartfelt thank is delivered to the steering committee, the academic reviewers, member of the organizing committee for the strong support, active participation, cooperation and hard works throughout in preparing and organizing this meaningful meeting and those who have contributed their untiring effort in making this seminar success.

Despite our best efforts, it is inevitable that there is a lack in organizing this seminar and profoundly apologize to all invited speakers, oral and poster presenters, attendants and committee members.

Finally, I would like to offer my best wishes for a highly enjoyable, successful, productive and fruitful conference.

Thank you

drh. Agung Budiyanto, M.P., Ph.D.

Chair person of the Organizing Committee
OPENING REMARKS FROM THE DEAN FACULTY OF VETERINARY MEDICINE

Distinguished Guest, Keynote Speaker, and Speakers

- drh. Pudji Atmoko, Ph D, Director Directorate General Animal Disease, Ministry of Agriculture
- Prof. Byeong Chun Lee DVM, Ph D, Kim Min Kyu, DVM, Ph D, and Goo Jang, DVM, Ph D, Seoul National University, Korea,
- and all the guest of this seminar

Assalamualaikum wr.wb.

Good Morning

On behalf of Faculty of Veterinary Medicine, Universitas Gadjah Mada, I wish to extend the very warmest of welcome to all Speakers from Indonesia, Korea, Malaysia, and participants to the seminar on “Advanced Technology on Veterinary and Life Sciences”. Also my word of welcome is especially aimed to drh. Pudji Atmoko, Ph.D, Director Directorate General Animal Disease, Ministry of Agriculture. We are very happy to have you with us and we are looking forward to your input into the seminar.

Dear Participants

The seminar on “Advanced Technology on Veterinary and Life Sciences” will discuss several interesting topic, such as the Snuppy and Dog Cloning: Last 5 year progress, Isolation and Differentiation of Mesenchymal Stem Cells Derived
CONFERENCE COMITTEE

Steering Committee
Prof. Dr. drh. Bambang Sumiarto, S.U., M.Sc.
(Dean Faculty of Veterinary Medicine, Universitas Gadjah Mada)
Prof. Dr. drh. Wayan T. Artama
(Vice Dean for the Academic Affairs Faculty of Veterinary Medicine Universitas Gadjah Mada)

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Sri Ratih Giant Puspitasari, Amd.
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drh. Woro Danur Wendo
Papers : Dr. drh. Aris Haryanto, M.Si.
Dr. drh. Sumartono, S.U., DEA.
Endah Choiriyah, S.I.P., M.Si
Consumption : Dr. drh. Agustina D.W., M.P.
Dr. drh. Tri Wahyu P., M.P.
drh. Ariana, M.Phil
                        drh. Sugiyono, M.Sc.

Equipment: Nawawi, S.E.
            Joko Kusno
            Haryadi
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Topic 1  : Basic Science
Sub Topic: Molecular Biology

1. The Possible Use of Sub Unit GAG-CA Gene (JC1) As Hybrization Probe Candidate
   Bambang Sutrisno, Penny Humaidah H., Asmarani Kusumawati, Hastari Wuryastuti and J. Sriwidada

2. Development of ELISA system using specific antigen LipL32 and LigA as effective diagnose tools for leptospirosis
   Dyah Ayu Widiasih, R. Isozumi, K. Yoshimatsu dan J. Arikawa

3. Molecular characteristic of Nucleoprotein gene of Rabies Virus isolated from Bali Indonesia
   Heru Susetya dan Nyoman Dibia

4. Detection of Jembrana Virus Using RT-PCR
   Narendra Yoga Hendarta and Asmarani Kusumawati

5. Molecular Epidemiology of Verectotoxigenic Escherichia coli (VTEC) in Livestocks in Yogyakarta
   Yatri Drastini, Setyawan Budiharta, Widya Asmara, Hastary Wuryastuti dan Langkah Sembiring

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Topic 2  : Basic Science
Sub Topic: Applied Science

1. Development of Catecholaminergic in the ventral mesenchepalon of longtailed macaque (Macaca fasicularis) during prenatal Period
   Tri Wahyu Pangestiningsih, Ariana, Djoko Pamungkas and Erni Sulistyawati
2. Pare Extract (Momordica charantia L) as an Antioxidant Potency For Reducing Blood Glucose Level on Pre-Diabetic Rat (Spague dawley) Model Stability of Sardine Fish Oil and Hydrolyzed Blood Protected to Increase Productivity Erni Sulistiawati, R.P. Agus Lelana, and Tri Wahyu Pangestiningsih

3. Acute Toxicity Of Cashew Nuts Shell Extract (Anacardium occidentale L) in Albino Rat: A Preliminary Study Harlita, Nur Handayani, NS., Sagi, M., Astuti, P.

