VOL 96 No. 07 JULY, 2019

Print ISSN 0019 - 6479 E - ISSN 0974 - 9365 ₹. 80

# THE INDIAN VETERINARY JOURNAL SINCE - 1924

# Journal of the INDIAN VETERINARY ASSOCIATION ESTD - 1922 Regd. No. Sl. No. 96/1967



No. 11, Pasump Nandanam, Che Tel. : +91 44 24: Email : ivj83@y. BIOL ONLINE : www.ivj.org.in



evar Salai (Chamiers Road), Jadu, India

# THE INDIAN VETERINARY JOURNAL

(Official organ of the Indian Veterinary Association)

Vol. 96

# July 2019

No. 07

# CONTENTS

#### GENERAL ARTICLES :

| Repiratory Diseases in Exotic Pigeons<br>Chemphius Anand Kumar, K. Senthilkumar, S. Hemalatha and M.G.Javathangaraj   | 09   |
|---|------|
| Intivalent Dengue Vaccines Induced Neutralization Antibodies in Rabbits   |      |
| La Rakhma Yustinasari, Rahayu Ernawati, Fedik Abdul Rantam, and Agus Widodo   | 10   |
| avito Evaluation of Entomopathogenic Fungi Metarhizium anisopliae and Beauveria bassiana<br>Azinst House Flies  |      |
| Ranland G.Ponnudurai  | 12   |
| Taxoplasmosis Occurrencein Stray Cat Frombanyuwangi City, Indonesia<br>Altra Yudhana and Ratih Novita Praja   | 14   |
| Perceived Constraints of Farmers in Dairy Farming in Kottayam District of Kerala<br>Ess Elizabeth Jacob and A.S Ambily  | 16   |
| Quality Improvement of Spermatozoa of Rooster Exposed to Heat Stress Treated with<br>Pumpkin Seeds (Cucurbita moschata) and Vitamin E   |      |
| St Elana Rochmi, Herinda Pertiwi and Diyantoro Diyantoro  | 19   |
| Canine Transmissible Venereal Tumour Cases in Tirunelveli Region of Tamil Nadu<br>W. Murugan, T. Sathlamoorthy, A. Ganesan, V. Kumar, Chhavi Gupta, S. Satheshkumar and R.Ramprabhu | 21   |
| Readomonas sp. and Bacillus sp. Culture in Whey Tofu: A Way to Increase Aquaculture Production<br>Wate Hastuti Satyantini, Nur Fauziyah Martiningsih, Adriana Monica Sahidu .       | 2.07 |
| Vira Nurmalia Dewi and Daruti Dinda Nindarwi  | 24   |
| Prevalence of Lentivirus Antibodies in Small Ruminants of South Gujarat   | 27   |
| Detary of Maize Oil on Folicullar Hierarchy and Visceras Weight of Quail (Cortunixcortunix japonica)<br>Herna Pertivi and Tri Bhawono Dadi  | 30   |
| Fresh Water Algae (Cladophoraglomerata) as a New Alternative Protein Sources for Awassi Lambs   |      |
| AL MM. Mani   | 32   |
| Recological Properties of Nasal Mucus of Calves During Periods of<br>Development of Bronchopneumonia  |      |
| Tuny N. Alyokhin, Maksim S. Zhukov, Ivan I. Kalyuzhny, Konstantin Kh. Papunidi,<br>Rahid M. Aslanov and Sergey Yu. Smolentsev   | 36   |
| Pretective Effect of Polygonum minus Leaves Ethanol Extract on Cadmium Chloride-Induced<br>Neration of Aortic Histopathology in Mice (Mus musculus)                                 |      |
| PutriAnggraheni Kusumaningrum, Lita Rakhma Yustinasari, Iwan Syahrial Hamid, Sri Agus Sudjarwo,<br>Kurcoro Puguh Santoso, Chairul Anwar and Agus Widodo                             | 39   |
| Elect of Polypropylene Plastic Residue on Histopathological Changes of the<br>Snal Intestine in Rattus Norvegicus   |      |
| UzRakhmaYustinasari, AuliaPuspa Amaris, Chairul Anwar, JokoLegowo, Hani Plumeriastuti,<br>KweHidayati and AgusWidodo  | 43   |
|   |      |