Topic 3 : Basic Science
Sub Topic: Applied Science

1. Mesenchimal stem cell: Potensial Drug For Regeneratif Medicine Yudha Heru Fibrianto

2. Concentration of Estradiol in Nilem (Osteocilus hasselti C.V.) under Photoperiods Manipulation Prayogo, N.A., G.E. Wijayanti, Murwantoko san P.Astuti


Topic 4 : Basic Science
Sub Topic: Basic Anatomy

1. Comparison Distribution of Various Lectins on The Stomach of the Java Fruit Bat (Rosettus sp.) and The Java Insect Bat (Myosit sp.) Ariana and Teguh Budipitojo

2. Immunohistochemical Study of The Ghrelin In The Stomach of Java Porcupne (Hystrix Javanica) Teguh Budipitojo, Motoki Sasaki, Ariana, Soehartini Jatman, and Nobuo Kitamura
3. Lectin histochemistry for Sialic Acid Receptor Detection in Rat Olfactory Property Indicate An Alternative Transmission Route of Avian Influenza Virus
   Woro D. Wendo, Dwi Liliek Kusindarta, M. Untoro, and Ditya Bayu Pangestya

4. Morphometrical Data of The Canines Dens in Male and Female Longtailed Macaque as the Guidance for The Manipulation and Treatments
   Hery Wijayanto, Tri Wahyu Pangestiningsih, Dwi Liliek Kusindarta, I Nengah Budiarsa and Alfian Galuh

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**Topic 5 : Preclinical Science**

**Sub Topic: Microbiology**

1. The Effectiveness of Disinfectan Treatment to Reduce Egg’s Bacterial Count
   Widagdo Sri Nugroho, Khrisdiana Putri, Damar Dwi Haryanto dan Marsudi

2. Surface Character of Coagulase Negative Staphylococcus From Dairy Milk: Surface Protein, Hydrophobicity And Hemaglutination
   Agnesia Endang Tri Hastuti Wahyuni

3. Isolation and Identification of Staphylococcus intermedius From Short-haired Dogs Dermatitis In Yogyakarta

4. Role of An Adhesin of Chlamydia pneumonia on the Inhibition Of Infected Cells
   Sri Murwani

5. Isolation and Identification of Dermatophytes From Cats In Yogyakarta
   Yanuartono, Soedarmanto I., Surya Amanu, G. Sakan, and A. Anita
Topic 6 : Preclinical Science
Sub Topic: Fishery

1. Identification Species of Myxobolus From Gill of Cyprinus Carpio in East Java
   Agus Priyono, Kurniasih, Rini Widayanti, Ayuda D. Nurekawati

2. Early In Vitro Oogenesis Of The Giant Gourami (Osphronemus gouramy Lac)
   Aulidya Nurul Habibah, Isdy Sulistyo, and Gratiana E. Wijayanti

3. Identification of Clinostomum complanatum (Digenea: Clinostomidae) in Freashwater Fish in Yogyakarta and Riau, Indonesia Based on Molecular Study Fish in Yogyakarta and Riau, Indonesia Based on Molecular Study Morina Riauwaty, Kurniasih, Joko Prastowo and Windarti

4. Histopathological Changes of Edwardsiellosis in Cyprinus carpio From Pontianak, West Kalimantan, Indonesia
   Siti Narwiyani, Kurniasih and Asmarani Kusumawati

5. Pathogenetic differences of Aeromonas salmonicida isolates collected from fresh water fish in four areas of Indonesia
   Riza Priyatna, Kurniasih, S. Indarjulianto and Surya Amanu

Topic 7 : Clinical Science
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1. Effects of Varying Egg Yolk Addition On Post-Thawing Sperm Function of Ettawa Crossbreed Goats
   Erif Maha Nugraha Setyawans

2. The Effect Addition of Red Ginger Phytobiotic to The Production and Blood Profile of Broiler Chicken
   Herawati

3. Phosphate Homeostasis In Pre-Ruminant And Young Ruminant Goats
   Irkham Widiyono
4. Profile Of Some Blood Chemistry Parameters In Crossbred Ettawa Goats
Irham Widiyono and Sarmin

**Topic 8** : Clinical Science
**Sub Topic**: Reproduction and Large Animal

1. Stability of Sardine Fish Oil and Hydrolyzed Blood Protected to Increase Productivity of Dairy Cattle
   *Ahmad Pramono, Kustono, Prabowo P.P., Dyah Triwidayati and Hari Hartadi*

2. Follicular Dynamic On Repeat Breeding Crosbreed Cows Examined Using Ultrasonography
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3. Physiological Functions of Betaine in Monogastric Animals
   *Adi Ratriyanto*

4. Nuclear Maturation Of Immature Porcine Oocytes After In Vitro Maturation Following Vitrication
   *Agung Budiyanto*
ISOLATION AND IDENTIFICATION OF DERMATOPHYTES FROM CATS IN YOGYAKARTA

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ABSTRACT

Dermatophytosis is a superficial cutaneous infection caused by one or more species of fungi from dermatophytes group. The most common dermatophytes in cats are Microsporum canis, Microsporum gypseum, and Trichophyton mentagrophytes. The aim of this study were to isolate and identify the presence of dermatophytes in cats in Yogyakarta region. Fifteen skin cat scrapings with clinically dermatitis lesions including alopecia, erythema, papules, pustules, scaly and crusty were used in this study. Identification of dermatophytes performed by macroscopic using modified sabouraud’s dextrose agar (SDA) medium and microscopic using lacto phenol cotton blue (LPCB), followed by slide culture methods. The results of this study showed that 10 of 15 samples were grew between fourth to eighth days post cultivation. On the upper side of the colony surfaces were looks like cottony with white color in the central and surrounded by yellow to light brown. On the reverse side, it appeared bright yellow - orange colors and colorless at the edges. By microscopic, the isolates had a septate and branched hyphae with big macroconidia, round rod-shaped, and had 6-12 cells. Small microconidia could be observed as single-cell oval along with the hyphae. Based on these results it can be concluded that 66.66% of cats suffering dermatophytosis caused by the fungus Microsporum canis.

Keywords: Isolation, Identification, Dermatophytes, Cat.

Introduction

Dermatophytosis is a superficial cutaneous infection with one or more of the fungal species in Dermatophyte genera. The most common fungal organisms identified in the cat with dermatophytosis are Microsporum canis, Microsporum gypseum, and Trichophyton mentagrophytes. These fungal organisms are adapted to colonize hair and the cornified layers of the skin where they can digest keratin protein for their nutrition (Outerbridge, 2006). Those three genera are the so-called
zoophylic strains and they are the most reported dermatophytes found worldwide (Olivares, 2003). Dermatophytosis has been widely found in around the world, particularly in areas of tropical and cold climates and high humidity conditions (Moriello, 2004). The disease is generally transmitted directly or indirectly from animal to animal or from animals to humans (Carter and Claus, 1986). *Microsporum canis* is most commonly isolated from cat dermatophytosis, but *Microsporum gypseum* and *Trichophyton mentagrophytes* are less common. Various reports cases of skin disease in cats abroad found that about 80% of cases of dermatophytosis caused by *Microsporum canis* and 20% are caused by *Microsporum gypseum* and *Trichophyton mentagrophytes* (Carter and Claus, 1986; Kahn and Line, 2007). However, there is less information about the incidence of cat dermatophytosis in Indonesia. The aim of this study were to isolate and identify the presence of dermatophytes in cats in Yogyakarta region.

**Materials And Methods**

The sample used in this study were skin scrapings from 15 cat's which clinically suspected as dermatophytosis in the region of Yogyakarta. The identification procedure based on the guidelines according to Al-Doory (1980) and Ellis (2010). Samples were cultivated in the modified sabouraud's dextrose agar (SDA), incubated at a temperature of 25-30 º C for 3 weeks and then the fungal identified by macroscopically and microscopically. Microscopic examination conducted on the observed positive fungal cultures, using lacto phenol cotton blue (LPCB) and a slide culture with Riddle methods.