# The Important Role of Macrobenthos and Phytoplankton: Biological Indicators in Tabuhan Island, East Java, Indonesia

Annur Ahadi Abdillah, Mochammad Amin Alamsjah, Mohammad Faizal Ulkhaq, Hapsari Kenconojati, Darmawan Setia Budi, Suciyono, Muhammad Hanif Azhar, Eka Saputra and Dita Wisudyawati

... 46

#### **CLINICAL ARTICLES :**

| Efficacy of Amikacin in the Management of Recurrent Staphylococcal Pyoderma in Dogs<br>A.Meena, P.Thirunavukkarasu, S. Kavitha and M.Ananda Chilra                               | -   | 49 |
|--|-----|----|
| Carprofen and Pentosan in Combination for Palliative Treatment of Hemangiosarcoma in a Dog:  |     |    |
| A Case Report<br>AmeWidada Steven Taufici en LitaRakhmaVustinasari EkaPramuthaHestianah  |     |    |
| Tri Bhawana Darti and Herinda Perilwi  | and | 50 |
| Extendion of Nexal Ferning Porting Porting   |     | -  |
| Praveen Kumar, Saurabh Tiwari, Arup Kumar Das and Mahesh Kumar   | *** | 52 |
| Dystocia due to Perosomus Elumbis Monster Fetus with Cleft Palate in a Mecheri Ewe<br>K.P. Prabhakaran, C. Srinivasan and S. Manokaran   |     | 54 |
| An Intrasplenic Myelolipoma in a Labrador Retriever<br>T.Mohanapriya, S. Hemalatha and R. Sridhar  |     | 55 |
| Mesotheliomain a Desi Hen-Case Report<br>C.Niranjana, Joanna Kollipillai, R.Bharathi and S.Kavitha   |     | 57 |
| Rectal Transmissible Venereal Tumor in a Dog<br>M. Shiju Simon and N. Pazhanivel   | +++ | 59 |
| Control of Lynxacarus radovskyi Infestation in Persian Cats in Kerala -A Case Study<br>Bindu Lakshmanan, Deepa Chirayath, R.Dhivyabharathi, T.P.Vipin and S.Ajithkumar           |     | 61 |
| Secondary Aspiration Pneumonia in a Calf<br>A.L. Mohammed Ismail, A. Gopalakrishnan, C.S. Arunaman, Mala Shammi, S. Kavitha,<br>D. Sumathi M.G. Javathangarai and Cecilia Joseph |     | 63 |
| Retrieval of Metallic Needle from Neck in a Kangayam Cattle<br>P.A.Ammu, R.Ravi, B. Sudhakara Reddy, K Mohanambal and G Vijayakumar  | -   | 65 |
| Repair of Uterine Tear by Vagino-Uterine Eversion Technique in a Non-Descript Doe<br>K.Ravikumar, M.Selvaraju, S. Prakash and V. Varudharajan                                    | -   | 66 |
| Management of Ring Womb in a Non-Descript Doe – A Case Report<br>A.Reshma, Imama hussain Gudur and A.J.Shankare Gowda  |     | 68 |
| A Rare Case of Congenital Megaoesophagus in a 50 Days Old Non-Descript Puppy   |     |    |
| S. Rajyalakshmi, P.A. Ammu, K.Mohanambal, S.Kathirvel and G.Vijayakumar  |     | 69 |
| SHORT COMMUNICATIONS :   |     |    |
| Cow Side Tests for Detection of Ketosis in Dairy Cows<br>R.Ravi, G.Vijayakuma, K.Mohanambal, S.Sivaraman and B.Sudhakara Reddy   |     | 7  |
| Theileriosis in Pattanam Sheep and its Therapeutic Management<br>K.Jayalakshmi, M.Venkatesan, M.Veeraselvam, S.Yogeshpriya and P.Selvaraj  |     | 7. |
| Successful Management of Paraphimosis in a Buffalo Bull<br>K. Ravikumar, V. Varudharajan, M. Selvaraju and S. Prakash  | -   | 74 |
| Dystocia Due to Dicephalus Fetal Monster in a Buffalo – A Case Report  |     | 1  |
| M.V.V.S.L.Narayana Rao   |     | 70 |
| Association News   |     | 7  |
|  | 94  |    |