**Results And Discussion**
The clinically expression of dermatophytosis strongly varies depending on both the host and fungal species. Whereas infection can be acute and chronic (Bond, 2010). In this study, the samples of skin scrapings could be taken from 15 cats with various clinical expression including lesions with a combination of alopecia, erythema, papules, and the skin scaly and crusty (Figure 1,a). Some of them showed light yellowish green fluorescence on examination using woods light (Figure 1,b).

![Figure 1: (a). Cats with lesions clinically suspected dermatophytosis and (b). Fluorescent hair under Woods light of a cat with lesions suspected dermatophytosis.](image_url)

In this investigation, 10 of 15 samples were relatively quick grew in modified sabouraud's dextrose agar (SDA) in fourth and eighth days and the colony of them could be mature between seventh to fourteenth days after cultivation. Macroscopically, the colony developed fairly-rapid forming cottony or woolly mycelium white to buff in color, and later becoming buff to brown in the center. The reverse side of the colony appeared bright yellow to orange in color, and colorless at the edges (Figure 2,a). Microscopically, the isolates had adapted and branched hyphae with big macroconidia, round rod-shaped, and had 6-15 cells. Small microconidia could be observed as a single cell oval a long with the hyphae...
All of these results lead to the identification of *Microsporum canis*, and in accordance with Al-Doory (1980 and Ellis (2010).

Moriello (2004) concluded that infection occur via direct transmission of infective spores to a susceptible host. Reservoirs of infection for animals include contaminated environments and objects, animals with subclinical or clinical infections, and animals that are mechanical carriers of spores on their hair coat. Mancianti et al. (2003) reported that infected cats appeared to be a striking source of contamination in their environment, and also provoke a massive airborne presence of viable fungal elements. Furthermore, Their results also indicate that cats were the source of spread of the *Microsporum canis* -Infected to owner.

![Figure 2: (a). Macroscopic colony of *Microsporum canis* in 14 days (b). *Microsporum canis* colony appearance on microscopic examination.](image)

**Conclusion**

Based on this research it can be concluded that 66.66% samples of skin scrapings cats suffering dermatophytosis caused by *Microsporum canis*. 
REFERENCES


PATHOGENETIC DIFFERENCES OF *Aeromonas salmonicida* ISOLATES COLLECTED FROM FRESH WATER FISH IN FOUR AREAS OF INDONESIA

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²Faculty of Veterinary Medicine Gadjah Mada University, Jogjakarta, Indonesia,

Abstract

The aim of study was to know the differences of pathogenicity of *Aeromonas salmonicida* infection from several areas in Indonesia. Four isolates of *Aeromonas salmonicida* have been isolated and identified from *Cyprinus carpio* (Pontianak), *O. gouramy* (Semarang), *C. macropomum* (Yogyakarta), and *O. niloticus* (Jambi). Each isolate was examined the value of LC₅₀ to fish, *Cyprinus carpio*, then they were observed their pathogenicity in fish, *Cyprinus carpio*. Each isolate was injected intramuscular 0.1 ml with concentration of their LC₅₀. The observation was for 7 days. The value of LC₅₀ of *Aeromonas salmonicida* from Pontianak (7.24 x 10² cell/ml), Semarang (2.29 x 10⁴ cell/ml), Yogyakarta (1.66 x 10³ cell/ml), Jambi (3.55 x 10³ cell/ml). Based on the histopathological changes showed that isolates of *Aeromonas salmonicida* from Pontianak and Jambi were more pathogen than two other isolates. Skin lesion (40%), liver congestion (60%), epicarditis (80%), and enteritis (50%) could be seen clearly at the fifth days after infection.

Keyword: *Aeromonas salmonicida*, pathogenicity, LC₅₀.

Introduction

Emmerich and Weibel found *Aeromonas salmonicida* (synonym of *Bacillus salmonicida* or *Bacterium salmonicida* or *Bacterium trutta*) from Trout fish in the hatcheries of Germany in 1894 (Cipriano and Bullock, 2001). *Aeromonas salmonicida* was one of the very pathogen *Aeromonas* for Salmonid aquaculture (Schachte, 1985; Austin and Austin, 2007). Other salmonid fish, like fresh water fish, cyprinids, especialy *Cyprinus carpio* could be infected by *Aeromonas salmonicida*...
subspecies nova or smithia (Schacte, 1985; Hastings, 1988; Inglis et al., 1993, Austin and Austin 2007).