Author and Subject Index

6

5

py have been used to treat the TVT but chemotherapy has been shown to be the most effective and easily available practical therapy Otter *et al.* (loc cit.). The chemotherapeutic agents such as vincristine, vinblastine, doxorubicin and cylophosphamide have been used. However, vincristine sulphate intravenous injection (0.025 mg / kg body weight) is considered to be the most effective therapy for canine TVT (Feldman and Nelson, 1996: Murugan *et al., loc cit.*).

### Summary

Proper examination of animals prior to mating in order to eliminate animals with patent infection from the breeding population and strict measures against mingling with stray animals will control the disease. TVT is curable in almost all cases with chemotherapy using vincristine injection. Sustained animal birth control programme of stray dogs shall decrease the incidence of TVT cases to a great extent.

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Indian Vet. J., July 2019, 96 (07) : 24 - 27

# *Pseudomonas* sp. and *Bacillus* sp. Culture in Whey Tofu: A Way to Increase Aquaculture Production

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(Received : February, 2019 46/19 Accepted : March, 2019)

## Abstract

The purpose of this study was to determine the growth of *Pseudomonas* sp. and *Bacillus* sp. grown together on skimmed milk media with the addition of liquid whey of tofu. The results showed that the optimum growth of *Pseudomonas* sp. and *Bacillus* sp. was found at addition 10% of liquid whey tofu and the increased

growth of *Bacillus* sp. faster than *Pseudomonas* sp. The optimum of exponential phase was done at 42 hours with the average number of *Bacillus* sp.  $23.54 \times 10^{10}$  CFU/ml and *Pseudomonas* sp. was  $67.8 \times 10^{9}$  CFU/ml.

**Key words**: *Pseudomonas* sp., *Bacillus* sp., Growing on Liquid Whey Tofu

*Pseudomonas* sp. is commonly used in the aquaculture because of it produces protease, lipase, and amylase enzyme to help the

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process of digestion (Dalahi *et al.*, 2014, Armada and Rhoda, 2016). While *Bacillus* sp. can also produce siderofor to inhibite pathogenic *Vibrio vulnificus* (Sugita *et al.*, 1998). This study purpose was to determining their growth by adding whey tofu in media. Whey tofu contains 0.05% carbohydrates, 9% proteins, 0.69% fat, and  $P_2O_5$  about 228.85ppm (Karina *et al.*, 2016, Asmoro 2008).

# Materials and Methods

Materials used in this research were *Pseudomonas* sp. and *Bacillus* sp. bacteria derived from intensive pond sediments, liquid whey tofu obtained from tofu factory at Pacar Keling, Surabaya, Indonesia. This study was conducted using Completely Randomized Design (CRD) with four treatments and five replications. The treatments used in this research were:

P0:10% TSB + 10% skimmed milk + 6% glucose (control)

P1 : 10% TSB + 10% skimmed milk + 6% glucose + 10% liquid whey tofu

P2 : 10% TSB + 10% skimmed milk + 6% glucose + 20% liquid whey tofu

P3 : 10% TSB + 10% skimmed milk + 6% glucose + 30% liquid whey tofu

TSB media was made by dissolving 30 grams in 1 L of aquadest water, then heated over hot plate until homogeneous. Liquid whey tofu was obtained from tofu factory at Pacar Keling, Surabaya, Indonesia. The liquid whey tofu used in this research were from mixture of a variety of other liquid wastes that flows from the process of making the tofu to the sewer / tub, for further discharge into the river or sewer. The liquid whey tofu was filtered using filter paper to separate the tofu waste with water, then sterilized using an autoclave at 121°C for 15 minutes at 1 atm pressure. *Pseudomonas* sp. and *Bacillus* sp. was done on 20 ml volume test tube with media filling only half of the total volume (10 ml).

*Pseudomonas* sp. and *Bacillus* sp. were calculated for the density using spectrophotometer in 550 nm (Suminto, 2008). *Pseudomonas* sp. and *Bacillus* sp. taken as much 1 ml and inserted into each treatment and incubated for  $1 \times 24$  hours at temperature of  $30-35^{\circ}$ C.

Calculation of bacterial growth in this study was done by calculating bacterial colonies used Total Plate Count (TPC) method (Waluyo, 2007). Data of *Pseudomonas* sp. and *Bacillus* sp. calculated using TPC method and then analyzed using ANOVA (Analysis of Variance) and Duncan test.

# **Results and Discussion**

Data of *Bacillus* sp. which were cultured on skimmed milk media by the addition of liquid whey tofu are presented in Table I.

P1 reaches the optimum point of exponential phase at  $42^{\text{th}}$  hours with a density of  $23.54 \times 10^{10}$  CFU / ml. At the P2 treatment the optimum point of exponential phase occurs faster than P1, that is at 36 hours with  $87.3 \times 10^{9}$  CFU / ml. The optimum point of exponential phase in P3 occurs at  $48^{\text{th}}$  hour with  $64.8 \times 10^{9}$  CFU / ml.

*Bacillus* sp. which were cultured in skimmed milk media with 10% (P1), 20% (P2) and 30% (P3) with additions of liquid whey tofu at the  $12^{\text{th}}$  hour showed different growth compared control treatment (P0). *Lactobacillus* 

**Table I**. Average number of *Bacillus* sp. bacteria from 1 to 48 hour.

| Treatment | Time of Calculation (CFU/mI) |                        |                        |                        |                       |                        |                         |                        |                          |                        |  |
|-----------|------------------------------|------------------------|------------------------|------------------------|-----------------------|------------------------|-------------------------|------------------------|--------------------------|------------------------|--|
| Treatment | 0                            | 1                      | 6                      | 12                     | 18                    | 24                     | 30                      | 36                     | 42                       | 48                     |  |
| P0        | 105                          | 43.6×10 <sup>8 a</sup> | 13.1×10 <sup>8 a</sup> | 20.7×10 <sup>8 b</sup> | 89.8×10 <sup>8a</sup> | 68.2×10 <sup>8 b</sup> | 72.5×10 <sup>8 b</sup>  | 27.0×10 <sup>9 c</sup> | 18.59×10 <sup>10 a</sup> | 99.6×10 <sup>9 a</sup> |  |
| P1        | 10 <sup>5</sup>              | 25.0×10 <sup>7 c</sup> | 10.4×10 <sup>8 a</sup> | 31.2×10 <sup>8 a</sup> | 71.8×10 <sup>8b</sup> | 71.7×10 <sup>8ab</sup> | 88.3×10 <sup>8 ab</sup> | 34.6×10 <sup>9 c</sup> | 23.54×10 <sup>10 a</sup> | 80.0×10 <sup>9 b</sup> |  |
| P2        | 10 <sup>5</sup>              | 31.0×10 <sup>7 d</sup> | 12.0×10 <sup>8 a</sup> | 26.2×10 <sup>8 b</sup> | 92.8×10 <sup>8a</sup> | 57.3×10 <sup>8 b</sup> | 71.0×10 <sup>8 b</sup>  | 87.3×10 <sup>9 a</sup> | 8.90×10 <sup>9 b</sup>   | 27.6×10 <sup>9 c</sup> |  |
| P3        | 105                          | 51.0×10 <sup>7 b</sup> | 87.0×10 <sup>7 a</sup> | 28.3×10 <sup>8 a</sup> | 14.0×10 <sup>8c</sup> | 97.6×10 <sup>8 a</sup> | 10.38×10 <sup>9 a</sup> | 58.3×10 <sup>9 b</sup> | 11.9×10 <sup>9 b</sup>   | 64.8×10 <sup>9 b</sup> |  |