Aeromonas salmonicida had a wide distribution of host, geography, and pathogenicity (Paterson et. al, 1980). Aeromonas salmonicida was bactery Gram positive, non motile, oxidase positive, produce brown pigment. These characters could help to diagnose this bactery ((Kimura, 1969; Elliot and Shotts, 1980; Teska et al, 1992).

Group 1 of A. salmonicida subspecies salmonicida was typical of derivate isolate from salmonid fish. It was homogenous taxon, respectively to biochemistry and genotypic characteristics (Gudmundsdottier et al., 2003). Group 2 of strain A. salmonicida subspecies achromogenes and masoucida, those two bactery were Gram negative, as specific syndrome of salmonid, or ulcer disease (Inglis et al., 1993) and Pasteurellosis (Hastein and Bullock, 1976), and it was called atypical strain now (Austin and Austin, 1987). Group 3 of strain A. salmonicida subspecies nova was atypical strain that related to disease from non salmonid fish.

Mechanism of pathogenicity of A. salmonicida consisted of 2 types, virulent and avirulent. Virulent type was auto-agglutination, could attach on the tissue culture faster by “A” layer than avirulent type. The pathognomonic leossion of Aeromonas salmonicida infection was a necrosis of muscle like a swollen subcutaneous, and called false furuncle. The swollen skin could burst and filled with blood, abscess and damaged tissues. Other symptoms was hemorrhage on the base of fins and near eyes. Aeromonas salmonicida had a capability to inhibit the inflammatory respons because A. salmonicida had a leucocidin which was leucocytolytic, could damage leucocyte including macrofag (Anonim, 1993).
Material and methods

*Cyprinus carpio* with 10-15 cm of size, 250 of fish were divided into five groups with fifty fish of each group. Isolates of *A. salmonicida* yang were isolated from Pontianak/isolate 1 (in *C. carpio*), Semarang/isolate 2 (in *O. gouramy*), Yogyakarta/isolate 3 (in fresh water bawal / *C. macropomum*), Jambi/isolate 4 (in *O. niloticus*) and isolate 5 was atypical isolate *A. salmonicida* subsp. smithia Austin et al. from Americans Type Culture Collection (ATCC) number: 49393. Isolation and identification of *Aeromonas salmonicida* were morphology, Gram staining, motility, fermentation, IMVlC, and biochemistry test.

The research consisted of two stage. The first stage was revirulency of isolates and detection of lethal consentrasi 50% (LC50) of *A. salmonicida*. The concentration of *A. salmonicida* was counted with Mc Farland method, in TSA for 24 hours. Test of LC50 of each isolate of *A. salmonicida* using four aquarium with ten fish of each aquarium. The infection of *A. salmonicida* by bathing with concentration of $10^6$, $10^5$, $10^4$ dan $10^3$ sel/ml for 48 hours. Symptoms, the number and time of death was observed every three hours. The result was analysed with Dragsted-Behrens method. The second stage was pathogenecity examination of four isolates of *A. salmonicida* in *Cyprinus carpio*. Each isolates experiment used 15 fish that were injected intramusculary 0,1 ml of LC50 concentration. Five fish were otopsied on 3, 5, and 7 days after infection for histopathological examination.
Results and discussion

All isolates from several areas of Indonesia (Pontianak, Yogyakarta, Jambi dan Semarang) and ATCC isolate were identified in Trypticase Soy Agar (TSA). Colony of Aeromonas salmonicida had no dark blue pigment in 20\(^0\)-25\(^0\)C for 3-4 days. Inoculation of

A. salmonicida using blood agar plate and TSA for 24 hours showed different density growth colony of both agar. Atypical isolate of A. salmonicida could grow better in blood agar plate than in TSA (Figure 1). Based on the identification of five isolates of A. salmonicida showed their similarity (Table 1).

Figure 1. Growth comparison of atypical isolate of A. salmonicida Between blood agar plate (left) and TSA (right).
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<th>Media</th>
<th>Atypical Isolate of <em>A. salmonicida</em></th>
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<td>Pontianak</td>
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<td>Sukrosa</td>
<td>+</td>
</tr>
</tbody>
</table>
The concentration of LC$_{50}$ of five isolates of *A. salmonicida* was a little variation, from $3.55 \times 10^2$ cell/ml to $2.75 \times 10^4$ cell/ml (Table 3-7).

### Table 3. Analysis data of death fish to get LC$_{50}$ of *A. salmonicida* from Pontianak.

<table>
<thead>
<tr>
<th>Aquarium</th>
<th>Concentration</th>
<th>$\Sigma N$</th>
<th>R</th>
<th>N-R</th>
<th>$\Sigma r$</th>
<th>$\Sigma (n-r)$</th>
<th>Total</th>
<th>$(\Sigma r)/T \times 100%$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A ($10^9$)</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>16</td>
<td>16</td>
<td>0.00%</td>
</tr>
<tr>
<td>B ($10^3$)</td>
<td>3</td>
<td>10</td>
<td>6</td>
<td>4</td>
<td>6</td>
<td>6</td>
<td>12</td>
<td>50.00%</td>
</tr>
<tr>
<td>C ($10^5$)</td>
<td>5</td>
<td>10</td>
<td>8</td>
<td>2</td>
<td>14</td>
<td>2</td>
<td>16</td>
<td>87.50%</td>
</tr>
<tr>
<td>D ($10^7$)</td>
<td>7</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>24</td>
<td>0</td>
<td>24</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

N : total number of fish; R: number of death fish; N-R: number of life fish; $\Sigma r$: accumulation number of death fish at concentration $\leq X$; $\Sigma (n-r)$: accumulation number of life fish at concentration $\geq X$; $(\Sigma r)/T$: percentage of death fish. Value of LC$_{50}$ was $7.24 \times 10^2$ cell/ml.
Table 4. Analysis data of death fish to get LC\textsubscript{50} of *A. salmonicida* from Yogyakarta

<table>
<thead>
<tr>
<th>Aquarium</th>
<th>Concentration</th>
<th>(\Sigma N)</th>
<th>(R)</th>
<th>(N-R)</th>
<th>(\Sigma r)</th>
<th>(\Sigma (n-r))</th>
<th>Total</th>
<th>((\Sigma r)/T) x 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A ((10^8))</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>17</td>
<td>17</td>
<td>0,00%</td>
</tr>
<tr>
<td>B ((10^3))</td>
<td>3</td>
<td>10</td>
<td>6</td>
<td>4</td>
<td>6</td>
<td>7</td>
<td>13</td>
<td>46,15%</td>
</tr>
<tr>
<td>C ((10^5))</td>
<td>5</td>
<td>10</td>
<td>7</td>
<td>3</td>
<td>13</td>
<td>3</td>
<td>16</td>
<td>81,25%</td>
</tr>
<tr>
<td>D ((10^7))</td>
<td>7</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>23</td>
<td>0</td>
<td>23</td>
<td>100,00%</td>
</tr>
</tbody>
</table>

Value of LC\textsubscript{50} was \(1,66 \times 10^3\) cell/ml

Table 5. Analysis data of death fish to get LC\textsubscript{50} of *A. salmonicida* from Semarang

<table>
<thead>
<tr>
<th>Aquarium</th>
<th>Concentration</th>
<th>(\Sigma N)</th>
<th>(R)</th>
<th>(N-R)</th>
<th>(\Sigma r)</th>
<th>(\Sigma (n-r))</th>
<th>Total</th>
<th>((\Sigma r)/T) x 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A ((10^8))</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>22</td>
<td>22</td>
<td>0,00%</td>
</tr>
<tr>
<td>B ((10^3))</td>
<td>3</td>
<td>10</td>
<td>2</td>
<td>8</td>
<td>2</td>
<td>12</td>
<td>14</td>
<td>14,29%</td>
</tr>
<tr>
<td>C ((10^5))</td>
<td>5</td>
<td>10</td>
<td>6</td>
<td>4</td>
<td>8</td>
<td>4</td>
<td>12</td>
<td>66,67%</td>
</tr>
<tr>
<td>D ((10^7))</td>
<td>7</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>18</td>
<td>0</td>
<td>18</td>
<td>100,00%</td>
</tr>
</tbody>
</table>