Description: P0: 0% liquid whey tofu, P1: 10% liquid whey tofu, P2: 20% liquid whey tofu, P3: 30% liquid whey tofu. The notation shown with different superscript letters in the same column shows the comparison between treatments having significant differences (p <0.05).

Pseudomonas sp. and Bacillus sp. Culture in Whey Tofu ...

| Treatment | Time of Calculation (CFU/mI) |                     |                     |                       |                       |                       |                       |                       |                        |                       |
|-----------|------------------------------|---------------------|---------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|------------------------|-----------------------|
|           | 0                            | 1                   | 6                   | 12                    | 18                    | 24                    | 30                    | 36                    | 42                     | 48                    |
| P0        | 10 <sup>5</sup>              | 34×10 <sup>7a</sup> | 92×10 <sup>6d</sup> | 67×10 <sup>7d</sup>   | 94×10 <sup>7c</sup>   | 37.1×10 <sup>8b</sup> | 15.8×10 <sup>8b</sup> | 35.9×10 <sup>9a</sup> | 46.2 ×10 <sup>9a</sup> | 30.1×10 <sup>9a</sup> |
| P1        | 10 <sup>5</sup>              | 10×10 <sup>6c</sup> | 35×10 <sup>7b</sup> | 20.0×10 <sup>8a</sup> | 15.4×10 <sup>8b</sup> | 59.7×10 <sup>8a</sup> | 45.8×10 <sup>8a</sup> | 3.3×10 <sup>9b</sup>  | 67.8×10 <sup>9a</sup>  | 57×10 <sup>8c</sup>   |
| P2        | 10 <sup>₅</sup>              | 41×10 <sup>7a</sup> | 17×10 <sup>7c</sup> | 10.8×10 <sup>8b</sup> | 10.7×10 <sup>8c</sup> | 34.5×10 <sup>8b</sup> | 83×10 <sup>7</sup>    | 16.0×10 <sup>9a</sup> | 43.4×10 <sup>9a</sup>  | 19.1×10 <sup>9b</sup> |
| P3        | 10 <sup>₅</sup>              | 60×10 <sup>6b</sup> | 71×10 <sup>7a</sup> | 13.3×10 <sup>8b</sup> | 35.3×10 <sup>8a</sup> | 67×10 <sup>7c</sup>   | 91×10 <sup>7c</sup>   | 23.7×10 <sup>9a</sup> | 20.7×10 <sup>9b</sup>  | 32.0×10 <sup>9a</sup> |

 Table II. Average number of Pseudomonas sp. bacteria from hour 1 to 48 hour.

Description: P0: 0% liquid whey tofu, P1: 10% liquid whey tofu, P2: 20% liquid whey tofu, P3: 30% liquid whey tofu. The notation shown with different superscript letters in the same column shows the comparison between treatments having significant differences (p <0.05).

*paracasei* cultured in liquid whey tofu is able to grow better than on media that contain only glucose (Le *et al.*, 2003).

Bacillus sp. which were cultured on the addition of 10% liquid whey tofu (P1) showed higher growth  $(23.54 \times 10^{10} \text{ CFU} / \text{ml})$  and were more stable compared to others. This is assumed because the content of N and P at the addition of liquid whey tofu 20% and 30% is too excessive, so *Bacillus* sp. unable to absorb N and P. and decrease the growth of bacteria (Zouari *et al.*, 2000).