Value of LC\textsubscript{50} was \(2,29 \times 10^4\) cell/ml
Table 6. Analysis data of death fish to get LC$_{50}$ of *A. salmonicida* from Jambi

<table>
<thead>
<tr>
<th>Aquarium</th>
<th>Concentration</th>
<th>$\Sigma N$</th>
<th>R</th>
<th>N-R</th>
<th>$\Sigma r$</th>
<th>$\Sigma (n-r)$</th>
<th>Total</th>
<th>$(\Sigma r)/T \times 100%$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A ($10^2$)</td>
<td>5</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>27</td>
<td>27</td>
<td>0,00%</td>
</tr>
<tr>
<td>B ($10^3$)</td>
<td>10</td>
<td>10</td>
<td>1</td>
<td>9</td>
<td>1</td>
<td>17</td>
<td>18</td>
<td>5,56%</td>
</tr>
<tr>
<td>C ($10^5$)</td>
<td>15</td>
<td>10</td>
<td>2</td>
<td>8</td>
<td>3</td>
<td>8</td>
<td>11</td>
<td>27,28%</td>
</tr>
<tr>
<td>D ($10^7$)</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>13</td>
<td>0</td>
<td>13</td>
<td>100,00%</td>
</tr>
</tbody>
</table>

Value of LC$_{50}$ was $3,55 \times 10^2$ cell/ml.

Table 7. Analysis data of death fish to get LC$_{50}$ of *A. salmonicida* from ATCC

<table>
<thead>
<tr>
<th>Aquarium</th>
<th>Concentration</th>
<th>$\Sigma N$</th>
<th>R</th>
<th>N-R</th>
<th>$\Sigma r$</th>
<th>$\Sigma (n-r)$</th>
<th>Total</th>
<th>$(\Sigma r)/T \times 100%$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A ($10^2$)</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>22</td>
<td>22</td>
<td>0,00%</td>
</tr>
<tr>
<td>B ($10^3$)</td>
<td>3</td>
<td>10</td>
<td>3</td>
<td>7</td>
<td>3</td>
<td>12</td>
<td>15</td>
<td>20,00%</td>
</tr>
<tr>
<td>C ($10^5$)</td>
<td>5</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>8</td>
<td>5</td>
<td>13</td>
<td>61,54%</td>
</tr>
<tr>
<td>D ($10^7$)</td>
<td>7</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>18</td>
<td>0</td>
<td>18</td>
<td>100,00%</td>
</tr>
</tbody>
</table>

Value of LC$_{50}$ was $2,75 \times 10^4$ cell/ml.

Pathological changes of *A. salmonicida* infection showed congestion and necrosis of skin, muscle inflammation, congestion, necrosis, and inflammation of liver, ren, spleen, gill, and heart at the fifth days after infection. All isolates of *A. salmonicida* could grow well in TSA or blood plate agar. Ishiguro, et al., (1986) reported that blood media was the best to grow atypical strain of *A. salmonicida*.
with the colony density reach $10^9$ cell/ml after 2-5 days of incubation at 25°C. Atypical isolate of *A. salmonicida* had beta-haemolysin character that could erythrocyte, lysis hemoglobin was as transparent zone surrounding the colony. Bootsma, et al. (1977); Austin dan Austin (2007) reported that blood media could be used to isolate a difficult and hemolytic bacteria. The pathological characteristic of *A. salmonicida* infection in fish was

Leucopenia, hemorrhage, necrosis, and degeneration of the muscle and other organs (Fuller, dkk 1977; McCarthy, 1980). *Furunculosis* caused inflammation of the skin of fish with chronic infection of *A. salmonicida* (Teska, dkk 1992).

**Conclusion**

The value of LC$_{50}$ from 5 isolates varied between $3,55 \times 10^2$ cell/ml (Isolate from Jambi) and $2,75 \times 10^4$ cell/ml (Isolate ATCC). The pathogenic isolates were from Jambi and Pontianak (LC$_{50}$ : $7,24 \times 10^2$ cel/ml).

Infection of *A. salmonicida* caused congestion and necrosis of skin, muscle inflammation, congestion, necrosis, and inflammation of liver, ren, spleen, gill, and hearth at fifth days after infection.

**References**