Growth of *Pseudomonas* sp. was less than *Bacillus* sp. The growth of *Pseudomonas* sp. which were cultured on skimmed milk media with the addition of liquid whey tofu shown in Table II.

At treatment P1, *Pseudomonas* sp. growth faster than P0. Increasing growth in P1 occured at 6<sup>th</sup> hour and the optimum point of exponential phase at 42<sup>th</sup> hour with cell count 67,8×10<sup>9</sup> CFU / ml. *Pseudomonas* sp. which was cultured on P2 showed increased growth in the first hour, but decreased at 6<sup>th</sup> hour. The optimum point of exponential phase at P2 occured at 42<sup>th</sup> hours with the number of cells as much as 43.4×10<sup>9</sup> CFU / ml. The optimum point of the exponential phase at P3 was more slowly than P1, P2 and P3, which occurred at 42<sup>th</sup> hour with 32.0×10<sup>9</sup> CFU / ml.

According Masangkay (2012) the presence of whey tofu in the culture medium could increase the growth of *Mycobacterium tuberculosis*. The addition of 10% (P1), 20% (P2) and 30% (P3) liquid whey tofu in this study also had significant effect on the growth of *Pseudomonas* sp. At  $42^{\text{th}}$  hour *Pseudomonas* sp. which

was cultured on the addition of 10% whey tofu (P1) showed the best treatment.

It was assumed that the nutrient concentration was too much or excessive, so that *Pseudomonas* sp. was unable to use the nutrients present in the media. Most hypertonic solutions (high nutrient levels) could inhibit bacterial growth because it caused plasmolysis of bacterial cells (Alawiyah, 2015).

Bacillus sp. grew faster than Pseudomonas sp. since the differences amount of bacterial cells occurred because of the amount of Carbon (C), Nitrogen (N), Phosphorous (P) and other elements contained in the culture medium different (Wulan *et al.*, 2006). Differences growth of bacteria between Bacillus sp. with Pseudomonas sp. which were cultured together assessed were due to the character of Pseudomonas sp. was non-fermentative bacteria (Suyono and Salahudin, 2011), while Bacillus sp. was fermentative (Whitman, 2009).

# Summary

The results of this study indicated that whey tofu could increase the *Pseudomonas* sp. and *Bacillus* sp growth. This was good finding since the culture of the two bacteria would increase the aquaculture production in the future.

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Indian Vet. J., July 2019, 96 (07) : 27 - 30

# Prevalence of Lentivirus Antibodies in Small Ruminants of South Gujarat

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(Received : October, 2018 365/18 Accepted : January, 2019)

#### Abstract

The study was undertaken to evaluate the presence of lentivirus antibodies in small ruminants of South Gujarat. Out of 416 goats tested 35 (8.41%) and from 127 sheep, 34 (26.77%) animals had antibodies against lentivirus by competitive ELISA. Seropositivity was higher in sheep as compared to goats and it was significant (p<0.05) statistically. Sex-wise analysis revealed, prevalence in male and female goats as 1.65% and 11.19%, respectively and the difference was significant (p=0.0015). Whereas in male sheep 29.27% and in female 25.58% animals were found to be positive and the difference was non-significant (p=0.661) at 95% level of confidence. While combining sheep

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and goats, prevalence in males was 8.64% and in females 14.44 % with an overall prevalence of 12.71 per cent. Overall, higher prevalence was recorded in females as compared to males.

**Key words**: Competitive ELISA; Goats; Lentivirus; Sheep

Small ruminant lentiviruses are slow growing retroviruses which infect a wide range of species including ruminants. They cause progressive degenerative diseases viz; Caprine-Arthritis Encephalitis (CAE) in goats and Maedi-Visna (MV) in sheep. CAE and MV are characterized by lifelong persistence of the causal agent in host monocyte and macrophages. Most infected sheep and goats do not exhibit clinical disease but remain persistently infected and are capable of transmitting the virus via